

# (R)evolution Stings Back: Rethinking Strategies for Conserving Local Biodiversity of the Western Honey Bee (*Apis mellifera*)

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## Abstract

The western honey bee (*Apis mellifera*) occupies an unusual position between domesticated livestock and wild organisms, creating persistent ambiguity in conservation policy. Most current conservation programmes prioritise controlled breeding, phenotypic stability, and lineage integrity, implicitly treating honey bees as populations dependent on continuous human management. While effective at maintaining recognisable breeding lines, such approaches largely bypass the evolutionary processes, natural selection, high colony turnover, and gene flow that historically generated honey bee diversity and local adaptation. Here, we argue that effective honey bee conservation should explicitly integrate managed protection with conservation through natural selection. We synthesise evidence that free-living colonies can persist as self-sustaining populations despite high mortality, and that these colonies provide essential opportunities to observe adaptation to pathogens, climate variability, and resource limitation under native selective regimes. We further emphasise the colony as a dynamic biological system in which host genetics, microbial communities, and pathogens jointly shape performance and evolutionary responses. Traditional and revived forest-based nesting systems, such as tree and log hives, are proposed as practical tools for linking honey bee conservation with broader biodiversity objectives. Finally, we outline a scalable monitoring framework that combines high-throughput phenotypic and genetic approaches to connect managed and free-living populations. We conclude that aligning conservation strategies with evolutionary processes is essential for preserving adaptive potential under rapid environmental change.

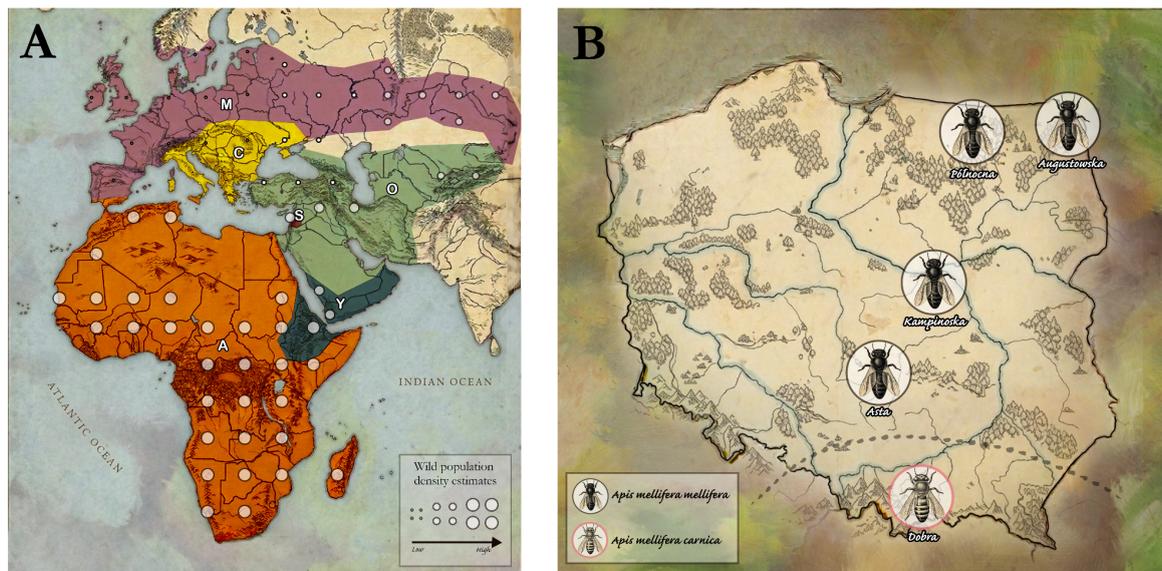
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## 1. Introduction

The western honey bee (*Apis mellifera*) is among the most economically and ecologically important insects worldwide [1], providing essential pollination services to both wild plant communities and agricultural systems [2,3]. It is also one of the most intensively studied animals, serving as a model organism in research on social behaviour [4], cognition [5], host–microbiome interactions [6,7], disease dynamics [8], and gene regulation [9]. Paradoxically, despite comprehensive biological knowledge, there remains considerable ambiguity about what honey bees are from a conservation perspective: a domesticated livestock species maintained almost exclusively in captivity, a wild organism shaped primarily by natural selection, or something in between.

Unlike fully domesticated animals such as cattle or domestic sheep, honey bees occupy a unique position along the domestication continuum [10–12]. Although humans have managed honey bees for millennia, primarily by providing artificial nesting sites and selectively propagating colonies, key aspects of their biology remain largely uncontrolled. Queens mate in flight with multiple males, colonies reproduce by swarming, and managed stocks frequently interbreed with feral or free-living colonies. As a result, honey bee populations across much of their native range consist of dynamic mixtures of managed and free-living populations rather than discrete domestic and wild gene pools.

This biology is central to understanding honey bee diversity. *Apis mellifera* exhibits exceptional phenotypic plasticity and ecological breadth, enabling it to colonise environments ranging from arid Mediterranean landscapes to boreal forests [13]. Over evolutionary time, adaptation to diverse climatic regimes, pathogens, and ecological constraints led to the emergence of numerous subspecies (currently estimated at 33; [14]), structured into seven major evolutionary lineages [13]. These lineages reflect deep phylogeographic divisions that arose in nature, long before modern apiculture, and are associated with distinct suites of morphological, behavioural, and life-history traits (Figure 1). Importantly, this diversity evolved under conditions of intense natural selection combined with high colony mortality, producing locally adapted populations characterised by continual turnover rather than long-term demographic stability.



**Figure 1.**

**A.** Evolutionary diversity and natural distribution of the western honey bee. Map of the native range of *Apis mellifera* showing major evolutionary lineages and approximate subspecies boundaries, including estimated densities of wild colonies (based on [87] and modified). Note that colony densities in Europe are likely substantially underestimated. Biome types that could have historically supported naturally occurring populations of *A. mellifera* are indicated. The map should be interpreted as a conservative approximation of areas suitable for the species prior to major anthropogenic influence.

**B.** Location of protected honey bee lines in Poland. The dotted line indicate the proposed boundary between *A. m. mellifera* and *A. m. carnica* (based on [40]). The Augustowska and Kampinoska lines are maintained within the boundaries of primeval forests.

The long-standing and intimate relationship between humans and honey bees has profoundly reshaped this evolutionary landscape. The widespread translocation of colonies beyond their native ranges, the global queen trade, managed mating practices, and selective breeding have increasingly blurred historical phylogeographic boundaries. While some consequences of these processes are well documented, including the emergence of Africanized honey bees in the Americas [15], pathogen spillover [16], and interactions with native pollinators [16–19], a more pervasive and less visible outcome is the progressive hybridisation and genetic homogenisation of local honey bee populations within their native range [20–22]. Today, many evolutionary lineages persist simultaneously in managed apiaries, semi-managed systems, and free-living colonies, often within the same landscapes [23–25].

Following the global spread of the parasitic mite *Varroa destructor* and other pathogens, which led to large-scale, global colony losses, it has been widely assumed that honey bees can no longer persist outside managed apiaries. This view has strongly influenced conservation strategies, implicitly treating honey bees as a fully domesticated species whose survival depends on continuous human intervention. However, recent findings challenge this assumption. Studies in North America [26], Africa [27] and Europe [28–31] demonstrate that free-living honey bee colonies can persist as self-sustaining populations, albeit with a significant annual mortality rates. In parallel, renewed interest in historical forest beekeeping practices has led to the reintroduction of tree beekeeping, the management of colonies in man-made tree cavities, in managed forests, particularly in

Central and Eastern Europe [32–35]. These developments highlight that honey bee populations exist along a continuum, from fully wild populations to those dependent on regular human management, which otherwise decline rapidly. This duality poses a fundamental challenge for conservation. Should efforts focus primarily on maintaining genetic diversity within managed breeding lines, analogous to the conservation of traditional livestock breeds? Or should conservation prioritise the protection of honey bees as evolving wild populations shaped by natural selection? In practice, most current conservation programmes emphasise controlled breeding, lineage purity and managed mating, often within administrative boundaries (Supplementary Table 1). While these approaches preserve identifiable lineages, they risk decoupling conservation from the evolutionary processes that originally generated and maintained honey bee diversity [36].

Here, we argue that effective honey bee conservation requires an explicit recognition of this hybrid biological reality [37]. We synthesise current conservation strategies across Europe and propose a complementary framework that integrates managed breeding with conservation through natural selection in wild and semi-wild colonies. By combining these approaches with high-throughput phenotypic and genomic monitoring and clearly articulated conservation goals, we aim to reconnect honey bee conservation with its evolutionary foundations and provide a realistic pathway for preserving adaptive potential in a rapidly changing world.

### **Box 1. Glossary:**

#### ***In situ* conservation**

Conservation of organisms within the environment in which they are subject to natural ecological and evolutionary processes. In the context of honey bees, *in situ* conservation should imply exposure to natural selection, including natural mating, pathogen pressure, climatic variability and seasonal resource limitations. Populations maintained exclusively in managed hives with supplemental feeding, routine chemical treatments, and controlled mating or artificial insemination experience substantially altered selective regimes and therefore only partially meet this definition [38].

#### ***Ex situ* conservation**

Conservation of genetic resources outside their natural selective environment, typically involving intensive human control. In honey bees, this includes breeding programmes based on artificial insemination, isolated mating stations, breeding nuclei and cryopreservation of semen. While essential for preserving genetic material, such programmes largely bypass natural selection. In light of recent evidence for the persistence of free-living colonies, many current honey bee conservation efforts function biologically as *ex situ* conservation, even when implemented within the species' native range [38].

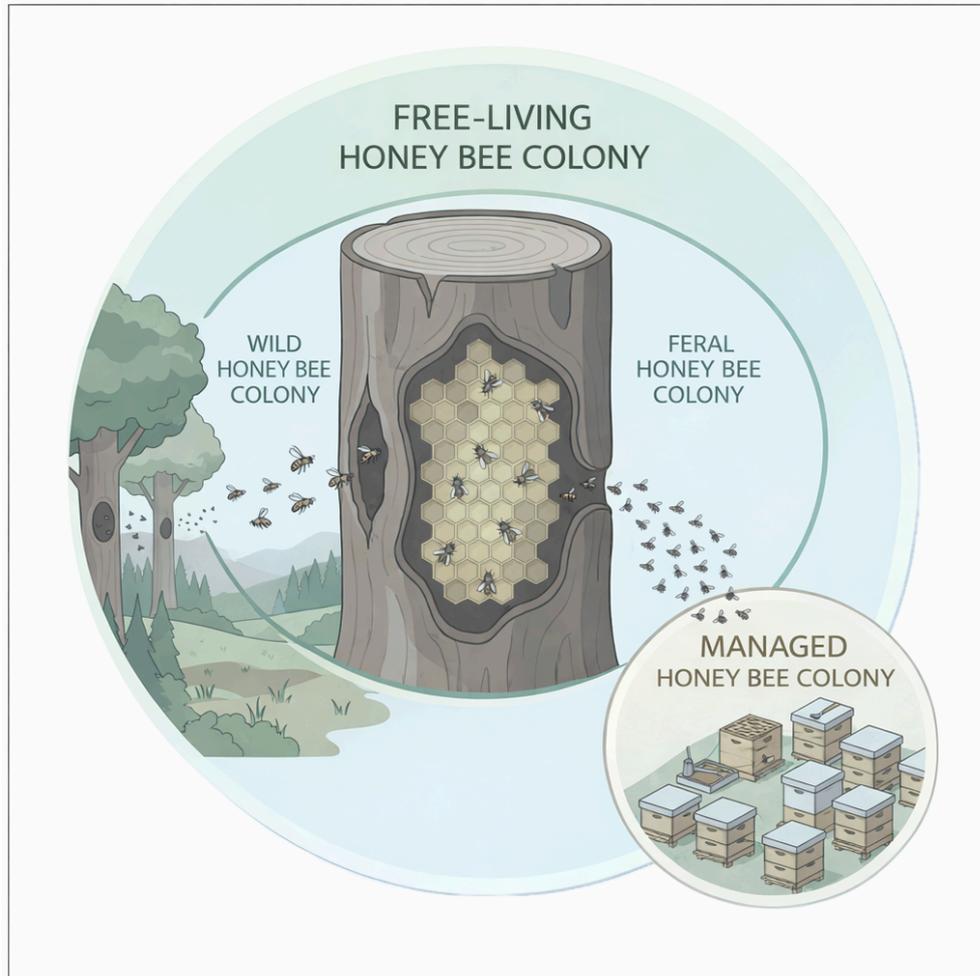
#### **Domestication**

A sustained evolutionary relationship between humans and nonhuman populations.

Traditionally, domestication has been defined as the intentional human control and management of organisms' reproduction, survival, and resources. In its modern, evolutionary formulation, domestication is defined as the adaptation of nonhuman populations to an anthropogenic niche created by human activity, independent of human intent or utility, such that domestic populations become symbiotically associated with humans and are unable to maintain self-sustaining populations outside human-modified environments [12].

### **Feralization**

An evolutionary and ecological process in which a previously domestic population transitions away from dependence on an anthropogenic niche and establishes self-sustaining populations in non-anthropogenic or minimally human-modified environments. Traditionally described as the reversion of domestic organisms to a "wild" state following the loss of human control, feralization is more precisely understood as population-level adaptation to novel environments outside the human niche, which may involve genetic, behavioural, and ecological change and does not imply a return to an ancestral wild condition [39]. In the honey bee context, it is crucial to determine whether we are referring to wild/feral colonies, as these can be blurred (Supplementary Figure 1). Hence, in our text, we are using the term free-living colonies to describe colonies operating that operate without human assistance.



**Supplementary Figure 1.**

Graphical representation of the relationships among free-living, wild, feral, and managed honey bee colonies. Free-living colonies are defined here as an umbrella category encompassing both wild and feral colonies, in contrast to managed colonies.

### **Evolutionary lineage**

A deep phylogeographic division within *Apis mellifera*, reflecting historical patterns of colonisation and isolation. Evolutionary lineages encompass multiple subspecies and are associated with broad-scale differences in morphology, behaviour, and ecological adaptation. Lineages represent evolutionary history rather than management units.

### **Subspecies**

Morphologically and genetically distinguishable populations within an evolutionary lineage, shaped by local adaptation and historical isolation. Under the International Code of Zoological Nomenclature, subspecies represent the only formally recognised rank below species and are assigned valid scientific names (trinomials, e.g., *Apis mellifera mellifera*). However, the decision to designate a subspecies is a taxonomic choice rather than a requirement, typically applied to geographic variants that do not normally interbreed but

remain capable of doing so. In honey bees, subspecies provide biologically meaningful reference units for describing local variation, but their boundaries are often subjective and may be blurred by hybridisation and introgression. Much of the current framework reflects the historical synthesis developed by Friedrich Ruttner (summarised in [40]), based primarily on morphometric and biogeographic criteria.

### **Breeding line**

A zootechnical category defined within a subspecies or artificially created by interbreeding various (often distinct) geographical subspecies, such as Buckfast bees, representing populations selected for specific traits such as productivity, docility or overwintering success. Breeding lines are products of artificial selection and do not necessarily reflect natural evolutionary processes.

### **Core microbiome**

A set of bacterial taxa consistently associated with honey bees across populations and geographic regions. In adult worker bees, the gut microbiome is dominated by five core bacterial lineages: *Snodgrassella*, *Gilliamella*, *Lactobacillus*, *Bombilactobacillus* and *Bifidobacterium*. In addition, several non-core but commonly present taxa, including *Bartonella*, *Commensalibacter* and *Frischella*, are frequently detected and may contribute to variation among colonies. The honey bee microbiome plays a crucial role in host nutrition, immune function and pathogen resistance. A comprehensive overview of the structure and functional significance of the core microbiome is provided by Motta and Moran [41]. For a broader evolutionary and ecological perspective on insect-symbiont interactions, see Łukasik and Kolasa [42].

### **Holobiont**

A functional biological entity consisting of a host organism and its associated community of microorganisms, including symbiotic, commensal and pathogenic taxa, whose interactions collectively influence host physiology, fitness and evolutionary responses [43,44]. In honey bees, the holobiont concept applies primarily at the colony level and encompasses the genetic background of the bees, their socially transmitted gut microbiome, and the assemblage of pathogens and parasites to which colonies are exposed [7,41]. Importantly, the composition of the honey bee holobiont is dynamic: microbial strains and pathogens can be gained or lost over ecological timescales and do not necessarily co-diversify with host lineages [42,45]. The term is therefore used here as an operational framework for understanding how interacting biological components shape colony performance and

adaptation under natural selection, rather than as a claim that the holobiont constitutes a single, indivisible unit of inheritance or selection.

### **Hive vs tree hive vs log hive**

A hive refers to a movable-frame beekeeping structure designed for intensive management and honey production. A tree hive denotes a cavity in a living or dead tree, either naturally formed or intentionally created by humans, as in traditional forest beekeeping practices. Tree hives expose colonies to natural microclimatic conditions, nesting constraints and selective pressures and may function as intermediate systems between managed and free-living colonies. A log hive represents an additional form of cavity-based nesting structure, consisting of a section of a hollowed log intentionally prepared and placed by humans, typically in forests or semi-natural environments. Log hives mimic natural tree cavities in terms of internal volume, insulation and material properties, while allowing some degree of human placement and harvesting, and therefore also constitute an intermediate system between fully managed movable-frame hives and naturally occurring tree cavities.

### **Hybridisation and introgression**

Hybridisation refers to interbreeding between honey bee populations belonging to different subspecies or evolutionary lineages. Introgression describes the persistence and incorporation of genetic material from one lineage into another through repeated backcrossing across generations. Hybridisation has occurred throughout the evolutionary history of *Apis mellifera*, particularly in regions where evolutionary lineages formed natural contact zones. However, extensive introgression driven by recent human-mediated bee movement, especially involving lineages without historical overlap, may disrupt locally adapted gene pools. Whether such introgressed variation is retained or eliminated ultimately depends on the strength and direction of natural selection acting in the native environment [46].

## **2. Current Protection Programs and Strategies**

Across honey bees' native range, a variety of programmes have been established to protect their native biodiversity ([47], Supplementary Table 1). These initiatives are typically organised at the national level and focus on maintaining designated subspecies through controlled breeding, mating isolation, and phenotypic assessment, reflecting a broadly shared understanding of conservation as the preservation of recognisable population types within administrative boundaries. In Supplementary Table 1, we thoroughly reviewed all available information on the conservation programs across the *Apis mellifera* native range.

While these programmes differ in scale and implementation, most rely on comparable tools, including breeding nuclei, isolated mating stations, artificial insemination, and selection based on morphological or behavioural traits. Molecular data are increasingly incorporated, but often remain limited in scope and resolution. As a result, conservation strategies tend to prioritise management stability and phenotypic conformity, rather than explicitly addressing the evolutionary processes that originally generated honey bee diversity.

Poland provides a representative case study illustrating both the strengths and limitations of current approaches. National conservation programmes have a long history, having been established in the 1970s, and currently focus on the protection of two subspecies (*A. m. mellifera* and *A. m. carnica*) and five recognised breeding lines (*A. m. mellifera*: Augustowska, Północna, Kampinoska, Asta; *A. m. carnica*: Dobra) [48]. These efforts have undoubtedly contributed to maintaining locally derived honey bee populations within the apicultural landscape. At the same time, the protected entities are defined primarily as breeding lines shaped by selective improvement and managed reproduction, rather than as populations evolving under natural selection.

A particularly illustrative example is found in the Augustowski Forest, one of the few remaining relatively undisturbed primaeval forest complexes in Europe. Within this region, a protected honey bee line (Augustowska), belonging to the dark European subspecies (*A. m. mellifera*), is maintained as part of national conservation efforts. However, the forest itself spans political borders, extending into Belarus and Lithuania, where comparable forest habitats persist. Despite this ecological continuity, conservation efforts remain fragmented. Collaboration with the Belarusian site is currently absent due to political reasons, and there is no formal framework for data sharing or coordinated monitoring with Lithuania, where honey bees are protected (though outside any dedicated program) in the Dzūkijos National Park. As a result, populations inhabiting a historically continuous landscape are treated as separate conservation units, with limited exchange of information, genetic material or analytical tools.

This example highlights a broader issue: current honey bee conservation operates largely within political and administrative boundaries rather than ecological or evolutionary ones. Consequently, even programmes aimed at protecting “local” bees may fail to capture the full spatial and genetic context of adaptation. Underlying these practical limitations is a more fundamental conceptual ambiguity: **what exactly are current programmes attempting to conserve?** In practice, three potential conservation targets can be distinguished.

(i) *The reconstruction or preservation of bees resembling historical populations.* While theoretically ideal for preserving local gene pools, this approach is largely unattainable in practice due to the lack of reliable historical genetic references.

(ii) *The maintenance of bees adapted to a particular local environment, even as that environment changes under ongoing climate change.* This strategy is well-suited for studying natural adaptation but is inherently associated with high colony mortality. This approach may be interpreted in two ways: either as facilitating the persistence or establishment of free-living colonies under natural environmental conditions, or as maintaining colonies in hives with minimal or no active management interventions, thereby allowing environmental selective pressures to operate with limited human influence.

(iii) *The preservation of populations derived from locally adapted bees but continuously improved through selective breeding and cross-breeding for apicultural performance.* To our knowledge, this is the dominant approach in current conservation programmes. While suitable as a source of genetic material for breeding, artificial selection obscures which components of the original adaptive gene pool are retained or lost.

While all three goals are legitimate, they are rarely stated explicitly, and most programmes implicitly pursue the third strategy. This implicit focus creates a mismatch in time scales: conservation programmes operate on short management cycles driven by breeding seasons, economic constraints and policy frameworks, whereas local adaptation and evolutionary resilience emerge over much longer periods shaped by natural selection. As a consequence, current efforts tend to stabilise phenotypes rather than preserve adaptive potential.

Finally, honey bee conservation remains largely uncoordinated at the continental scale. There is limited harmonisation of monitoring methods, little exchange of genetic reference material, and no shared baseline for assessing admixture or genetic integrity. In the absence of common tools and reference populations, programmes often operate in isolation, protecting phenotypes without robust or comparable assessments of underlying genetic structure. While such efforts have been successful in maintaining managed honey bee diversity within national frameworks, they remain conceptually disconnected from the evolutionary processes that originally shaped *Apis mellifera*. Addressing this gap requires complementary strategies that operate across ecological rather than political boundaries and explicitly integrate natural selection into conservation frameworks. At the international level, the conservation of animal genetic resources formally falls within the mandate of the Food and Agriculture Organisation of the United Nations (FAO) [49], which recognises honey bees within its frameworks and promotes principles such as transboundary cooperation,

documentation of genetic diversity and long-term sustainability. In practice, however, honey bee conservation remains largely decentralised, with limited alignment between national programmes and these international guidelines, leaving FAO initiatives as a conceptual umbrella rather than an operational platform for coordinated conservation of honey bee biodiversity.

### 3. Free-living colonies as a complementary strategy for *in situ* conservation

As already stated, current honey bee conservation strategies are predominantly centred on managed populations maintained through controlled reproduction and selective breeding. While effective for stabilising specific phenotypes, such approaches only partially capture the evolutionary processes that historically shaped *Apis mellifera*. Free-living colonies (defined here as colonies reproducing without routine human intervention) offer a complementary conservation framework in which natural selection, rather than management objectives, remains the dominant evolutionary force.

A key concern with natural reproduction is uncontrolled gene flow from managed bees, which can potentially alter the genetic composition of local populations. Hybridisation between individuals of different subspecies or evolutionary lineages can lead to the incorporation of introduced genetic variants into local populations. However, the evolutionary significance of such gene flow depends strongly on historical context. Genetic exchange between lineages that formed natural contact zones in the past does not necessarily conflict with conservation goals. For example, the presence of genetic components associated with the C evolutionary lineage in north-eastern Polish populations traditionally assigned to the M lineage, as reported by Oleksa et al. [28], may reflect long-standing biogeographic dynamics. Classical work by Ruttner [40] indicates that both lineages were historically present in this region, with their boundaries likely shifting in response to environmental change.

In contrast, the detection of alleles associated with the African A lineage in north-eastern Poland, documented by Oleksa et al. [50] a decade after his original work, represents a different scenario. There is no evidence that bees of this lineage historically contributed to gene pools in these regions, suggesting recent, human-mediated introductions. From a conservation perspective, such gene flow poses a clearer challenge to the maintenance of locally adapted populations.

An open and largely unexplored question is whether natural selection acting under native environmental conditions in wild populations can mitigate the effects of such hybridisation by removing maladaptive introgressed alleles. The efficiency of such filtering

may be constrained in bees, which have been suggested to experience reduced effective population sizes and relaxed purifying selection associated with their life-history traits [51]. If selection nonetheless operates effectively, free-living colonies may function as evolutionary filters, retaining locally adaptive genetic variation while eliminating genetic components poorly suited to the local environment. Crucially, this filtering process requires that natural selection operate without continuous human intervention. Allowing free-living colonies to persist and reproduce under native environmental conditions is therefore not a liability but a prerequisite for evaluating the long-term evolutionary consequences of hybridisation.

### 3.1 Natural selection, swarming and adaptive genomic change

Free-living honey bee colonies persist under continuous, untreated selective pressures, including climate variability, nutritional limitation and pathogen exposure. Importantly, these pressures act on colonies with full reproductive autonomy. Swarming (the primary reproductive strategy of honey bees) often produces multiple daughter colonies, many of which fail [26,52]. This high turnover, far from being a sign of maladaptation, represents a fundamental life-history trait that evolutionarily enabled honey bees to colonise diverse and often harsh environments. Under such conditions, adaptation emerges not through colony longevity but through differential reproductive success across generations. Monitoring wild populations, therefore, enables conservation efforts to focus on adaptive genomic changes, rather than static genetic compositions. Genomic regions associated with survival, disease resistance or behavioural traits can be tracked over time, providing direct insight into evolutionary responses to local selective regimes.

The Gotland “Bond Project” [53] provides a well-known example of this process, demonstrating that honey bee populations can evolve resistance to *Varroa destructor* and resilience to viral infections [54] in the absence of treatment. However, it is crucial to recognise that this experiment involved an isolated population in which both host and parasite underwent evolutionary change. While informative, such systems represent specific evolutionary trajectories and highlight the need to study multiple wild populations across heterogeneous landscapes to capture broader patterns of adaptation.

Together, these considerations point toward a future in which free-living honey bee colonies are not only conserved but systematically observed as natural experiments in evolution. Rather than relying on isolated case studies, networks of free-living colonies distributed across environmental gradients could be monitored over time to identify parallel and lineage-specific adaptive responses. By combining repeated genomic sampling with ecological and phenotypic data, it becomes possible to observe adaptation *in statu nascendi*

and to assess the tempo and mechanisms by which honey bees respond to novel selective pressures.

Crucially, free-living colonies need not remain isolated from applied apiculture. Individuals or genetic material sampled from naturally surviving populations can be incorporated into breeding programmes in a controlled manner, providing a reservoir of adaptive variation shaped by natural selection. Artificial selection can then proceed independently toward economically and socially desirable traits, such as reduced aggression, diminished swarming tendency or improved manageability. At defined intervals, survivors from these breeding programmes may be reintroduced into *ex situ* conservation lines (if no wild population exist), allowing adaptive traits identified in the wild to be combined with traits favoured in managed systems. In this way, natural and artificial selection are not treated as opposing forces, but as complementary processes operating on different time scales and serving distinct, yet interconnected, conservation goals.

### 3.2 Microbiome dynamics as an overlooked component of adaptation

The honey bee gut microbiome has long been regarded as one of the best characterised and most stable symbiotic systems among insects. Honey bees' microbiome (see Glossary) is transmitted socially shortly after adult emergence and is consistently detected in healthy individuals across populations. The core microbiome is largely conserved across corbiculate bees, although some lineages have lost specific taxa and is thought to have originated early in the evolution of this clade, around 80 million years ago, underscoring its deep evolutionary association with social bees [55].

This apparent stability, however, conceals substantial evolutionary and ecological dynamics occurring below the genus level and observed in other insects characterised by ancient symbiosis with bacteria [42]. While the taxonomic composition of the microbiome is broadly conserved, recent comparative genomic analyses across multiple *Apis* species demonstrate that honey bee gut symbionts do not strictly co-diversify with their hosts. Instead, symbiont gain and loss, host switching and lineage-specific turnover of bacterial strains play a major role in shaping these communities. Using a phylogenomic framework, Prasad et al. [56] showed that signals of host-symbiont co-diversification are weak and often indistinguishable from patterns expected by chance, in marked contrast to mammalian systems, where co-diversification is pronounced. Even within core bacterial genera shared across all honey bee species, only subsets of strains show phylogenetic congruence with host lineages, indicating repeated acquisition, loss and exchange over evolutionary time.

Taken together, these findings suggest that the honey bee microbiome represents a dynamic genetic reservoir rather than a static, co-evolved entity. Within the holobiont

concept, functional traits encoded by microbial strains, including metabolic capabilities, immune modulation and pathogen resistance, may therefore be gained or lost on ecological timescales, potentially contributing to colony-level performance under local environmental conditions. Understanding the adaptive significance of such dynamics requires systems in which microbiome composition is shaped primarily by natural ecological processes rather than routine human intervention.

In this respect, wild colonies provide a critical contrast to managed hives. Managed bees are routinely exposed to chemical treatments against *Varroa destructor*, antibiotics (banned in the EU), artificial diets and commercial probiotics, all of which can perturb microbial community structure and obscure natural host-microbiome interactions. In free-living colonies, by contrast, microbiome assembly is driven by natural foraging, environmental exposure, social transmission and colony-level selection, allowing the fitness consequences of microbial variation to be expressed under native selective regimes.

An important and largely unexplored question concerns the extent of microbiome exchange among sympatric corbiculate bees, including honey bees and bumblebees. Shared floral resources may facilitate interspecific transmission of microbial strains, with potential implications for both local adaptation and disease dynamics. Whether such exchanges buffer colonies against environmental stress, contribute to adaptive flexibility, or promote pathogen spillover remains unknown. Addressing these questions requires access to wild colonies embedded within natural pollinator communities, reinforcing their value not only for honey bee conservation but also for understanding the broader ecological and evolutionary dynamics of pollinator-associated microbiomes.

### 3.3 Tree hives as multi-trophic biodiversity hotspots

Tree hives, carved directly into the trunks of living trees, and log hives, carved into logs and subsequently attached to living trees, represent artificial cavities that closely mimic natural tree hollows. Such cavities are a limiting resource in many forest ecosystems, particularly in managed forests where old, senescent trees and standing dead wood are routinely removed for economic or safety reasons. While those hives are primarily carved to provide nesting sites for honey bees, their ecological function extends far beyond a single species.

Cavity structures of this type provide suitable nesting and refuge sites for a wide range of organisms. In addition to honey bees, tree/log hives can be occupied by other social insects, including wasps and hornets, as well as a diverse assemblage of invertebrates such as spiders, mites, beetles and saproxylic insects associated with decaying wood. These invertebrate communities contribute to local food webs, nutrient cycling and overall forest biodiversity. Importantly, the ecological role of tree/log hives is not

limited to invertebrates. They may also serve as nesting or shelter sites for vertebrates, including cavity-nesting birds and small mammals such as dormice, particularly in landscapes where natural cavities are scarce.

Field observations as well as historical descriptions indicate that only a part of the installed tree hives or log hives are occupied by honey bee colonies at any given time. For instance, at the end of the 18th century in Białowieża Forest (North-east Poland), among 6601 tree hives, only 632 were occupied (8.7% overall occupancy, 0.2 colony per square kilometre) [57]. Whereas modern estimates from Kotlina Kłodzka (South-western Poland), where 1000 loghives have been established, show that up to 25% loghives below 600 meters above sea level are occupied (unpublished data), and in Spała (Central Poland) between 2012 and 2022, the mean occupancy was equal to 45% [32]. This would be consistent with estimates from the Shulgan-Tash Nature Reserve in Southern Ural, where the occupancy is estimated at around 30% [58]. Consequently, the majority of tree hives remain available as potential nesting or refuge sites for other organisms. From an ecological perspective, this represents a natural turnover of occupying species, with different taxa interacting and replacing one another over time, highlighting the broader value of tree/log hives as dynamic, multi-species habitat structures.



## Figure 2.

Tree and log hives as multi-trophic biodiversity hotspots. Artificial cavities created as tree hives or log hives provide nesting and refuge sites for diverse forest organisms in addition to honey bees. These structures support vertebrates, including dormice (*Glis glis*, *Dryomys nitedula*), pine marten (*Martes martes*) and cavity-nesting birds (*Parus major*), as well as invertebrates such as wasps (*Vespula vulgaris*) and hornets (*Vespa crabro*), saproxylic beetles (*Dendrophilus punctatus*, *Dermestes lardarius*) and Lepidoptera (*Scoliopteryx libatrix*, *Aglais io*). By providing stable cavity habitats where natural hollows are limited, tree and log hives function as biodiversity hotspots supporting both vertebrate and invertebrate communities.

Viewed in this light, tree/log hives can be considered biodiversity hotspots that enhance structural complexity in both old-growth forests and intensively managed production forests. In the latter, where suitable natural cavities are often absent, those hives may partially compensate for habitat loss by providing long-lasting, spatially stable nesting opportunities (which distinguish them from nest boxes aiming to host mainly honey bees). Importantly, this function aligns honey bee conservation with broader forest biodiversity goals, reducing potential conflicts between pollinator protection and the conservation of wild fauna. As such, the deployment of tree/log hives has the potential to act as a low-cost, multifunctional conservation tool that benefits honey bees while simultaneously supporting wider ecosystem integrity.

### 3.4 Risks, controversies and the need for active monitoring

The inclusion of free-living honey bee colonies in conservation strategies remains contentious. A central and ongoing debate concerns the potential negative effects of honey bees on wild pollinators, particularly through competition for floral resources and pathogen transmission. This concern is well-founded in the context of managed beekeeping, where high densities of colonies are often concentrated within single apiaries. Coupled with the honey bee's evolutionary strategy of intensive resource accumulation and long-term storage of nectar and pollen, such densities can result in strong competitive pressure on other pollinators and local floral communities.

However, it is essential to distinguish between managed apiaries and free-living honey bee colonies. The latter are naturally dispersed across landscapes and occur at substantially lower densities. Empirical estimates suggest an average natural density significantly varies across the current honey bee worldwide range and is estimated to be the lowest in Europe (0.26/km<sup>2</sup>) and is followed by Northern America (1.4/km<sup>2</sup>), Oceania (4.4/km<sup>2</sup>), Latin America (6.7/km<sup>2</sup>) and Africa (6.8/km<sup>2</sup>) [10], orders of magnitude below those typical of managed systems. At such densities, honey bees function as native components of pollination networks rather than as dominant exploiters of floral resources. While human activities have dramatically increased honey bee abundance in many regions,

the species itself evolved as part of native ecosystems and historically coexisted within diverse pollinator communities. Conservation strategies that aim to restore or mimic natural colony densities should therefore not be equated with intensive beekeeping practices.

A second major concern, particularly among beekeepers, is that free-living colonies may act as reservoirs of pathogens and parasites, posing a threat to managed bees. While this perception is widespread, empirical evidence demonstrating that free-living colonies represent a disproportionate or unmanageable source of disease for apiaries remains limited [59]. Importantly, disease dynamics in free-living colonies differ from those in managed systems: colonies experiencing severe pathogen pressure often collapse, and their nests are rapidly cleared by scavengers and decomposers, reducing the persistence of infectious agents within cavities. This contrasts with managed colonies, which are often maintained despite high pathogen loads through chemical intervention, potentially altering pathogen evolution and transmission dynamics.

Nonetheless, the inclusion of free-living colonies in conservation frameworks should not rely on a purely hands-off approach. Active monitoring is essential. Free-living colonies designated as conservation units should be regularly assessed for health status, pathogen presence and genetic composition. Where monitoring indicates a demonstrable risk to surrounding managed populations, targeted intervention (such as colony removal and cavity disinfection) may be needed. Such measures ensure that conservation goals do not conflict with apicultural practice and help maintain trust among stakeholders.

Taken together, these considerations underscore a central requirement for integrating free-living colonies into honey bee conservation: the availability of robust, scalable tools for genetic, health and microbiome monitoring. Without such tools, risks cannot be reliably assessed or managed. The development of high-throughput, cost-effective monitoring approaches, therefore, represents a prerequisite for responsible implementation and forms the focus of the following section.

## **4. Tools for modern conservation monitoring**

Effective conservation monitoring requires tools that are scalable, reproducible over long time frames and informative about biologically meaningful variation. Although methodological development, their caveats, costs and level of laboratory difficulty deserve their own review, here we propose a technology-oriented framework that reflects both analytical resolution and practical feasibility. This progression moves from high-throughput, low-cost screening approaches toward increasingly detailed genome-scale analyses, with intermediate tools serving as bridges rather than stand-alone solutions.

## 4.1 High-throughput amplicon-based screening

Amplicon-based approaches provide a practical foundation for large-scale conservation monitoring. Their core strength lies in the ability to process large numbers of samples in parallel while maintaining low per-sample costs and relatively simple analytical workflows. When designed thoughtfully, amplicon-based assays can simultaneously capture information on host genetic affiliation, symbiont composition and pathogen presence, making them well-suited as first-line tools.

For host affiliation, phenotypic approaches such as wing morphometrics remain an important complementary component. Wing venation patterns have long served as biologically meaningful proxies for lineage and subspecies affiliation [60]. Recent advances in computer vision and machine learning enable automated landmark detection and pattern recognition, dramatically increasing throughput and reducing operator bias. AI-assisted morphometric pipelines also allow decentralised image acquisition, including contributions from beekeepers and conservation practitioners, while maintaining centralised processing and quality control. This combination expands spatial and temporal coverage but introduces new challenges, notably variation in image quality and acquisition protocols. These limitations can be mitigated through standardised imaging guidelines, automated quality filtering and continuous model retraining using curated reference datasets. Fortunately, such a tool is now being developed and tested ([61,62], Krajewski et al., in prep., ).

At the molecular level, targeted amplicon sequencing offers a flexible entry point for both host and microbial screening. Microsatellite markers, despite well-known limitations [63,64], illustrate the value of targeting highly informative loci. When coupled with high-throughput sequencing rather than fragment-length scoring, microsatellite alleles can be treated as sequence variants, preserving compatibility with established analytical frameworks while substantially increasing resolution and throughput [65]. Importantly, conservation-relevant inference typically operates at the colony rather than the individual level. Honey bee colonies function as superorganisms [66], and bulk DNA extractions from multiple workers allow cost-effective reconstruction of allele presence and approximate frequency distributions without individual genotyping [67,68].

The same logic extends naturally to symbiont and pathogen monitoring. Multi-locus amplicon sequencing targeting bacterial 16S rRNA regions, fungal markers and pathogen-specific loci enables parallel assessment of microbial community composition and pathogen presence within a single workflow. Recent studies have demonstrated the feasibility of such multi-target designs, including simultaneous amplification of multiple bacterial 16S regions together with host mitochondrial markers [69], as well as combined host, bacterial and fungal profiling [70]. The incorporation of synthetic spike-ins further allows

estimation of absolute microbial abundances, addressing a key limitation of relative-only community profiling [71]. Absolute load is particularly relevant in conservation contexts, where changes in total microbial or pathogen burden may carry biological significance independent of compositional shifts[72].

Several caveats warrant explicit acknowledgement. Amplicon-based approaches are inherently reduced-representation methods and are sensitive to primer choice, amplification biases and marker resolution [73]. Pathogen detection may be uneven across taxa, and low-abundance organisms can escape detection [69]. However, these limitations are technical rather than conceptual. Iterative marker optimisation, inclusion of positive controls and periodic validation against genome-scale data provide realistic paths toward improving robustness while preserving scalability.

## 4.2 Bridging approaches: ddRAD-seq and SNP arrays

Reduced-representation genomic approaches, such as ddRAD-seq and SNP arrays, have historically played a central role in advancing honey bee population genomics [74,75]. These methods enabled genome-wide sampling of variation at a time when whole-genome sequencing remained prohibitively expensive, and they contributed substantially to our understanding of population structure, admixture and selection.

In contemporary conservation monitoring, however, their limitations are increasingly apparent. ddRAD-seq datasets are sensitive to protocol-specific choices, including restriction enzymes, size-selection windows and bioinformatic filtering, which complicates comparability across studies and time points [76,77]. Allele dropout due to polymorphisms at restriction sites introduces non-random missing data, particularly problematic when comparing genetically divergent populations or tracking introgression [78,79]. SNP arrays, while technically robust and cost-effective, suffer from ascertainment bias and limited flexibility, constraining their ability to capture rare, novel or population-specific variation [80,81].

These considerations do not negate the value of ddRAD-seq or SNP arrays for specific applications. Rather, they suggest that such approaches are best viewed as transitional tools that helped bridge the gap between marker-based studies and genome-scale sequencing, rather than as long-term platforms for conservation monitoring.

## 4.3 Genome-scale approaches: genomes and metagenomes

Whole-genome sequencing (WGS) provides the most comprehensive view of genetic variation, capturing single-nucleotide polymorphisms, structural variants and signatures of selection across the genome [82,83]. Declining sequencing costs increasingly position WGS

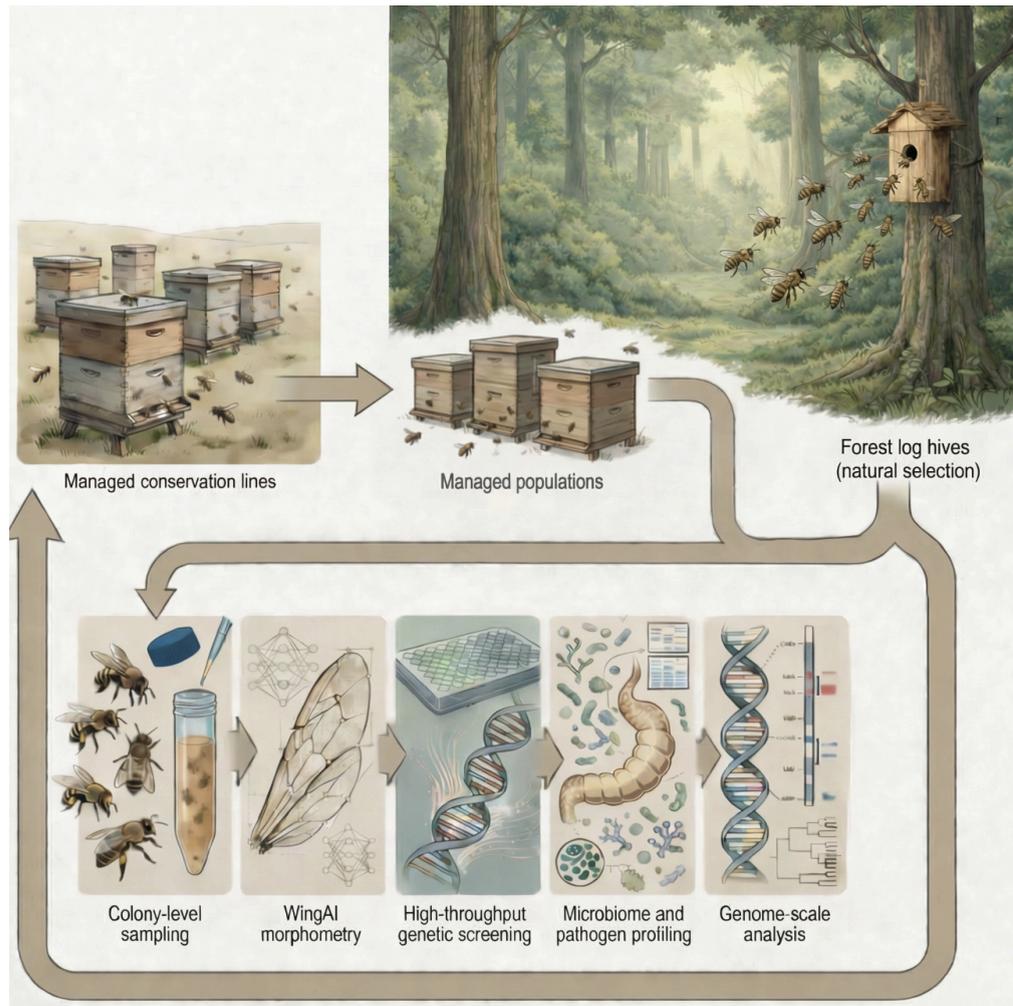
as a central component of conservation genomics, but its effective use requires careful integration with lower-resolution screening tools.

Shallow WGS offers a pragmatic entry point for defining conservation units and assessing genetic structure among populations or management lines. In Poland, for example, multiple breeding lines of *Apis mellifera mellifera* are currently maintained under conservation programmes. Shallow genome sequencing can objectively test whether these lines represent genetically differentiated units or form a largely homogeneous subspecies, directly informing conservation priorities. In this role, WGS functions as a decision-support tool rather than a routine monitoring assay.

Beyond single-reference analyses, pangenome frameworks provide a more realistic representation of genomic diversity by incorporating core and accessory variation across populations or lineages [84]. Reliance on a single linear reference genome risks obscuring structural variants, gene presence-absence polymorphisms and lineage-specific sequences, particularly in evolutionarily diverse taxa. Hybrid genome assembly strategies combining long reads for structural resolution with short reads for polishing offer practical routes toward constructing high-quality pangenomes [83,85]. While resource-intensive, such infrastructure investments yield long-term benefits by improving mapping accuracy and interpretability for all downstream analyses.

Genome-scale approaches also introduce important trade-offs. Individual-level sequencing maximises resolution but scales poorly, whereas pooled sequencing (Pool-Seq) provides cost-effective estimates of allele frequencies at the colony or population level (refs). Pooling reduces sensitivity to rare variants and limits haplotype inference, but aligns well with the colony-level focus of conservation monitoring. Similarly, metagenomic sequencing that captures host, symbiont and pathogen genomes simultaneously offers unparalleled biological insight but demands substantially higher sequencing depth and analytical complexity.

These limitations underscore the importance of selective deployment. Genome-scale and metagenomic analyses are most effective when guided by prior screening that identifies populations, colonies or symbiont variants of particular interest. Used in this way, they represent a natural analytical endpoint that refines and contextualises patterns detected through scalable amplicon-based monitoring rather than replacing it.



**Figure 3.**

Conceptual framework for integrating managed honey bee conservation with natural selection and high-throughput monitoring. Protected breeding lines are maintained within managed populations, while subsets are introduced into forest log hives to experience natural selective pressures. Both managed and forest-resident colonies are monitored using a shared, multi-level analytical pipeline, with progressively fewer samples subjected to increasingly detailed analyses. Information gained from these complementary pathways informs conservation and breeding decisions, aligning preservation efforts with ongoing evolutionary processes.

## 5. Proposed implementation and conclusions

We argue that effective honey bee conservation should rest on two complementary pillars. The first encompasses established protection and breeding programmes based on managed populations, controlled mating and long-term maintenance of recognised lineages. These efforts have been instrumental in preserving phenotypic and genetic diversity and will continue to play a central role in conservation. However, their objectives must be stated explicitly, including whether the primary aim is the preservation of historical genetic identity,

the maintenance of breeding resources, or the enhancement of traits relevant to contemporary apiculture.

The second pillar involves conservation through exposure to natural selection within native environments. Allowing colonies to persist, reproduce and fail under natural ecological pressures offers a unique opportunity to observe how behaviour, physiology, microbiomes and genomes respond to pathogens, climate and resource variability. Such approaches have already yielded important insights, for example, in Gotland settings, demonstrating their value for understanding adaptive processes that are difficult to capture in fully managed systems. However, the implementation of such an approach needs prior activities. First, we should begin by determining whether a stable, self-sustaining free-living population exists in the focal region. This requires assessing colony density, long-term nest occupancy and genetic structure to distinguish wild populations from transient feral colonies repeatedly replenished by managed apiaries. If a wild population is present, conservation should prioritise *in situ* habitat protection, safeguarding nesting sites, establishing buffer zones limiting the introduction of non-local subspecies, and minimising artificial selection so that adaptive processes can continue under natural conditions. If no stable population exists, but the environment retains ecological potential, a rewilding-oriented strategy may be implemented: establishing minimally managed apiaries based on locally derived or historically native stock, promoting natural selection under semi-natural conditions, and gradually facilitating the formation of self-sustaining populations through swarming and provision of near-natural nesting structures. In both cases, the primary objective is long-term ecological resilience rather than strict maintenance of predefined genetic identities.

Crucially, these two pillars shouldn't operate independently. Instead, their integration offers a powerful framework in which protected lineages can be evaluated under natural selective regimes, and naturally selected traits can be identified, characterised, and, where appropriate, reintegrated into managed populations. The analytical approaches outlined in this work provide the tools necessary to monitor such dynamics in a systematic and scalable manner.

Poland offers a particularly suitable context in which to implement and test this integrated approach. On the one hand, the country has a long-standing and well-developed honey bee protection programme. On the other hand, it retains substantial forest infrastructure, including large and relatively intact forest complexes such as the Augustów Forest and, to a lesser extent, Kampinos National Park. Importantly, recent initiatives have already begun to explore the reintroduction of honey bee colonies into forest environments, creating a foundation upon which more coordinated efforts can be built [86].

We propose that these currently separate activities be brought together through the establishment of forest-based log hives colonised with material derived from protected

breeding lines. By monitoring both managed and forest-resident colonies over time, it becomes possible to track how genetic composition, behaviour, symbiotic communities and pathogen profiles change under contrasting selective regimes. Such an approach would allow direct comparison between conservation focused on preservation and conservation that explicitly incorporates natural selection.

Naturally, implementing this vision requires a robust genomic and analytical foundation, including the establishment of reference genomes, pangenomes and standardised monitoring pipelines. While this represents a substantial investment, it also presents a rare opportunity to move beyond conceptual discussions and to develop a living, evolving conservation framework. More broadly, the approach outlined here is not intended as a universal blueprint, but as a demonstration that by aligning evolutionary thinking with modern monitoring tools, honey bee conservation can become both more realistic and more forward-looking.

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### Supplementary Table 1.

Summary of conservation programs and strategies for *Apis mellifera* across its native range, including conservation approach, target subspecies, and key measures. Methods are classified as in situ, ex situ, or combined approaches. "No data found" indicates lack of available information.

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