



CRISPR-based gene silencing for Varroa resistance in honey bees and its implications for host–parasite G×E interactions

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Antalya, Türkiye

PROBLEM

Main cause of colony losses

Varroa destructor is the most destructive ectoparasite of honey bees, feeding on developing brood and adult bees and causing severe colony losses worldwide.



Current Varroa control methods

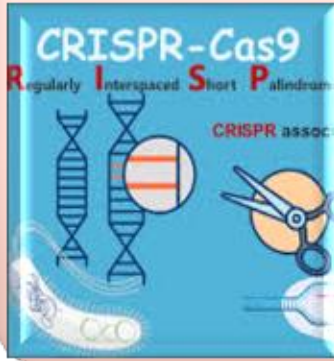
- 🧪 Chemical Treatments
- 🍋 Organic Acids
- 🔥 Thermal Control
- 🔧 Mechanical Control
- 🐝 Behavioral Selection

- ⚠️ Repeated applications required
- ⚠️ Varroa develops resistance
- ⚠️ Residues in hive products
- ⚠️ Risks to bees, humans, and the environment



All current methods treat the mite after infestation. None prevents host recognition.

Idea



Targeting the host–parasite interaction rather than the parasite itself.

Instead of killing Varroa after infestation, we aim to prevent host recognition before infestation.

Our CRISPR-Based Approach

Prevent host recognition before infestation

Heritable across generations

Potentially long-lasting

Chemical-free

Scalable through queen breeding

- **Research Hypothesis**
- Varroa uses chemical cues to recognize hosts
- **Why JHAMT?**
- Linked to Varroa attraction

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ORIGINAL RESEARCH

Ecology and Evolution
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Juvenile hormone pathway in honey bee larvae: A source of possible signal molecules for the reproductive behavior of *Varroa destructor*

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Chedly Kastally^{2,4}  | Daniel S. Dezmirean¹ | Jarkko Routtu²  | Adriana Aurori^{1,5} 

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Abstract

The parasitic mite *Varroa destructor* devastates honey bee (*Apis mellifera*) colonies

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Title: Identification of methyl farnesoate from the hemolymph of insects

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- NYASEMBE, VINCENT - International Centre Of Insect Physiology And Ecology
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> Science. 1987 Jan 9;235(4785):202-5. doi: 10.1126/science.235.4785.202.

Identification of a juvenile hormone-like compound in a crustacean

H Laufer, D Borst, F C Baker, C C Reuter, L W Tsai, D A Schooley, C Carrasco, M Sinkus

PMID: 17778635 DOI: 10.1126/science.235.4785.202

Abstract

Juvenile hormone (JH) has central roles in the regulation of insect development and reproduction but has not previously been identified in other arthropod classes. The hemolymph of a crustacean, *Libinia emarginata* (Leach), has now been analyzed for JH-like compounds. Samples contained 0.003 to 0.030 nanogram of JH III per milliliter and 10 to 50 nanograms of methyl farnesoate per

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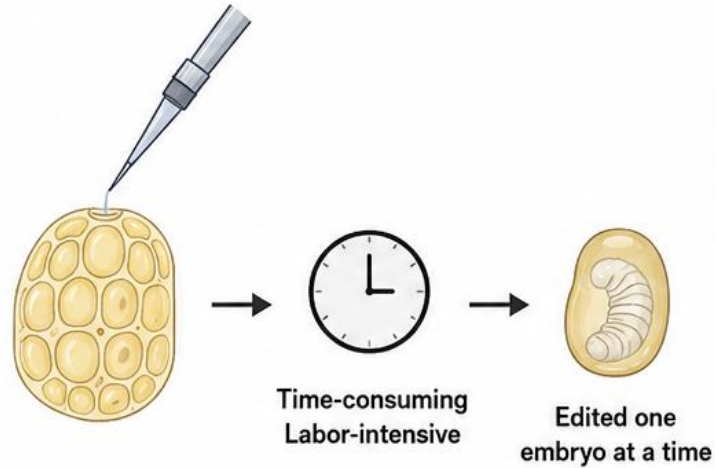
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Why CRISPR?

Conventional Method

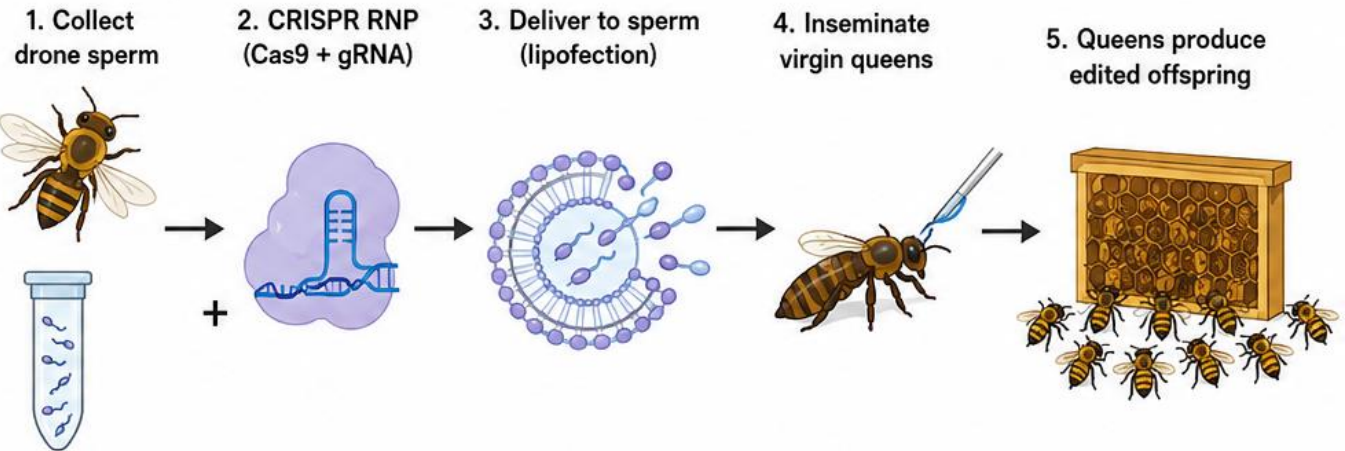
Embryo Microinjection



Technically demanding
Difficult to scale
Low throughput

Our New Strategy

Sperm-Mediated CRISPR



Edits millions of sperm at once



One sperm prep can inseminate multiple queens

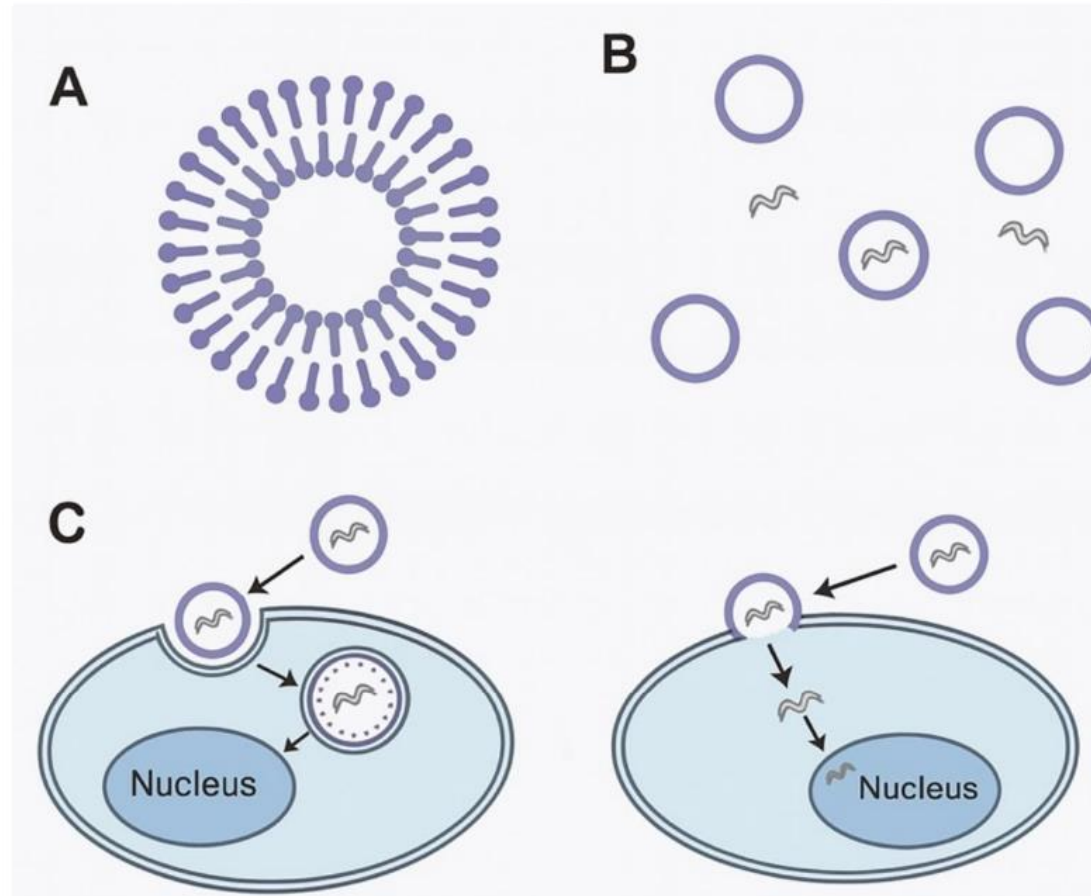


Each queen produces thousands of edited offspring



Scalable and practical for breeding programs

Why lipofectamin?



Delivery of genetic material into the cell via liposomes. **a** Liposome vesicle with a phospholipid bilayer structure; **b** Fusion of liposomes carrying genetic material with the cell membrane and direct release of DNA into the cells; **c** Endocytosis-mediated uptake of liposome-encapsulated genetic material by the cells (Carter and Shieh 2015)

Experimental Workflow

sgRNA design

Cas9 preparation

Sperm pool

Sperm transfection

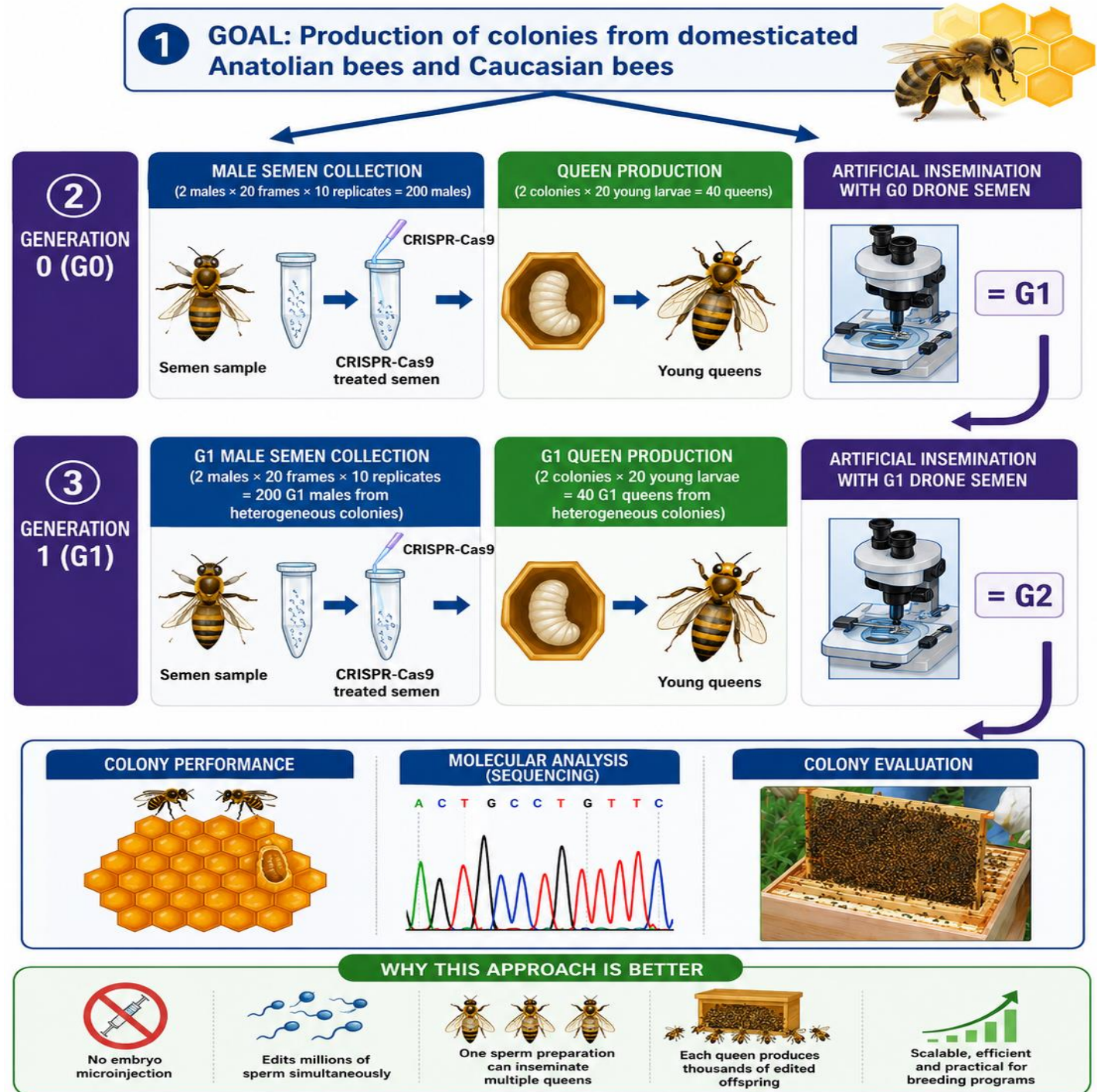
Queen insemination

Colony establishment

Genotyping

Behavioral assays

Field validation



sgRNA Design and Target Selection

Target gene: JHAMT (Gene ID: 724216). Sequence obtained from the NCBI database

Candidate CRISPR target sites identified using CHOPCHOP

On-target efficiency and off-target risk evaluated

Selected 20-nt target sequence: 5'-AGTTTGAGGACGAAATAACG-3' in Exon 5

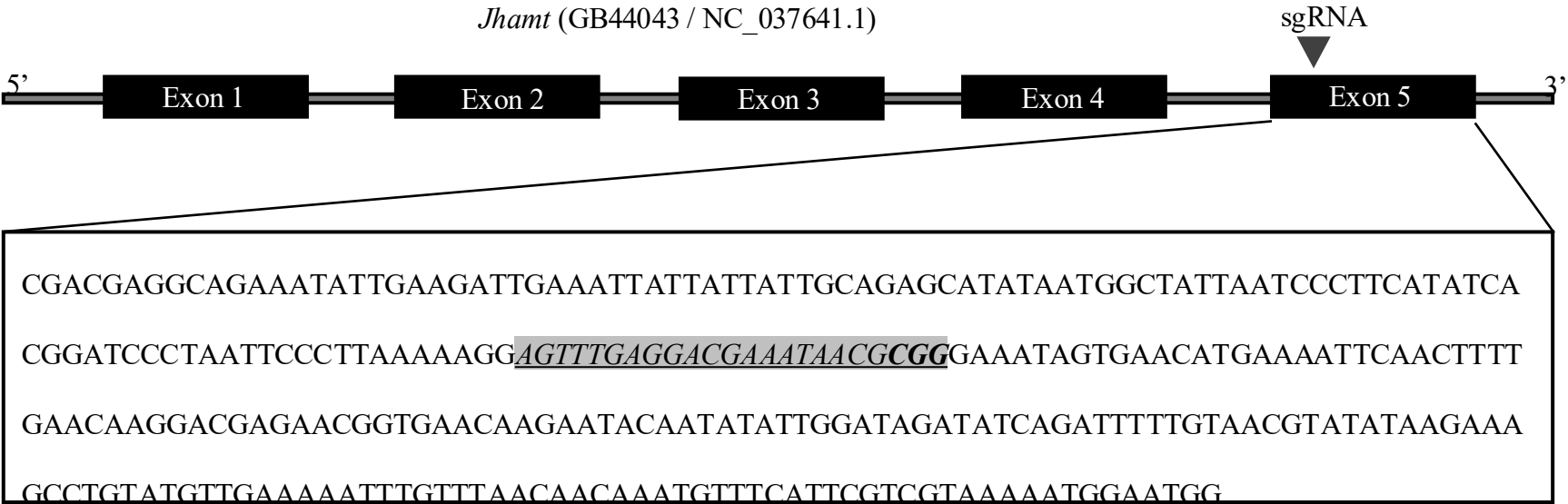
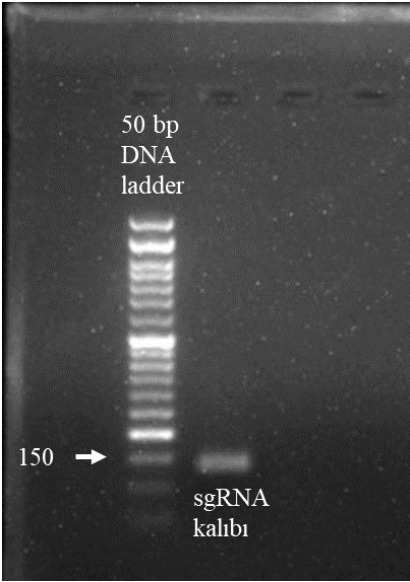
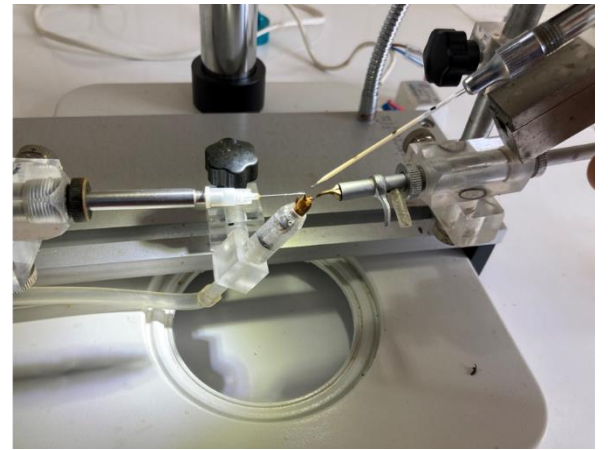


Figure. Location and design of the sgRNA target region on the JHAMT gene. Black boxes indicate exon regions, and the area indicated by triangles shows the location of the sgRNA. The sgRNA sequence is also underlined. Gray bases on the sequence represent the PAM sequence.



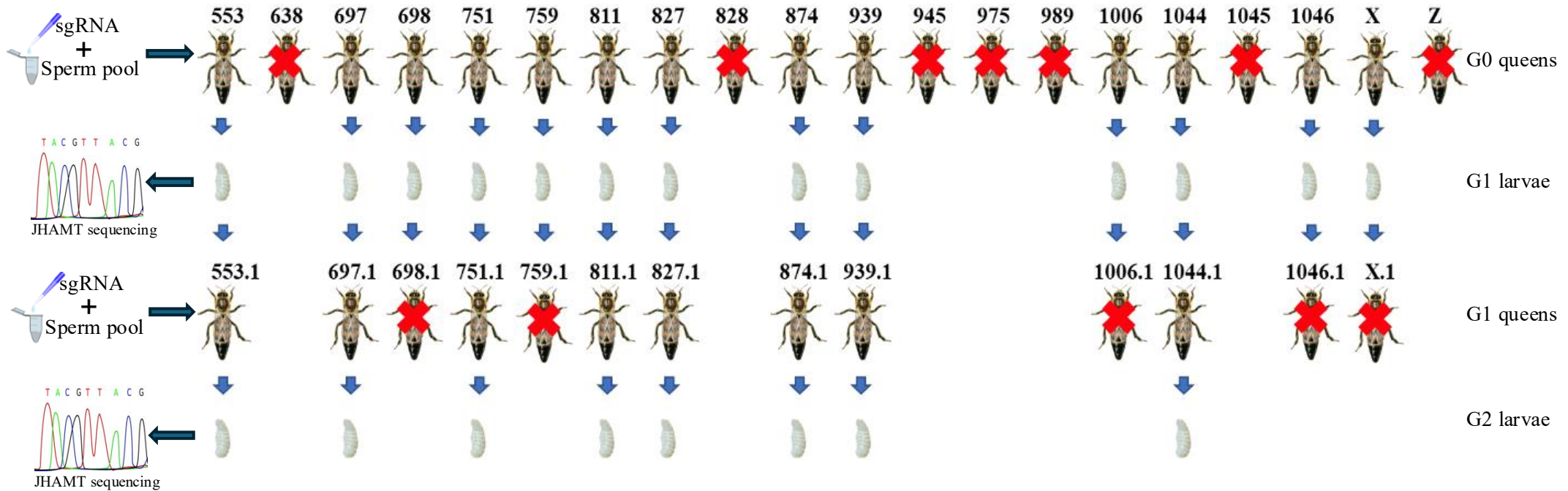
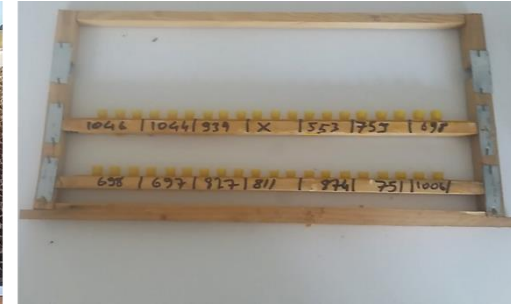


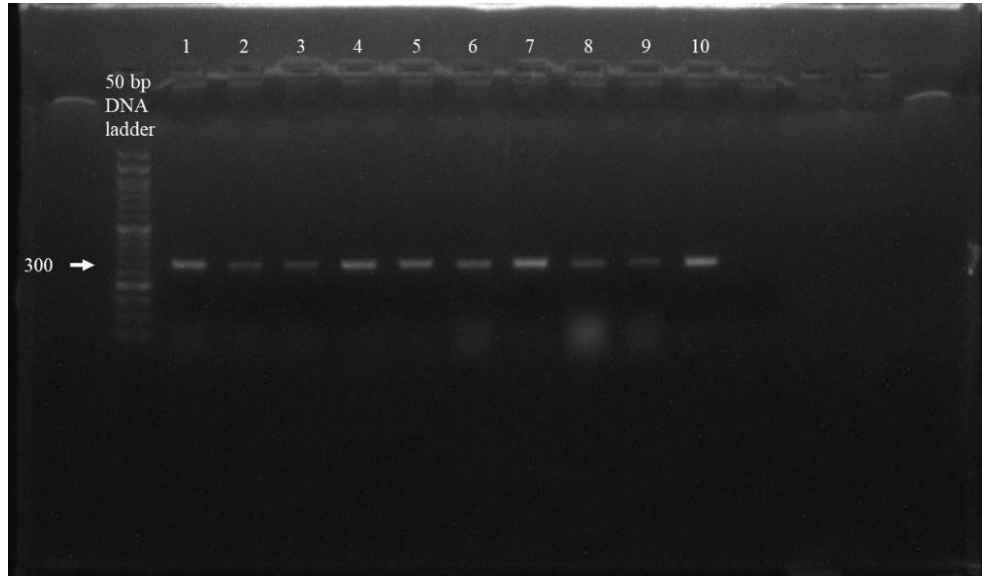
Figure . A visual representation illustrating the pedigree of the queens and larvae utilized in the research. The numbers and letters on the queen bee figures represent the registration information of the queen bees. Non-experimental bees are highlighted in red X.



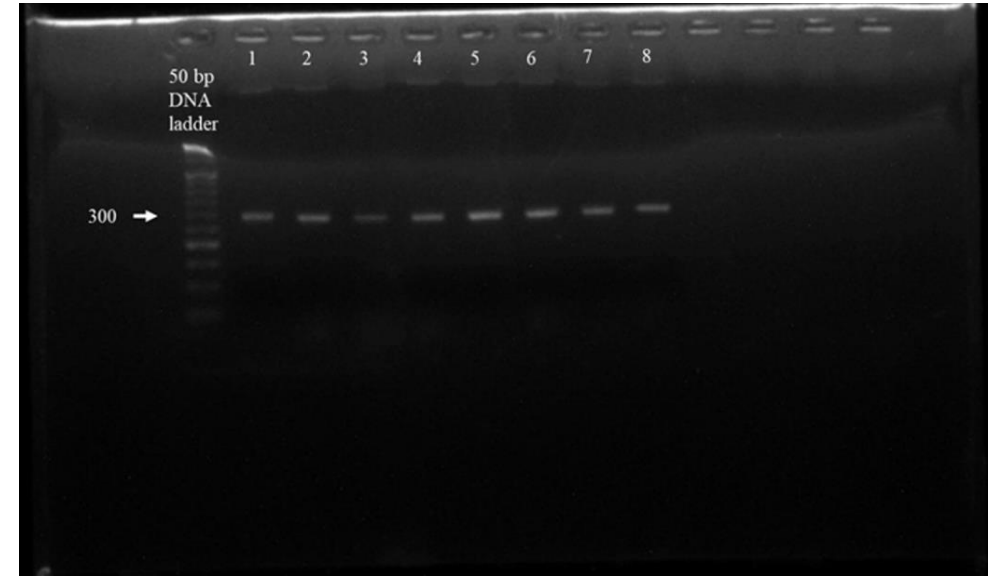
Rearing G1 queen bees in queen cell cups



Phenotypic appearance of honeybee colonies resulting from gene-editing experiments. Photographs show brood combs from A (colony developed from untreated sperm) and B–D (gene-edited colonies derived from queens inseminated with liposome-assisted CRISPR-Cas9-treated sperm)



Agarose gel image of amplicons obtained by PCR amplification of the JHAMT gene region of G1 generation larvae.



Agarose gel image of amplicons obtained by PCR amplification of the JHAMT gene region of G2 generation larvae.

Table 1. Different insertions and deletions (indels) identified through genotyping analysis in gene-edited honey bee workers.

G	ID	Sekans	R ²	FS (%)	AR (%)	In (+) / Del (-)
C	C	CCCTTAAAAAGGAGTTTGAGGACGAAAT AACGCGG GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	1.00	0	100	0
G1	751	CCCTTAAAAAGGAGTTTGAGGACGAAAT AACGCGG GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.68	0	100	+4 / -4
G1	811	CCCTTAAAAAGGAGTTTGAGGACGAAAT AACGCGG GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.68	0	100	+4 / -4
G1	874	CCCTTAAAAAGGAGTTTGAGGACGAAAT AACGCGG GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.68	0	100	+4 / -4
G1	827	CCCTTAAAAAGGAGTTTGAGGACGAAAT CGAGAA GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.82	10.5	89.5	+6 / -3
		CCCTTAAAAAGG-----CAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.82	10.5	10.5	0 / -44
G1	939	CCCTTAAAAAGGAGTTTGAGGACGAAATA -----AGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.65	100	82.9	0 / -46
		CCCTTAAAAAG-----ACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.65	100	17.1	0 / -47
		CCCTTAAAAAGGAGTTTGAGGACGA -----GAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.42	45.5	54.5	0 / -39
G1	1044	CCCTT -----ACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.42	45.5	26.9	0 / -40
		CCCTT -----TAGATATCAGATT	0.42	45.5	18.5	0 / -101
G2	827*	CCCT -----TGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.47	60.6	43.1	-44
		CCCTTAAAAA -----TCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.47	60.6	39.4	-45
		CCCT -----TATCAGATT	0.47	60.6	17.5	-106
G2	939*	CCCTTAAAAAGGAGTTTGAGGACGAAATA -----GAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.66	19.9	80.1	-45
		CCCTTAAAAAGG -----ACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.66	19.9	19.9	-46
G2	1044*	CCCTTAAAAAGGAGTTTGAGG ----- ACGCGG GAAATAGTGAACATGAAAATTCAACTTTTGAACAAGGACGAGAACGGTGAACAAGAATACAATATATTGGATAGATATCAGATT	0.83	100	100	-8

*Gray highlight indicates the target, bold nucleotides indicate PAM sequence, Nucleotides with double lines below indicate insertions and deletions, solid dashed lines indicate the sequence region subjected to deletion. G: Generation, ID: individual code, FS (%): Percentage of frameshift mutations, AR (%): Rate of alleles, In (+): Number of insertions / Del (-): Number of deletions.

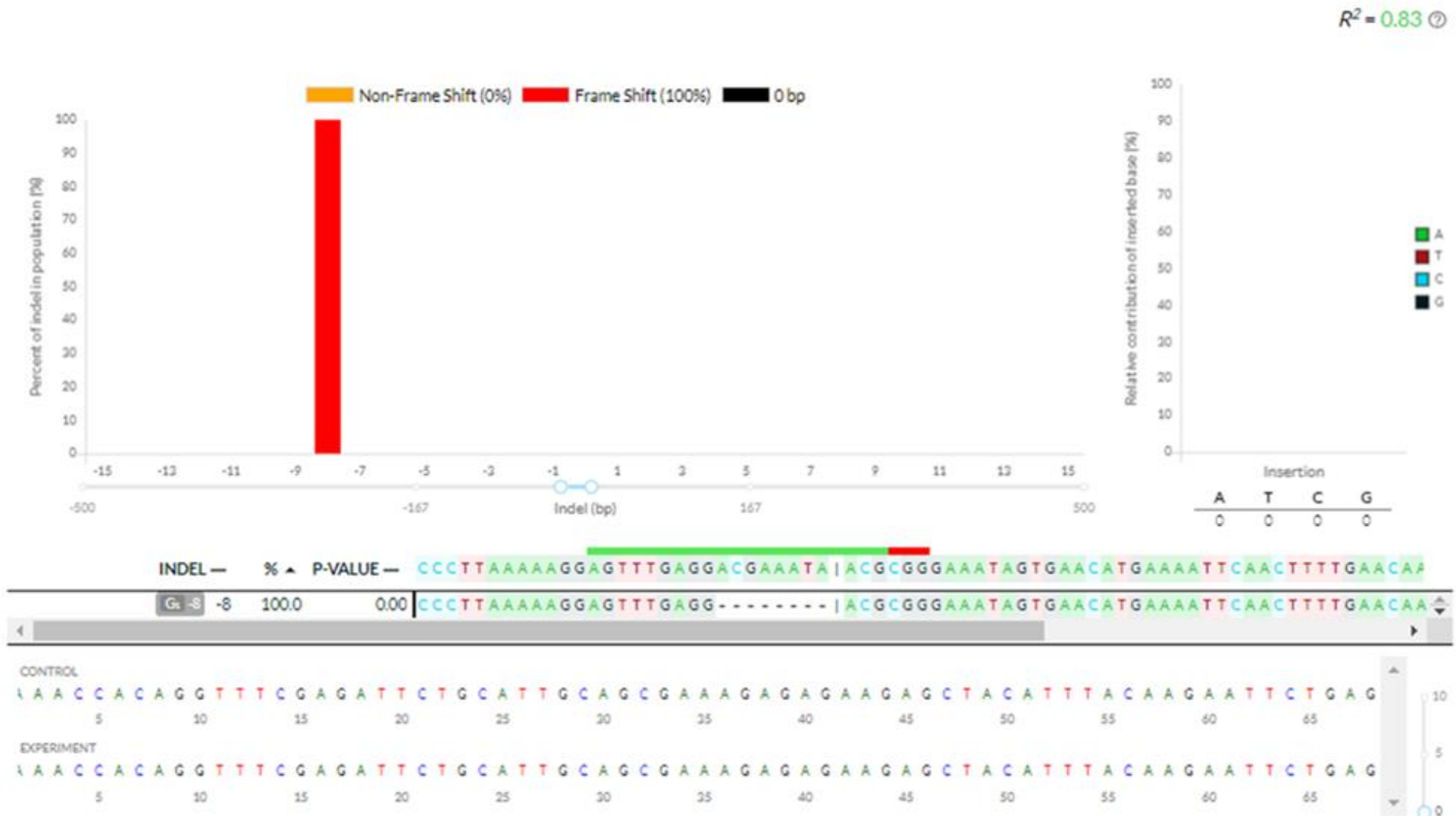


Figure . DECODR software result obtained from the sequences of individuals descended from hive number 1044 (100% rate -8 deletions/frame shift)



Removing Varroa mites from the eyes of male bees using tweezers.

Scoring

1 = Preferred mutant larva

0 = Preferred wild-type larva

? = No clear preference

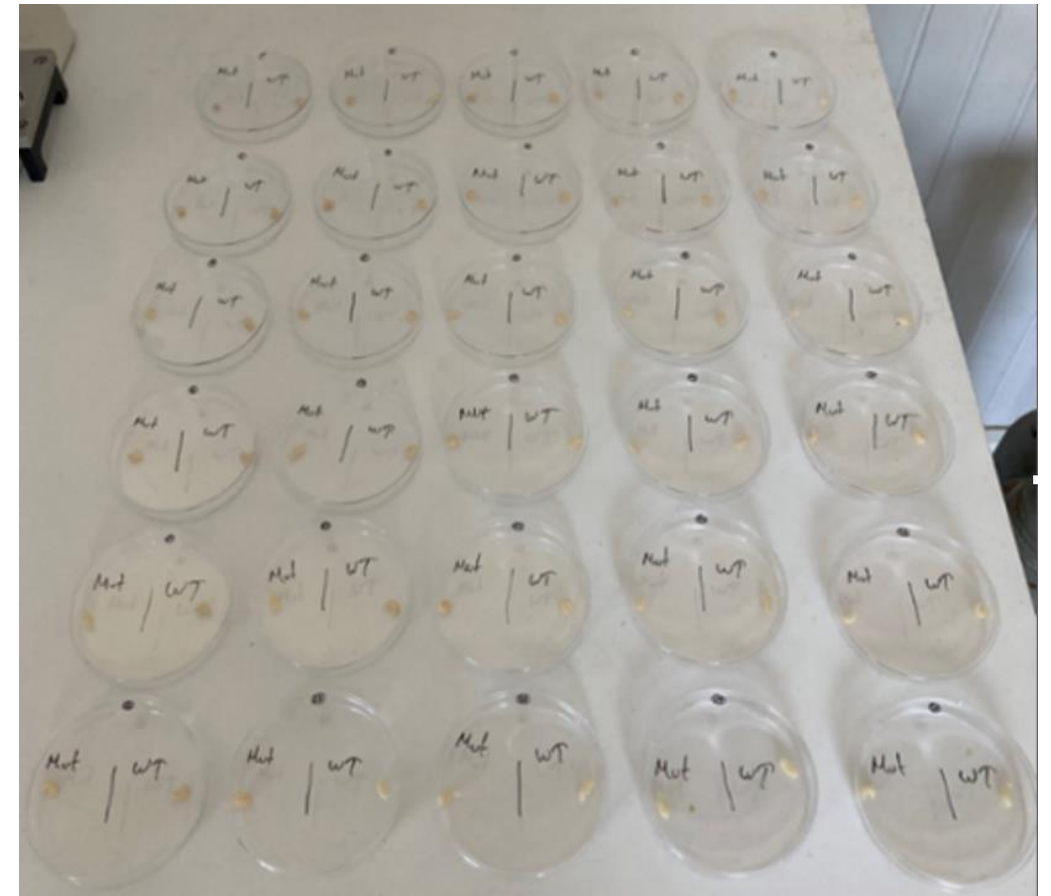
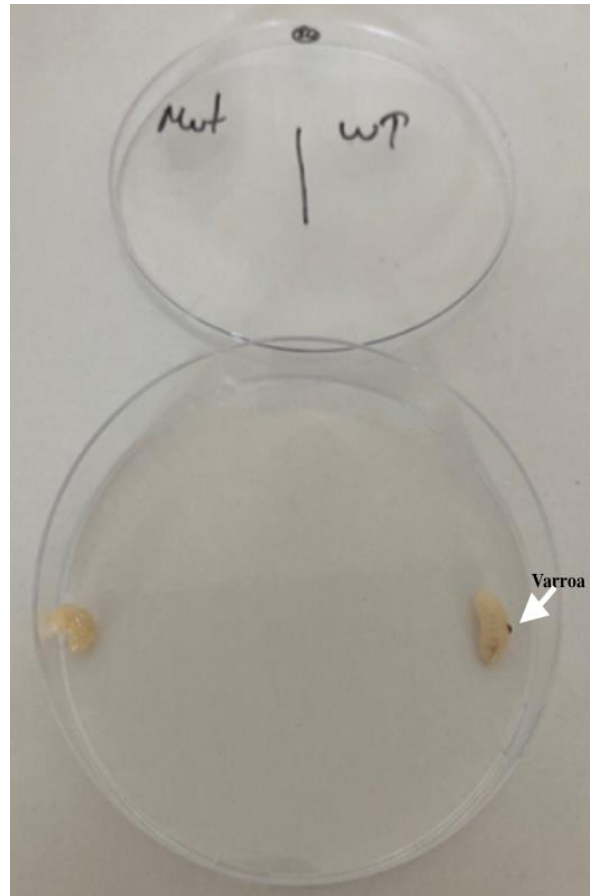


Figure 3. Petri containers in which Varroa host tests were performed. Mut: mutant larvae, WT: wild type larvae. Conditions during assay: 34.5°C and 60–70% relative humidity
Total number of assays: 30

Does disruption of *JHAMT* actually affect *Varroa* behavior?

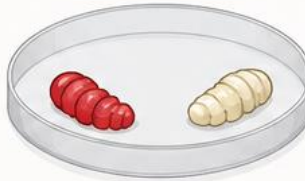
Would *Varroa* still prefer these mutant larvae?

LABORATORY HOST PREFERENCE ASSAY

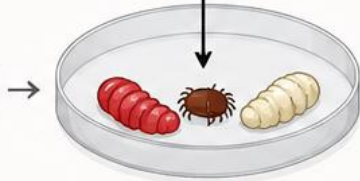
One **mutant larva** and one **wild-type larva** were placed in a Petri dish.

A single adult female *Varroa* mite was released in the center.

The mite's position was recorded every 15 minutes for 1 hour.



Mutant larva **Wild-type larva**



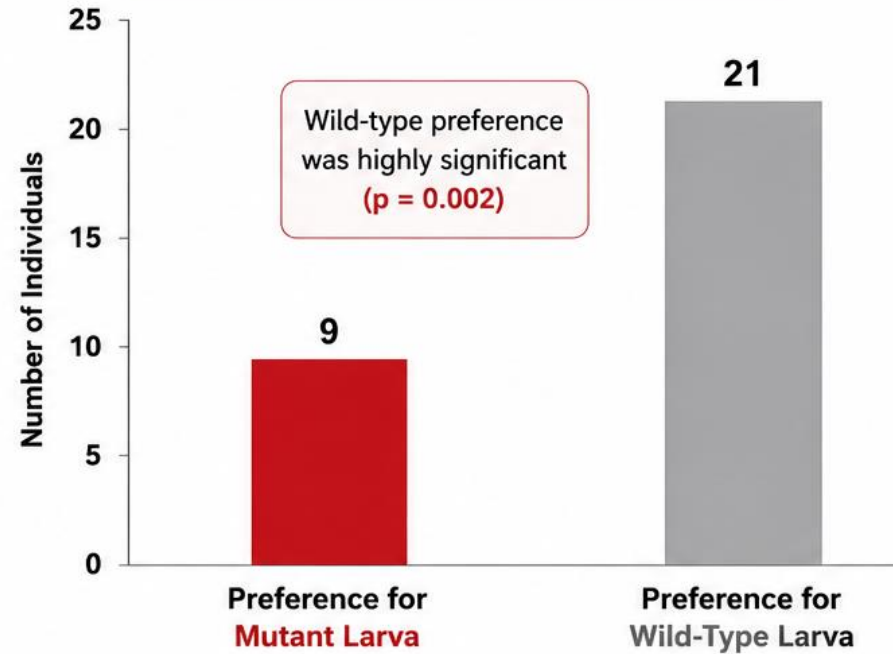
15 min intervals



x30

In total, **30 independent assays** were conducted.

VARROA LARVAL PREFERENCE RESULTS



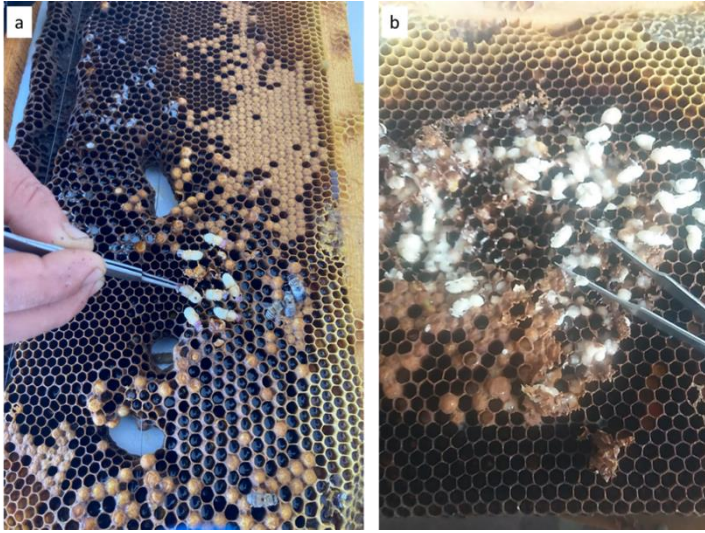
Out of 30 assays, *Varroa* mites preferred the wild-type larva in 21 cases and the **mutant larva** in 9 cases.



Disrupting *JHAMT* significantly reduced larval attractiveness to *Varroa*.

This is the first direct evidence supporting our hypothesis.





One honeycomb sample was taken from each of the gene-modified colonies and the control group colonies, and the presence of mite-infested larvae and pupae was examined in 100 randomly selected cells from each honeycomb in the laboratory . In this way, the effect of the JHAMT gene on the preference of Varroa in vivo conditions was also determined.

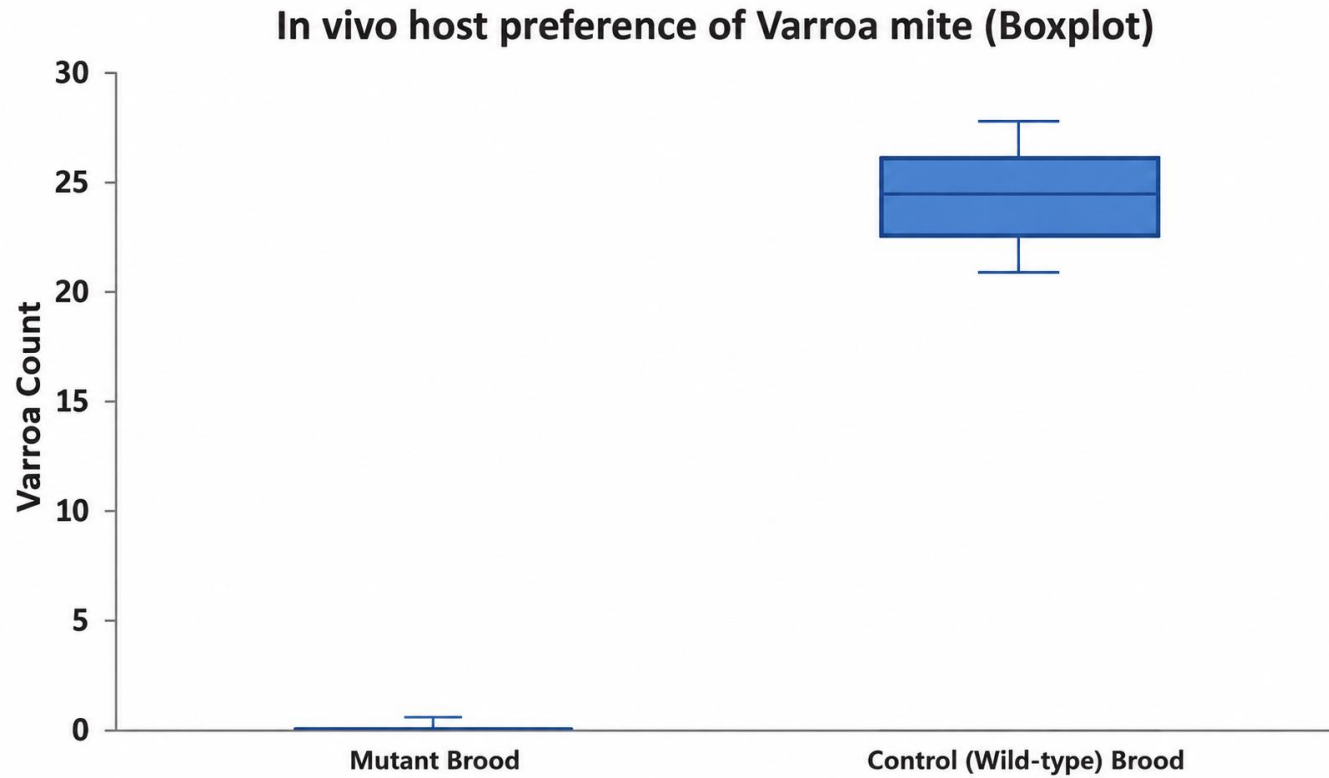


Figure Box plot diagram showing Varroa's host preference in vivo conditions.

This difference was statistically significant, with a **p-value of 0.037**.

Biological Interpretation

JHAMT plays a functional role in the chemical communication system used by Varroa to locate and select brood.

NORMAL (JHAMT FUNCTIONAL)

JHAMT produces chemical signals attracting Varroa.



Varroa detects the signal and is **attracted**.



Result:
High mite attraction and infestation

JHAMT DISRUPTED (THIS STUDY)

Disruption of **JHAMT** alters or reduces host chemical signals.



Varroa cannot properly detect the signal and is **less attracted**.



Result:
Reduced mite attraction and infestation

This approach is fundamentally different from current control methods.

Current Control Methods



Aim: Kill the parasite
Directly target and eliminate Varroa mites.

VS.



This Study

Aim: Prevent host recognition
Interfere with Varroa's ability to recognize and select the host.

We are not trying to kill the parasite.
We are preventing host recognition.
That is the key conceptual innovation of this work.

Summary of Key Findings

Our study provides proof of concept for a novel host-based strategy for Varroa control.

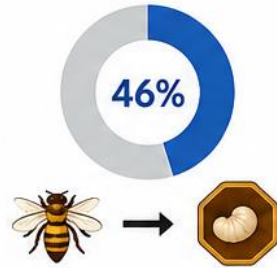
1

We developed the first sperm-mediated CRISPR genome editing method for honey bees.



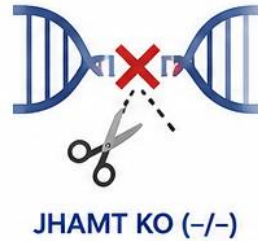
2

Forty-six percent of established colonies produced edited offspring.



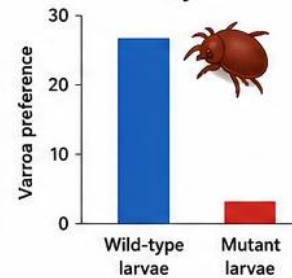
3

We obtained one homozygous JHAMT knockout line.



4

Varroa significantly preferred wild-type larvae in laboratory assays.



5

No Varroa mites were detected in mutant brood under colony conditions.



Advantages of This Novel Approach

1

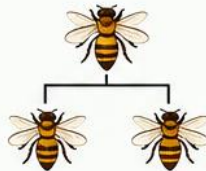
Chemical-free



No chemicals are used.

2

Heritable



The trait is passed on to future generations.

3

Environmentally friendly



Safe for bees, beekeepers and the environment.

4

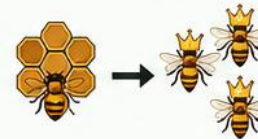
Integrable into queen breeding programs



Can be incorporated directly into existing breeding schemes.

5

Easily propagated once established



Resistant lines can be propagated using routine queen production.

6

Reduces costs and labor



May substantially reduce treatment costs and labor requirements.



This work opens the door to sustainable, long-term Varroa control through host-based genetic resistance.

Future Work

Although the results are highly promising, several important steps remain.

These include:

1

Large-scale field validation



Test the approach under real-world conditions across multiple environments and seasons.

2

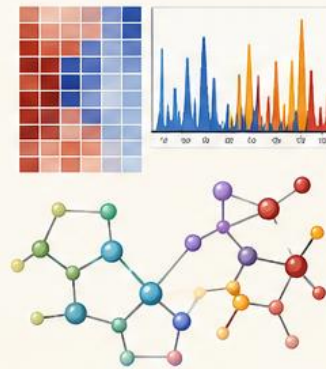
Whole-genome sequencing



Evaluate potential off-target effects to ensure precision and safety.

3

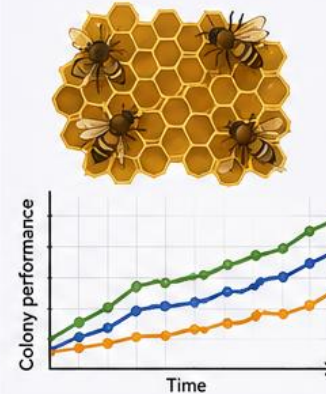
Transcriptomic and metabolomic analyses



Understand molecular changes and pathways associated with JHAMT knockout.

4

Long-term colony performance studies



Assess the impact on colony health, productivity, and survival over multiple generations.

5

Regulatory and biosafety assessments



Ensure compliance with regulations and evaluate environmental and biosafety aspects.



These studies will help determine how rapidly this technology can be implemented in commercial breeding.





Targeted Gene Editing in Honey Bees Using Liposome-Based CRISPR-Cas9

Berkant İsmail Yıldız¹ · Kemal Karabağ¹

Received: 22 January 2025 / Accepted: 15 August 2025
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Abstract

Genome editing technologies have revolutionized molecular manipulation of gene functions across diverse organisms. In this study, we introduce a novel liposome-mediated delivery system for targeting the Juvenile Hormone Acid Methyltransferase (JHAMT) gene in honey bees (*Apis mellifera anatoliaca*). This approach leverages CRISPR-Cas9 transfection, overcoming the technical challenges of microinjection in honey bees, such as low survival rates and high mortality. The study involved artificial insemination of



(19) **TÜRK PATENT**
TÜRK PATENT VE MARKA KURUMU

(21) Başvuru Numarası
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(43) Başvuru Yayın Tarihi
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(11) Başvuru Yayın No.
TR 2023 019280 A2

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(10) TR 2023 019280 B

(51) Buluşun tasnif sınıfı
C12N 15/00

(30) Rüçhan Bilgileri (32) (33) (31) (74) Vekil
GİZEM TEKE KARSLI (GRUP OFİS MARKA PATENT A.Ş.)
ATATÜRK BULVARI NO:211/11 KAVAKLIDERE/ANKARA

(71) Patent Sahibi
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PINARBAŞI MAH. DUMLUPINAR BLV. TIP FAKÜLTESİ 444 F 1 Konyaaltı Antalya TÜRKİYE

(72) Buluşu Yapanlar
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KIZILSARAY MAH. 69 SK. ATALAY TUZCUOĞLU SİTESİ ATALAY TUZCULAR NO: 1 İÇ KAPI NO: 1 MURATPAŞA / ANTALYA Muratpaşa Antalya TÜRKİYE
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(54) Buluş Başlığı
Bal arılarında crispr-cas9 yöntemi kullanılarak varroa destructor paraziti dirençli jhamt geni susturulmuş genotip üretim yöntemi.

(57) Özet
Buluş, CRISPR-Cas9 teknolojisi aracılığıyla JHAMT (Gene ID: 724216) gen üzerinde bulunan SEQ ID NO:1 nükleotid dizilimine sahip hedef bölgenin SEQ



From Scientific Discovery to Commercial Innovation

— Patent-Protected Technology Commercialized Through BeeGenesis Global A.Ş. —



PATENT PROTECTION



TR 2023 019280 B



Patented technology for the production of Varroa-resistant honey bees using CRISPR-Cas9.



COMPANY ESTABLISHMENT



BeeGenesis
Global A.Ş.



Antalya
Teknokent



Established
in 2024



Biotechnology for
Beekeeping



PARTNERSHIP & SUPPORT



ANTALYA
TEKNOKENT



TÜBİTAK

KEY ACHIEVEMENTS



Scientific
Publication



Gold Medal
(TEKNOFEST ISIF'25)



Patent
Protection



Company
Establishment



Our
Mission



SCIENCE

• INNOVATION

• SUSTAINABILITY

• IMPACT





🎓 Expertise

Animal breeding, population genetics, molecular genetics

📊 Projects

33 projects (TÜBİTAK, TAGEM, Ministries+)

📖 Publications

43 SCI/SCI-E articles, total 170 papers, conference papers, 3 book chapters

🧬 Innovation

Varroa-resistant genetic line (CRISPR-based), ISIF2025 Gold medal



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