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Competition assessment protocol



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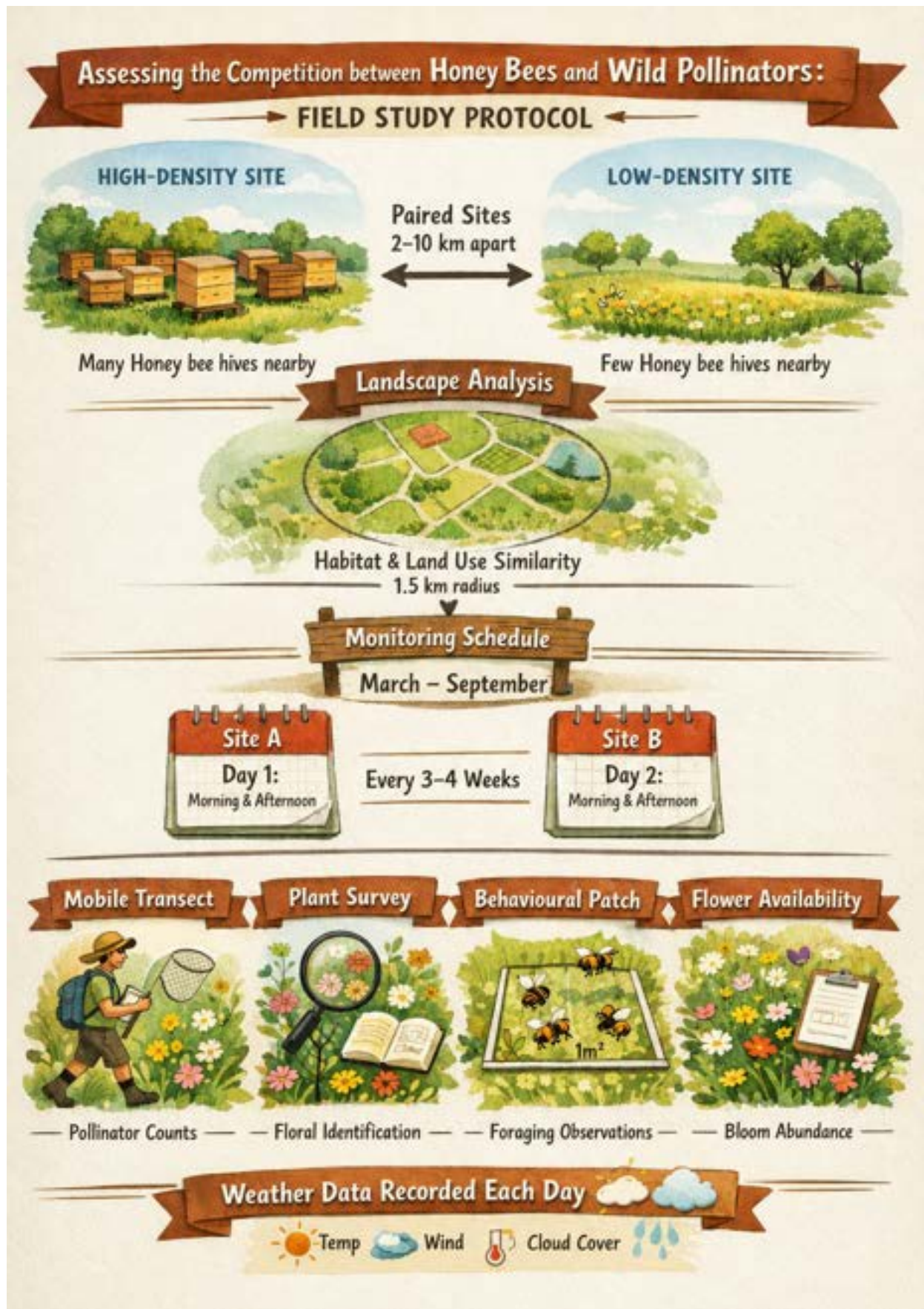
This protocol was developed within Work package 8 – Analysis of plant-pollinator-pathogen networks to assess the impact of beekeeping on wild pollinators. It supports a pan-European field study on the effects of honey bee colony density on wild pollinator abundance, diversity, and foraging behaviour, taking into account land use and floral resource availability.



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Task 8.1 - Assessing the competition between honey bees and wild pollinators

FIELD PROTOCOL



STUDY DESIGN

Site selection:

Monitoring sites are chosen to capture contrasting conditions of **honey bee colony density** while maintaining similar landscape and environmental features. Two types of sites are established:

- **High-density sites:** Areas with a high number of honey bee colonies, including possibly the institutional apiary, which provides logistical advantages and precise hive data.
- **Low-density sites:** Areas with fewer honey bee colonies in the surrounding landscape.

Sites are paired at **2–10 km distance** to ensure independence while keeping comparable conditions.

Colony density thresholds are set according to national context, with actual honey bee abundance verified during field surveys.

A **landscape analysis** within a 1.5 km radius ensures similarity between paired sites, using the CORINE Land Cover classification system. Georeferencing, hive numbers, habitat types, and land-use percentages are recorded to support subsequent analyses.

Timing:

- Sites are monitored **monthly from March to September**.
- Each site is visited on **two separate days per month**, within the same predefined sampling week.
- Monitoring includes **morning and afternoon sessions**, adjusted for weather and logistical constraints.
- A minimum interval of approximately three weeks is maintained between visits to each site.

MONITORING PROTOCOL

Each monitoring day includes **four complementary actions**, with date, time, and weather conditions recorded:

1. Mobile Transect

- A 200 m × 1.5 m transect is surveyed to record all pollinators visiting flowers, including honey bees and wild species.
- Transects are surveyed twice per day (morning and afternoon) under suitable weather conditions.
- Insects difficult to identify are captured temporarily for closer examination; specimens may be released or preserved according to ethical standards.
- Observed pollinators are classified into morphotypes and functional groups for further analysis.
- Transects are georeferenced to allow spatial tracking of pollinator activity.

2. Plant Survey

- Flowering plants within the transect are identified to species level whenever possible, or otherwise at genus/family level.
- Abundance is estimated using predefined presence classes: **Very rare, Rare, Occasional, Frequent, Dominant**.
- Morning surveys capture the main flowering species; afternoon checks account for flowers that open or close during the day.
- Uncertain taxa are labelled systematically (e.g., “Asteraceae sp.1”) to allow consistent plant–pollinator network analyses.

3. Behavioural Patch Observations

- A **1 m² quadrat** is placed in a sunny area near the transect to observe **wild pollinator foraging behaviour**. Honey bees are not followed.
- Observers record:
 - Number of floral units visited per plant species
 - Duration of each visit
 - Any aggressive interactions between insects (contact, disturbance, displacement, fights)

- Observations focus on the plant species most visited by honey bees, to detect potential competitive effects.
- Pollinators are identified at least to functional group, and when possible, to family, genus, and sex.

4. Total Flower Availability Assessment

- **Standardized plots:** Ten 1 m² plots along the transect are surveyed to count floral units for all species present.
- **Random walk survey:** A 30-minute survey within ~100 m radius around the transect captures additional plant diversity and abundance.
- For both methods, abundance classes are recorded, and unidentified taxa are noted at genus/family level for consistency.

Pathogen Sampling (Italy Only)

- Conducted at the **two Italian sites** three times per year (April, June, September).
- Based on transect observations, the most visited flowering plant species and its two most abundant pollinator taxa (plus honey bees) are sampled.
- Insects and flowers are collected, stored in sterile tubes with cooling packs, and transported to the lab for storage at –80 °C.
- Sampling aims to study potential pathogen transmission between managed and wild pollinators.

Functional group	Morphotype	Size / body length	Details and (examples)
Bees	Honey bee	~12 mm	(<i>Apis mellifera</i>)
	Bumblebee		(<i>Bombus</i>)
	Small wild bee	< 12 mm	Smaller than a honey bee (<i>Lasioglossum, Hylaeus</i>)
	Medium wild bee	~12 mm	Same size of a honey bee (some <i>Andrena, Halictus</i> spp., several Megachilidae)
	Large wild bee	> 12 mm	Bigger than a honey bee (<i>Xylocopa, Anthophora</i>)
Flies	Small hoverfly	< 12 mm	Smaller than a honey bee (<i>Sphaerophoria, Episyrphus balteatus</i>)
	Large hoverfly	≥ 12 mm	Same size or bigger than a honey bee (<i>Eristalis tenax, Volucella zonaria</i>)
	Small bee fly	< 12 mm	Smaller than a honey bee (<i>Bombylius minor</i>)
	Large bee fly	≥ 12 mm	Same size or bigger than a honey bee (<i>Bombylius major</i>)
	Other fly	–	Not a bee fly or hoverfly (<i>Musca domestica, Calliphora</i> spp.)
Lepidopterans	Small butterfly	< 30 mm (wingspan)	(<i>Polyommatus icarus, Lycaena phlaeas</i>)
	Medium butterfly	30–60 mm (wingspan)	(<i>Vanessa atalanta, Pieris brassicae</i>)
	Large butterfly	> 60 mm (wingspan)	(<i>Papilio machaon, Nymphalis polychloros</i>)
	Small moth	< 10 mm	(<i>Crambus</i> spp.)
	Large moth	≥ 10 mm	(<i>Noctua pronuba, Autographa gamma</i>)
	Sphingidae	–	(<i>Macroglossum stellatarum, Hyles euphorbiae</i>)
Beetles	Small beetle	< 5 mm	(<i>Anthrenus</i> spp.) pollen beetles excluded (<i>Meligethes, Brassicogethes</i>)
	Medium beetle	5–10 mm	(<i>Oedemera nobilis</i>)
	Large beetle	> 10 mm	(<i>Cetonia aurata, Trichius fasciatus</i>)
Wasps	Social wasp	–	(<i>Vespula vulgaris, Polistes dominula</i>)
	Solitary wasp	–	(<i>Sphex, Ammophila, Pompilus</i> spp.)



Honey bees



Bumblebees



Wild bees



Hoverflies



Bee flies



Other flies



Butterflies



Moths



Hawk moths



Beetles



Social wasps



Solitary wasps