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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
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Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

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Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This study aimed at reconstructing the full chain of factors affecting temporal division of labour in honey bees. To this purpose we maintained under artificial conditions for different times, newly emerged honey bees fed with different diets, and then observed their behavior/gene expression after introduction into the bee colony. Treatment factors were therefore: honey bee age at the time of introduction into the colony and diet. Experiments were normally repeated at least three times.

Research sample

In this case, a research sample is a group of honey bees (*Apis mellifera*). Given the replicated design, and the large number of individuals per experimental group per replicate, the samples should represent any bee exposed to a given treatment. All experiments involved *Apis mellifera* worker bees, sampled at eclosion from the hives of the experimental apiary of the University of Udine (46°04'54.2"N, 13°12'34.2"E). Previous studies indicated that the local bee population consists of hybrids between *A. mellifera ligustica* and *A.m. carnica*.

Sampling strategy

Sample size (i.e. number of individual bees per experimental group per replicate) was roughly pre-determined based on previous experience and published literature. In general, the biggest possible sample size was used in each replicate and at least three replicates of each experiment were carried out on different dates. Sampling of newly emerged honey bees involved the collection of several brood combs from the experimental apiary, the transfer of the combs in a net box which was maintained inside an incubator at 34.5 °C, 75% R.H., for a few hours, until a convenient number of adult bees emerged from the sealed brood cells.

Data collection

Data collection involved the periodic observation of honey bee behaviour or the quantification of the expression level of some selected honey bee genes. Honey bee behavior was recorded using pencil and paper by the authors. Gene expression was estimated using qRT-PCR according to standard procedures that are described in detail in the methods section.

Timing and spatial scale

Experiments were carried out in Udine, Italy, during the Summer months (June-August) in the period 2021-2025.

Data exclusions

No data were excluded from the analyses.

Reproducibility

All experiment were replicated a convenient number of times. No replicates were excluded.

Randomization	Allocation of individual bees to experimental groups was random.
Blinding	In general, the person in charge of scoring the results of an experiment did not know the treatment that was applied to the sample.
Did the study involve field work?	☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	The study did not involve laboratory animals.
Wild animals	All experiments involved <i>Apis mellifera</i> worker bees, sampled at the larval stage or before eclosion, from the hives of the experimental apiary of the University of Udine (46°04'54.2"N, 13°12'34.2"E). Previous studies indicated that the local bee population consists of hybrids between <i>A. mellifera ligustica</i> and <i>A.m. carnica</i> .
Reporting on sex	Only workers bees (functionally sterile females of the species <i>Apis mellifera</i>) were used in the experiments.
Field-collected samples	For all the experiments, honey bees were collected from the experimental hives of the University of Udine, which are maintained according to standard beekeeping procedures. For the collection of newly emerged adult bees, brood frames were transferred to the lab and bees allowed to spontaneously emerge from cells in a net box maintained at 34.5 °C, 75% R.H., dark.
Ethics oversight	No ethical approval was required in this case.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.