

Simultaneously applying Darwinian selection for honey bee survival in 8 European countries

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Introduction

A sustainable approach to bee health is to foster natural resistance to *Varroa* (*V. destructor*) in European honey bees (*A. mellifera*). The Darwinian Black Box protocol propagates colonies left untreated against *Varroa* and selects for reproduction, growth, and survival. It has shown long-term persistence in the Netherlands, though its broader applicability remains to be tested.

Objectives

Testing the Darwinian Black Box protocol across different climates and subspecies.

→ evaluate its potential as a sustainable beekeeping strategy.

We focus on identifying early indicators of success (survival):

- Genetic traits
- Developmental patterns
- Behaviour

Methods

Locally adapted *A. mellifera* subspecies (Map) are managed using the

Darwinian Black Box protocol (see right):

• **In brief:** Genetically diverse colonies → split into 4 nukes (T1–T6) → virgin queens mated on isolated islands (T6–T8) → winter survivors (T) propagated annually (2024–2027) → selection on survival, reproduction, growth

Monitoring: Automated hive weight data (BEEP, NL); annual worker genetic analyses; responsiveness to vibrational stimuli (see poster: Knocking on a honeybee hive); colony-level aggression assays.

• **Analysis:** Regional comparisons to test robustness & limits of DBB and identify early signatures of colony success.



From North – South: Norges Biokontakt For  ning (NB), Stichting Wageningen Research (WR), Martin-Luther-Universit  t Halle-Wittenberg (MLU), Universitatea de Stiin  a Agricola si Medicin   Veterinar   (USAMV), Universiteit Gent (UGENT), Nottingham Trent University (TNTU), Universidade de Coimbra (UCOI), Instituto Regional de Investigaci  n y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Instituto Polit  cnico de Bragan  a (IPB).

The Darwinian Black Box Protocol

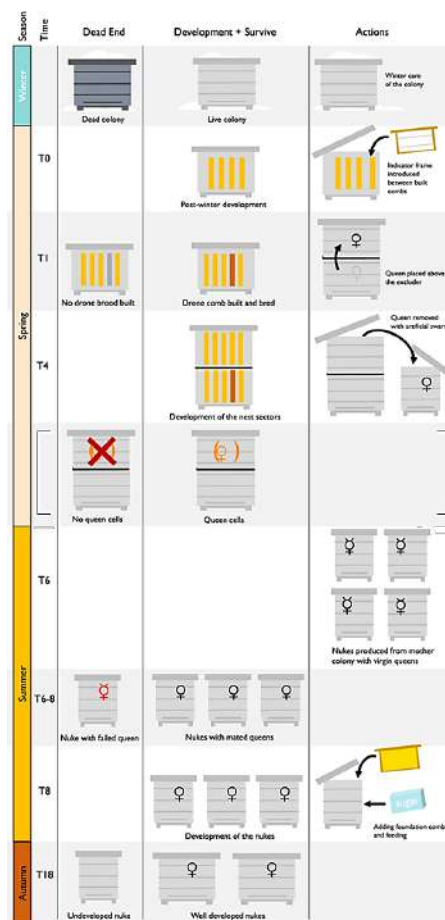


Figure from: Blacqu  re, T., Boot, W., Calis, J., Moro, A., Neumann, P., & Panziera, D. (2019). Darwinian black box selection for resistance to settled invasive *Varroa destructor* parasites in honey bees. *Biological Invasions*, 21(8), 2519–2528.

Preliminary Results (2024–2025, 1st year of selection)

Partner	2024					2025						
	#mother colonies	#splits	#colonies autumn	#wintering	#survivors	% mortality	#surviving mother lines	#splits	#final splits	% success mother lines		
IRIAF	32	108	54	41	3	90	2	11	11	8		
USAMV	30	74	57	51	26	49	26	25	66	83		
UCOI	18	72	7	2	0	100	0	40	40	0		
IPB	23	92	57	38	21	45	21	53	53	65		
TNTU	22	34	34	27	18	33	12	54	58	55		
UGENT	25	100	76	47	0	100	0	69	63	0		
NB	24	65	57	57	54	5	24	69	69	88		
WR	25	96	80	70	11	84	8	24	20	24		
MLU	25	100	79	66	16	76	10	50	70	40		
Mean mortality across partners:					64.6%					Mean success motherlines:		40.3%

Darwinian Black Box across Europe : First-Year Insights

- Low 1st-year survival in some regions
- Regional adaptations: fewer splits (larger nukes), multiple splitting rounds, stimulation of extra queen production, broader genetic base.
- Additional challenges: Asian hornet (*Vespa velutina*) and extreme weather.

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The ectoparasitic mite *Varroa destructor* remains the biggest biotic threat to European honey bees (*Apis mellifera* L.). The majority of beekeepers rely on varroa treatments to maintain their colonies, but effectiveness is limited for a number of reasons, including pesticide resistance or continued brood production throughout the winter, which limits the effectiveness of miticides and results in continued high colony losses. A potentially more sustainable solution focuses on breeding honey bee lineages with natural resistance to *V. destructor*. Observations of African and European populations surviving without treatment highlight the beneficial role of adaptation in addressing the varroa problem. In response, a method has been developed to establish sustainable populations of untreated European by splitting the fittest colonies: the Darwinian Black Box (DBB) protocol. A notable case demonstrate 16 years of survival without varroa treatment, likely due to naturally selected resistance traits as well as beekeeping practices incorporated into the DBB protocol. However, the effectiveness of this approach across diverse climatic conditions and honey bee populations remains uncertain.

In this study we apply the DBB protocol across eight European countries using locally adapted *A. mellifera* subspecies. Colony performance and survival are monitored, and data are collected to identify early signatures of surviving DBB colonies. This includes genetic markers as well as colony development patterns captured through an automated hive weight monitoring tool (BEEP, NL). Additionally, we assess the potential correlation between colony responsiveness to vibrational stimuli and performance within the DBB protocol.

By assessing the broader application of this method and identifying early indicators of success, we will determine the effectiveness and limitations of the DBB protocol as a practical tool for sustainable beekeeping. This research has the potential to provide beekeepers with a viable, pesticide-free strategy, fostering resilient honey bee populations and supporting a sustainable future for apiculture in Europe and beyond.