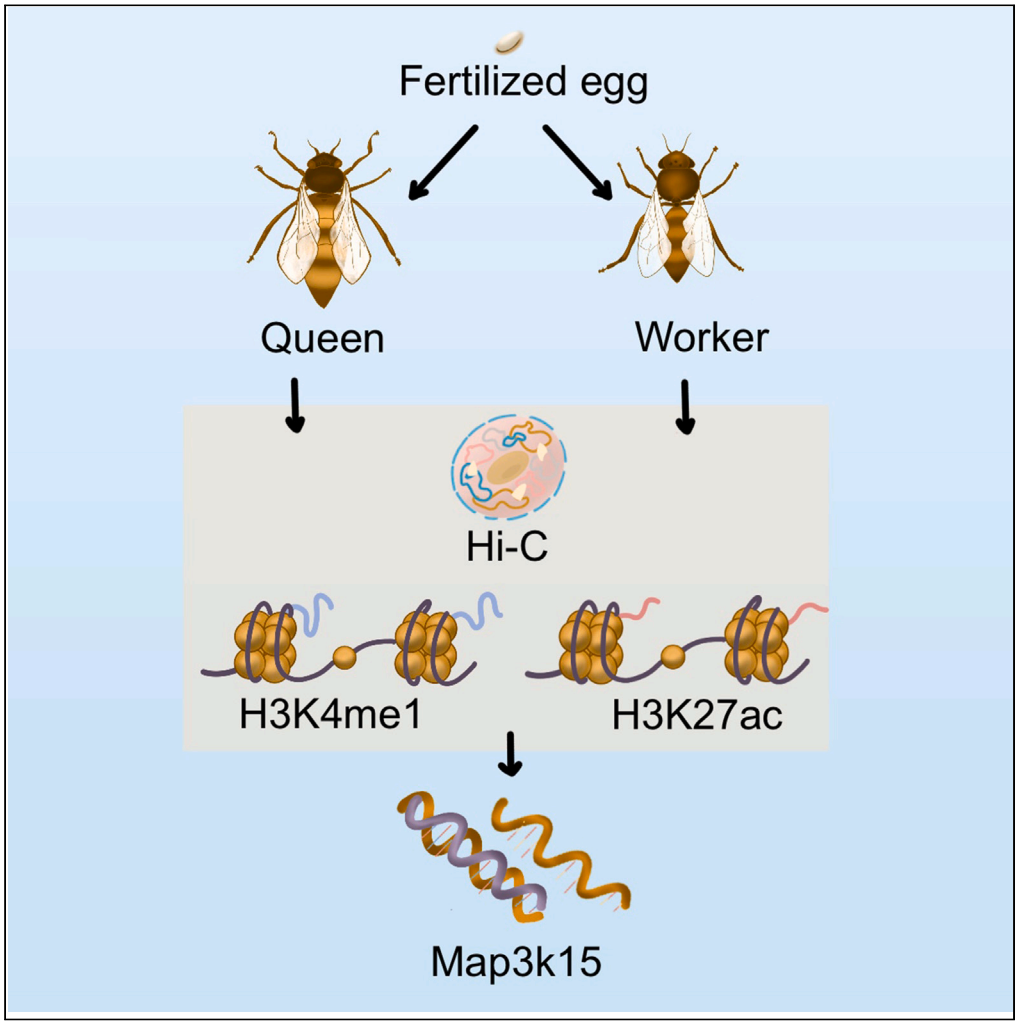
iScience

CellPress
OPEN ACCESS

Article

Phenotypic dimorphism between honeybee queen and worker is regulated by complicated epigenetic modifications



Meng Jie Jin, Zi Long Wang, Zhi Hao Wu, ..., Xiao Bo Wu, Wei Yu Yan, Zhi Jiang Zeng

bees1965@sina.com

Highlights

Multiple epigenetic modifications contribute to queen–worker phenotypic dimorphism

Queen contains more chromosome interactions and unique H3K27ac modifications than worker

Worker has more H3K4me1 modifications than queen

Jin et al., iScience 26, 106308
April 21, 2023
https://doi.org/10.1016/j.isci.2023.106308

iScience

CellPress
OPEN ACCESS

Article

Phenotypic dimorphism between honeybee queen and worker is regulated by complicated epigenetic modifications

Meng Jie Jin,[1,2,3] Zi Long Wang,[1,2,3] Zhi Hao Wu,[1,2] Xu Jiang He,[1,2] Yong Zhang,[1,2] Qiang Huang,[1,2] Li Zhen Zhang,[1,2] Xiao Bo Wu,[1,2] Wei Yu Yan,[1,2] and Zhi Jiang Zeng[1,2,4,*]

SUMMARY

Phenotypic dimorphism between queens and workers is an important biological characteristic of honeybees that has been the subject of intensive research. The enormous differences in morphology, lifespan, physiology, and behavior between queens and workers are caused by a complicated set of factors. Epigenetic modifications are considered to play an important role in this process. In this study, we analyzed the differences in chromosome interactions and H3K27ac and H3K4me1 modifications between the queens and workers using high-throughput chromosome conformation capture (Hi-C) and Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) technologies. We found that the queens contain more chromosome interactions and more unique H3K27ac modifications than workers; in contrast, workers have more H3K4me1 modifications than queens. Moreover, we identified *Map3k15* as a potential caste gene in queen–worker differentiation. Our results suggest that chromosomal conformation and H3K27ac and H3K4me1 modifications are involved in regulating queen–worker differentiation, which reveals that the queen–worker phenotypic dimorphism is regulated by multiple epigenetic modifications.

INTRODUCTION

Honeybees are eusocial insects that have a division of labor and an advanced information exchange system.[1–3] They are economically valuable pollinators that are essential for the ability of many crops and wild plants to produce seed. In addition, they can maintain an ecological balance through pollination.[4,5]

The queens and workers develop from a similar genetic background but show enormous differences in morphological characters, lifespan, reproductive ability, and behavior. The queen has an approximately 10-fold longer lifespan compared with the workers and is dedicated to reproducing offspring.[6] Usually, the queen has 150–180 ovarian tubes per ovary and can lay up to 2,000 eggs per day.[7,8] In contrast, the workers are usually sterile; with 3–26 ovarian tubes per ovary.[7] Moreover, both queen and larval pheromones can inhibit the development of worker ovaries.[9,10] The workers have a behavioral maturity period where they perform different tasks as they age. They serve as housekeepers when they are young and conduct such tasks as keeping eggs warm, cleaning the hive, and feeding larvae among others and eventually change to foraging behavior.[11,12]

The study of queen–worker differentiation has been the focus of concern in honeybees over a long period of time. The mechanism of caste differentiation is still not totally understood despite the large number of studies that have been conducted. The existing findings suggest that many factors contribute to the differentiation of castes in honeybees, including the size of the larval developmental space (worker cell and queen cell),[13,14] food quality and quantity,[15–18] hormones,[6,14,19,20] differences in gene expression,[21–26] DNA methylation,[20,23,27–29] histone modifications,[30–32] microRNAs,[33–35]and poly(A) tail.[36] To date, many signaling pathways have been reported to be involved in honeybee caste differentiation, including FoxO, mTOR, MAPK, Hippo, Hedgehog, Wnt, TGF-beta, Toll and Imd, longevity regulation (multiple species), dorsoventral axis formation, and insect hormone biosynthetic signaling pathways.[23,37–40]

[1]Honeybee Research Institute, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, P.R.China

[2]Jiangxi Province Honeybee Biology and Beekeeping, Nanchang, Jiangxi 330045, P. R. China

[3]These authors contributed equally

[4]Lead contact

*Correspondence: bees1965@sina.com

https://doi.org/10.1016/j.isci.2023.106308

Epigenetic modifications play an important role in the queen–worker differentiation of honeybees, and DNA methylation is crucial. In addition, histone modifications also have an influence on caste differentiation. There are four types of core histone modifications (H2A, H2B, H3, and H4), and the regulation of gene expression by H3 has been more widely studied.[41,42] It has been shown that H3K4me3, H3K27ac, and H3K36me3 differ extensively in the whole genome between worker and queen larvae and that H3K27ac can influence the caste differentiation of honeybees.[43]

In addition, we hypothesize that chromosomal interactions have an impact on the queen–worker differentiation of honeybees. Studies have shown that chromosomal interactions can dynamically regulate gene expression.[44–46] In higher eukaryotes, chromosomes are folded in three-dimensional (3D) structures within the nucleus, and extensive interactions exist within the same chromosome and between different chromosomes.[47–49] Recent advances in the genome-wide localization of chromatin interactions, such as Hi-C,[44,46] have facilitated the identification of important 3D genomic features, such as genome-wide chromatin loops, topologically associated domains (TADs), and A/B compartments.[44,46,50,51] Considering that chromosomal interactions extensively regulate individual development by influencing gene expression, we hypothesize that differences in chromosomal interaction are one of the important reasons for queen–worker differentiation.

Here, we studied the epigenetic regulatory mechanisms behind the phenotypic differences between queens and workers using multi-omics data, such as (Hi-C, ChIP-Seq, and RNA-Seq). We systematically investigated the differences in patterns of chromosome interaction and histone modification (H3K27ac and H3K4me1) between the two castes; and identified the A/B compartments, loops, TADs, and H3K27ac and H3K4me1 modifications related to queen–worker differentiation. To our knowledge, this is the first report of comprehensive insights into the epigenetic regulation mechanisms of queen–worker differentiation.

RESULTS

Data quality control

Three omics, including Hi-C-seq, ChIP-Seq and RNA-Seq, were performed on newly emerged queens and workers of western honeybee (*Apis melllifera*) to study the epigenetic mechanism of honeybee caste differentiation. In Hi-C-seq, a total of 180.13 G and 180.45 G clean reads were obtained from queens and workers, respectively, with an average of 600.97 M clean reads per sample, and the Q30 of each sample was higher than 89.42% (Table S1). In ChIP-Seq, the clean reads of each sample were between 2.75-and 4.28 G, and the Q30 of each sample was higher than 86.83% (Table S2). In RNA-Seq, the clean reads of each sample were from 5.97 G to 7.59 G, and the Q30 of each sample was higher than 92.71% (Table S3). These results indicated that the sequencing quality of these three omics were high enough and reliable.

The queens contain more chromosome interactions than the workers

There is a significant difference in chromosome interaction between the queens and workers (Figures 1A and 1B). Compared with the workers, the queens have a significantly larger number of *cis*- and *trans*-interactions (Figure 1B). A total of 2,165 bins were identified in both queens and workers, and 78 bins had the A/B compartment switched in the queen versus worker comparison (Table S4). We analyzed the changes in expression fold changes (queen/worker) of the genes associated with the switch of A and B compartments. We found that in the queen versus worker comparison, the B-to-A related genes were transcriptionally activated overall in the queen (the mean of log2[queen/worker] >0), whereas the A-to-B related genes were transcriptionally repressed overall in the queen (the mean of log2[queen/worker] <0) (Figure 1C). The loop numbers in queen and worker were also comparable, and most of them were unique to each other (Figure 1D). We found that there were 243 differentially expressed genes (DEGs) in the queen loops by co-analyzing the loop data with the RNA-Seq data (Figure 1E), and 73 DEGs unique to the queen loops were enriched in multiple signaling pathways (Figure S1A), of them, the insect hormone biosynthesis and apoptosis signaling pathways could be related to the growth and development of the queens. In addition, there were 78 unique DEGs in the worker loop, which are enriched in pathways associated with growth and development, immune function lifespan and caste differentiation in honeybees (Figures 1E and S1B). In addition, the queen and worker have approximately similar amounts of TADs (373 versus 385) (Table S5). Of the TAD coverage regions in the genome, 69.36% are common to both queens and workers; 15.11%

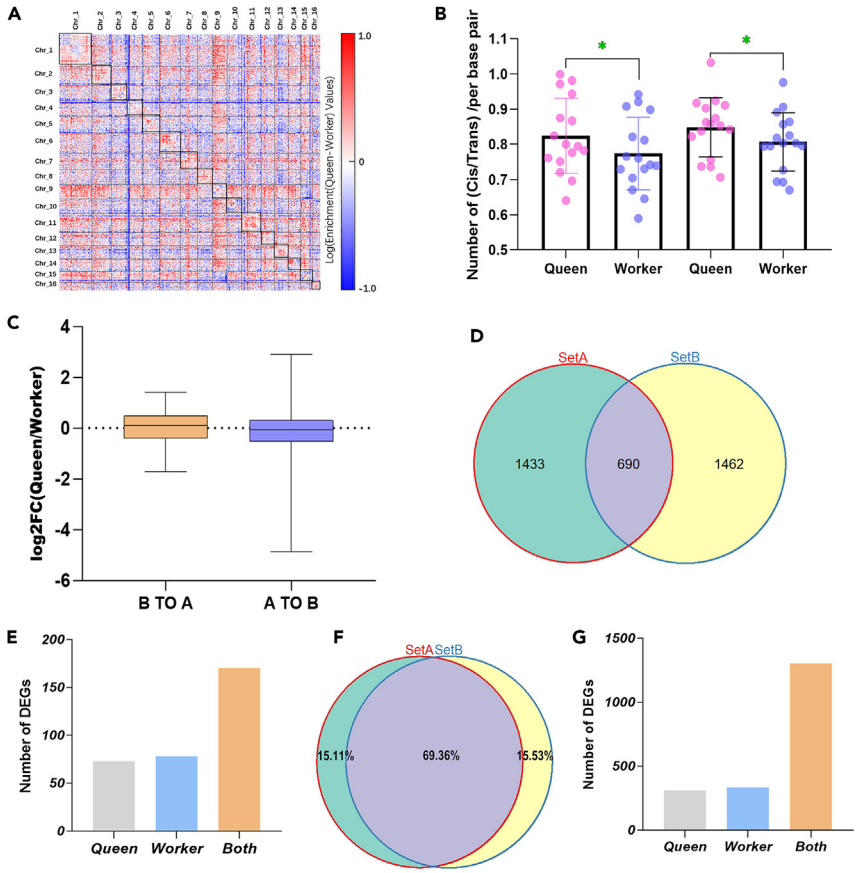


Figure 1. Hi-C analysis of queen and worker

(A) Interaction map of queen versus worker (observed/control). The sixteen chromosomes (chr) o *A. mellifera* are presented from left to right and top to bottom. Chromosomes are separated by black lines.

(B) The numbers of *cis* and *trans* interactions in queen and worker. Data were presented as mean ± SE. p <0.01 by t-test.

(C) The expression fold changes (queen/worker) of genes associated with A/B compartment switches (Unpaired t-test with Welch's correction). Data were presented as mean ± SE. p <0.05.

(D) The numbers of chromatin loops in queen and worker.

(E) The numbers of DEGs (queen versus worker) paired with chromatin loops.

(F) The percentages of unique TAD regions in queen and worker.

(G) The numbers of DEGs in the unique and shared TAD regions.

are unique to the queens, and 15.53% are unique to the workers (Figure 1F). Moreover, 311 and 333 genes were identified in the queen unique and worker unique regions, respectively (Figure 1G).

The queens contain more unique H3K27ac modifications than the workers

The distribution of H3K27ac between queens and workers is shown in Figure 2A. We found that H3K27ac is enriched around the transcriptional start sites (TSS) of the genes in both the queen and worker castes (Figure 2B), which is similar to previous studies of honeybee larvae.[43] There were 3,601 differential peaks between the queens and workers, and more peaks were up-regulated in the queen, which were distributed in the intron and promoter regions (Figure 2C). The genes associated with these differential peaks were enriched in the pathways associated with growth and development, ovarian development, and lifespan regulation in honeybees (Figure 2D, Table S6). Compared with the workers, the queens have exceptionally more unique H3K27ac peaks that are primarily distributed in the intron and promoter regions (Figure 2E, Table S7). The queen unique peak-related genes were enriched in the pathways related to differences between the queens and workers in body size, longevity, immunity, and ovarian development, such as the Hippo, Wnt, MAPK, Hippo, FoxO, TGF-beta, Notch, mTOR, Dorsoventral axis formation and longevity regulating signaling pathways (Figure S2A). In contrast, the worker unique H3K27ac peaks were few and

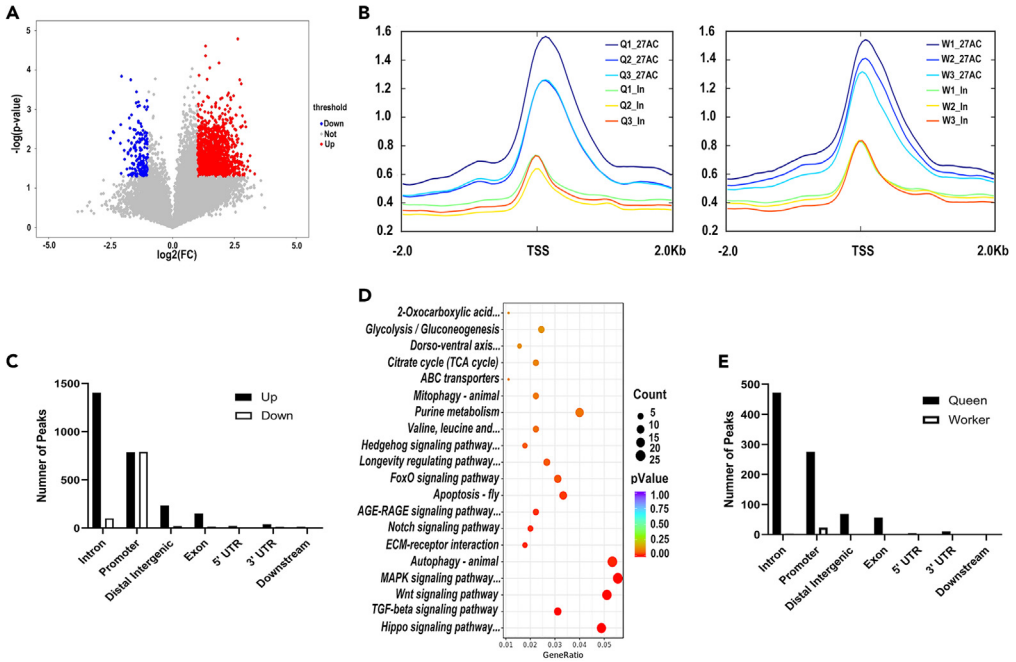


Figure 2. The queen and worker show caste-specific differences in the enrichment of H3K27ac that correlate with differential gene expression

(A) A volcano plot of the difference in enrichment between queen and worker against the negative log pvalue for H3K27ac. Regions in gray fall below the genome-wide threshold of significance ($p > 0.05$). Regions in red (queen) and blue (worker) are those that reach genome-wide significance ($p \leq 0.05$) and have a greater than two-fold difference in enrichment between queen and worker.

(B) Plots of the ChIP-Seq enrichment above input around the TSS (± 2 kbp) of genes profiled across queen (left) and worker (right). There are three replicates performed for each ChIP-Seq experiment.

(C) The distribution of differential peaks between queen and worker in the genome region. The black bars indicate peaks up-regulated in the queen compared with the worker; the white bars indicate peaks down-regulated in the queen.

(D) The top 20 significantly enriched KEGG pathways of differentialH3K27ac peak-related genes between queen and worker.

(E) The distribution of unique peaks in queen and worker in the genome region.

enriched in pathways related to metabolism (Figure S2B). Moreover, silencing the expression of the histone acetylase gene *p300* in honeybee larvae by RNAi resulted in a significant reduction in body weight, body length and the content of H3K27ac of the newly emerged bees in the RNAi group compared with the control group (Figure S3). In conclusion, we hypothesize that H3K27ac histone modification is important in shaping the differentiation between the queens and workers.

The workers have more H3K4me1 modifications than the queens

The distribution of H3K4me1 between the queens and workers is shown in Figure 3A. Compared with that of the queens, the H3K4me1 of the workers were more widely distributed throughout the genome. In addition, the workers have more up-regulated peaks (Figures 3A and 3B), and the genes associated with these differential peaks are enriched in pathways related to the formation of differences between the queens and workers. (Figure 3C, Table S8). There were 2,908 and 1,979 unique peaks in the queens and workers, respectively, which were primarily enriched in intron and promoter regions (Figure 3D). The queen unique peak-related genes were enriched in the Wnt, MAPK, Hippo, TGF-beta, FoxO, Dorsoventral axis formation, Notch, Hedgehog, mTOR, and Phototransduction signaling pathways (Figure S4A). These pathways are associated with body size, ovary development, lifespan, regulation and caste differentiation in honeybees. Pathway enrichment of the worker unique peak-associated genes exhibited results similar to those of the queens (Figure S4B). We analyzed the correlation between the levels of methylation of the peaks located in the promoter region and the expression levels of the corresponding genes and found a significant negative correlation between them (Figure 3E). Moreover, silencing the expression of the histone methylase gene *setd1* led to a significant increase in body length and reduction in H3K4me1 content of the newly emerged

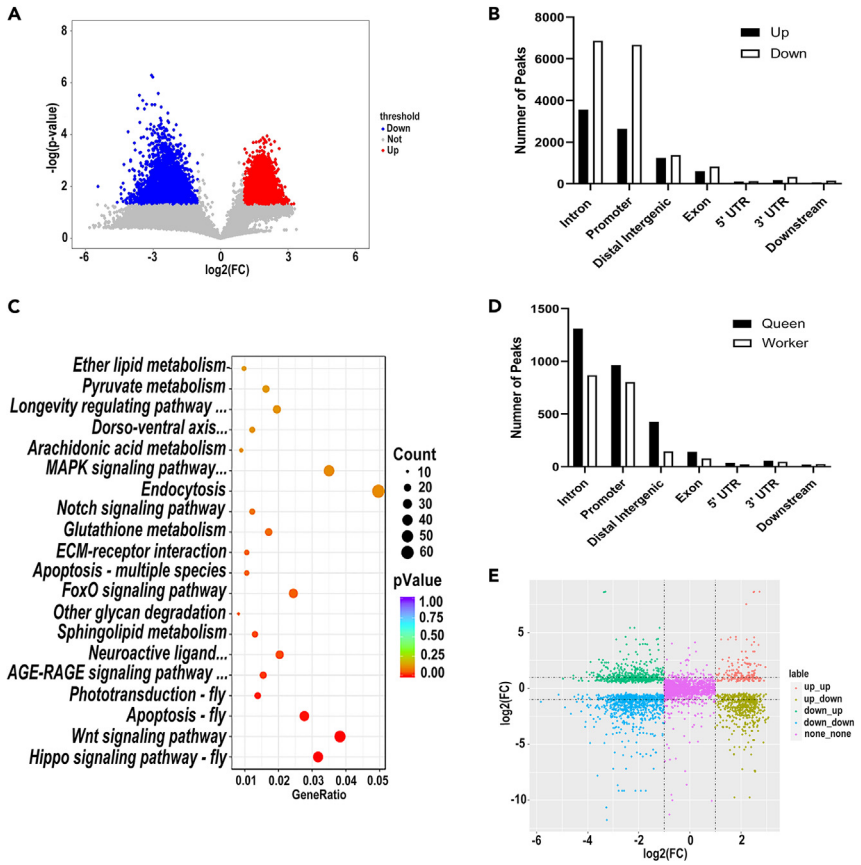


Figure 3. The queen and worker show caste-specific differences in the enrichment of H3K4me1 that correlate with differential gene expression

(A) A volcano plot of the difference in enrichment between queen and worker against the negative log pvalue for H3K4me1. Regions in gray fall below the genome-wide threshold of significance ($p > 0.05$). Regions in red (queen) and blue (worker) are those that reach genome-wide significance ($p \leq 0.05$) and have a greater than two-fold difference in enrichment between queen and worker.

(B) The distribution of differential peaks between queen and worker in the genome region. The black bars indicate peaks up-regulated in the queen compared with the worker; the white bars indicate peaks down-regulated in the queen.

(C) The top 20 significant enriched KEGG pathways of differential H3K4me1 peak-related genes between queen and worker.

(D) The distribution of unique peaks in queen and worker in the genome region.

(E) A scatterplot of the promoter ChIP-Seq H3K4me1 log2 (fold change) for peaks related genes (xaxis) and log2 (fold change) (yaxis) for RNA-Seq FPKM between queen and worker.

bees in the RNAi group compared with those in the control group (Figure S5). In conclusion, we hypothesize that H3K4me1 histone modification is important in shaping the differences between the queens and workers.

Association analysis of Hi-C, H3K27ac and H3K4me1 with DNA methylation

In this study, 839 A/B compartment switch-related genes, 1,868 differential H3K27ac peak-related genes and 5,383 differentially H3K4me1 peak-related genes were identified (Tables S9, S10, and S11). The distributions of CpG Observe/Expect (O/E) in the genomic regions of these genes showed a bimodal pattern (Figures 4A–4C), which is consistent with the findings of previous studies.[52,53]

We compared these genes with the 381 differentially methylated genes (DMG) reported by Lyko et al.,[52] and found that 25 A/B compartment switch-related genes and 72 differential H3K27ac peak-related genes and 223 differential H3K4me1 peak-related genes were overlapped between these two studies (Figures 4D–4F, Tables S12, S13, and S14).

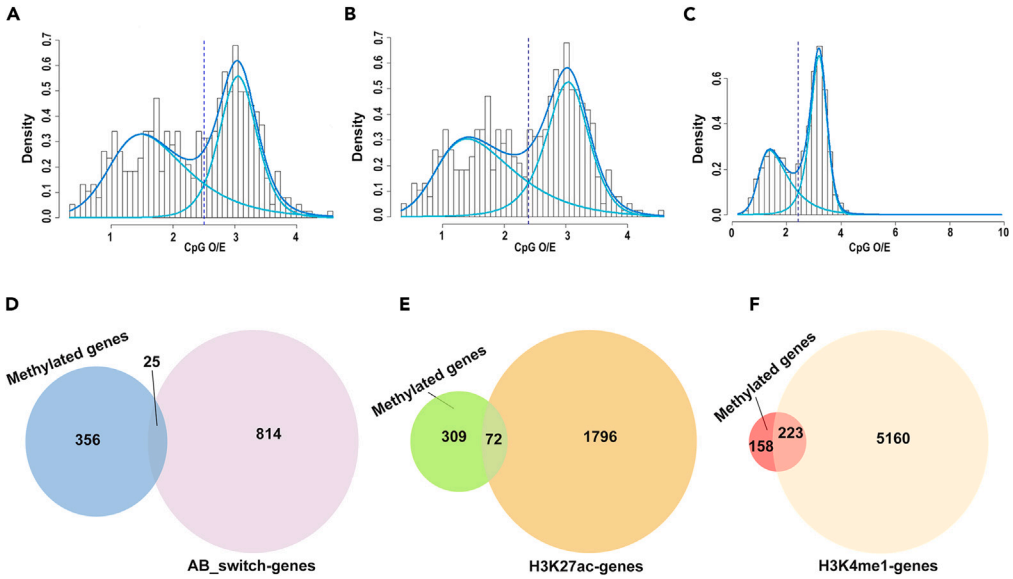


Figure 4. Association analysis of Hi-C, H3K27ac, and H3K4me1 with DNA methylation

(A–F) The upper part indicates the distribution of CpG O/E in genes associated with A/B compartment switches (A) differential H3K27ac peaks (B) and differential H3K4me1 peaks (C) The lower part indicates overlapped genes between the DMEs reported by Frank Lyko et al. and the A/B compartment switch related genes (D), differential H3K27ac peak related genes (E) and differential H3K4me1 peak related genes (F).

Map3k15 is a potential caste gene in queen–worker differentiation

There were 26 differential genes between the queens and workers identified in the integrated analysis of the Hi-C, H3K27ac and RNA-Seq, and 74 differential genes in the integrated analysis of the Hi-C, H3K4me1 and RNA-Seq (Figures 5 and S6). Four genes of these differential genes were chosen to verify their function in queen–worker differentiation using RNAi (Table S15). After knocking down the expression of *Map3k15* gene (GenBank: loc408533) by RNAi (Figure 6A), the newly emerged bees in the RNAi group had significantly reduced body weight and body length compared with the control group although other worker specific traits such as typical pollen baskets were not observed (Figures 6B and 6C). They were obviously smaller in size (Figure 6D), but the other three genes had no significant effect on body weight and body length of the newly emerged bees after RNAi (Figure S7). These results suggest that *Map3k15* has a significant effect on the queen–worker differentiation and is a potential caste gene in honeybees.

DISCUSSION

The dimorphism of queens and workers caused by external environmental factors is a typical epigenetic model.[15] To explore the genetic mechanism behind this phenomenon, previous studies have focused on the differences in transcriptome, proteome, and DNA methylation[54] between the queens and workers during the larval stage. In contrast, only a few studies to date have been conducted to resolve the molecular mechanisms that underlie the differences between adult queens and workers, and the results showed that there were significant differences in DNA methylation between them.[55] In this study, epigenetic differences between the newly emerged queens and workers were compared for the first time using Hi-C, ChIP-Seq, and RNA-Seq technologies. A combined multi-omics analysis was used to reveal differences between them, thus, providing a complex model of genome-wide epigenetic regulation in queens and workers. We found that there were significant differences in chromosomal interactions and H3K27ac and H3K4me1 modifications between the queens and workers. This suggests that developmental differentiation between these two female castes is regulated by multiple epigenetic modifications.

Hi-C sequencing showed that the queens contain more chromosome interactions compared with those of the workers, and A/B switches can significantly affect gene expression. An increasing number of studies have shown that the intra- and inter-chromosomal interactions are relatively common events in regulating gene

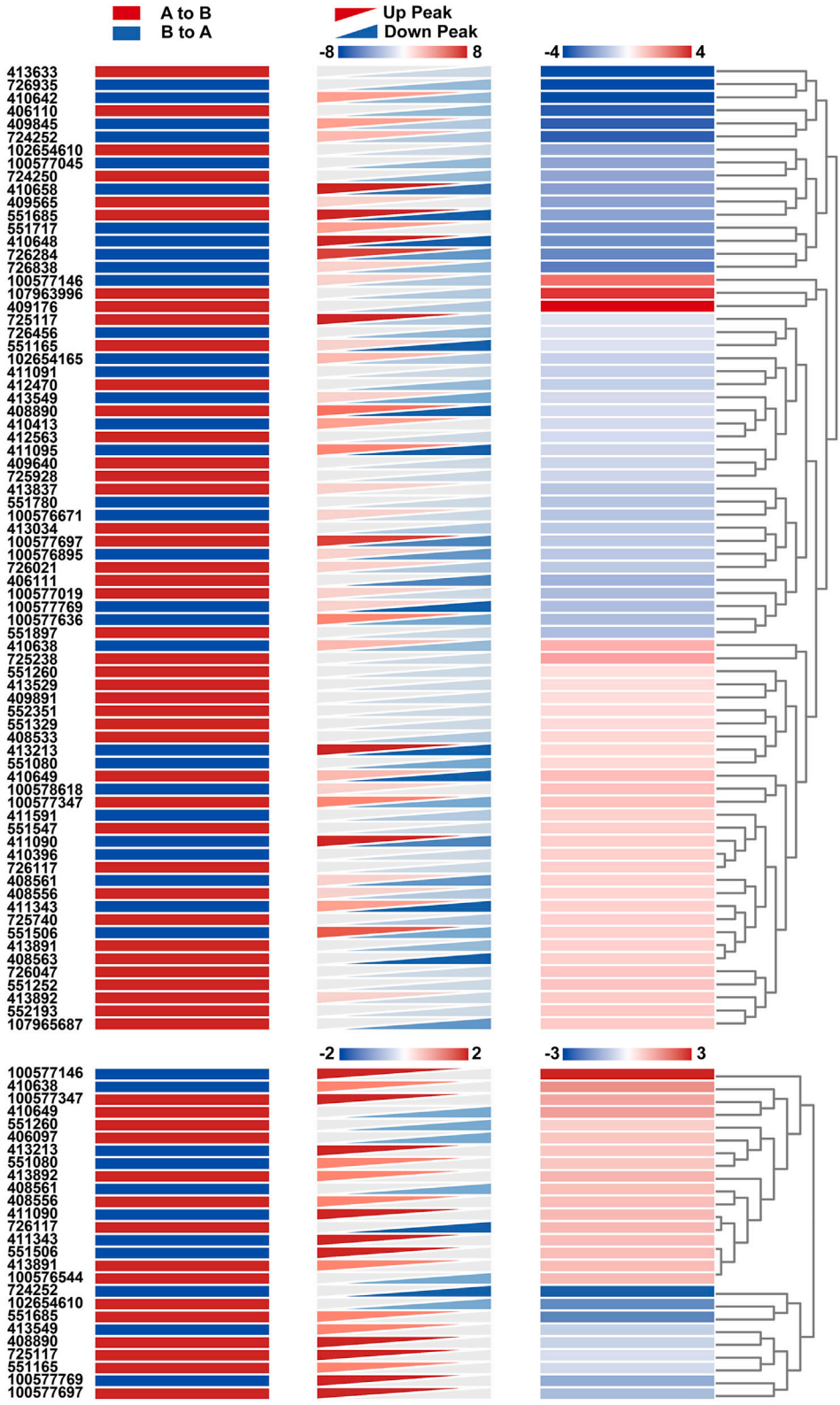


Figure 5. Heatmap of three omics

The genes in the upper part are differential genes in group Hi-C, H3K4me1, and RNA-Seq. The genes in the lower part are differential genes in group Hi-C, H3K27ac and RNA-Seq.

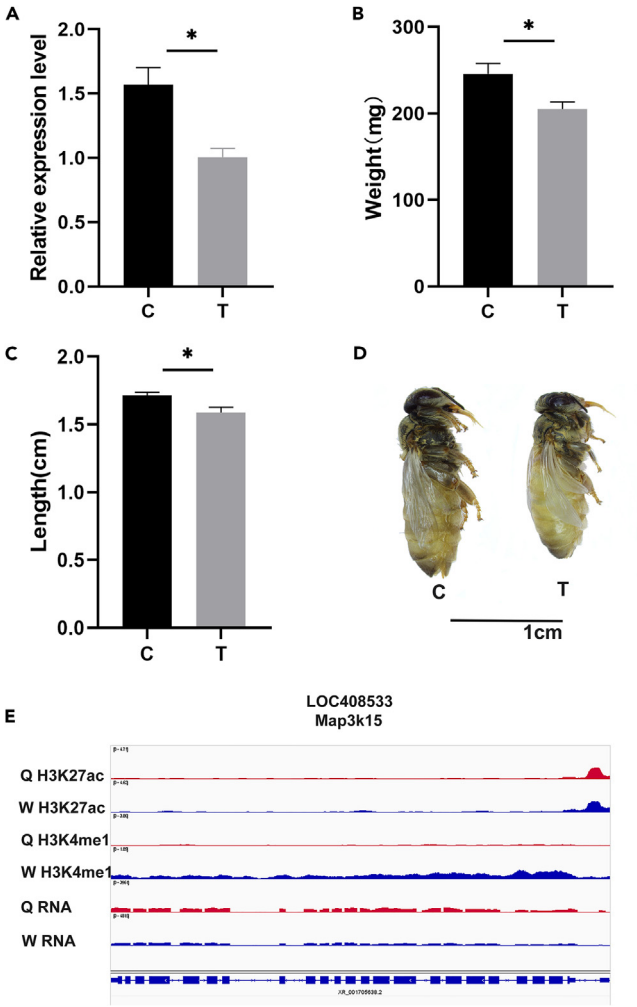


Figure 6. *Map3k15* has a significant effect on caste differentiation of honeybees

(A) The expression change of *Map3k115* after RNAi. Data were presented as mean ± SE. $p < 0.05$ by t-test.

(B) Birth weight of bees between siRNA group and control group. Data were presented as mean ± SE. $p < 0.01$ by t-test.

(C) Body length of bees between siRNA group and control group. Data were presented as mean ± SE. $p < 0.05$ by t-test.

(D) The morphology of bees in the RNAi group and control group.

(E) Expression and histone modification of *Map3k115*.

expression. For example, heart failure is associated with the reduced stability of chromatin interactions around disease-causing genes.[56] Our results suggest that chromatin conformation is involved in queen–worker caste differentiation by regulating the expression of related signaling pathway genes.

In this study, we found that the queen and worker each contained a large number of unique chromatin loops. Typically, chromatin loops enable two regions of the chromosome that are far apart to interact with each other.[46,57] Studies on chromatin structure have shown that regulatory elements, including enhancers, promoters, and insulators, can often form DNA loops to regulate the expression of related genes.[58–60] Our results suggest that these queen- and worker-unique loops could contain a large number of enhancers, promoters, or insulators to regulate transcription of genes located in the loops.

We found that the queens have many more unique H3K27ac peaks compared with those of the workers. In contrast, a previous study at the larval stages showed that the worker larvae had more specific peaks compared with those of the queen larvae. This could suggest that H3K27ac modification is dynamic over the time course of honeybee development. The H3K27ac modification is a robust mark of active enhancers and promoters that are strongly correlated with gene expression and transcription factor binding.[61] We

found that the unique peaks of the queens were primarily distributed in the intron and promoter regions, suggesting that H3K27ac modifications in the promoter region of these related genes that are unique to the queens lead to the development of queen-specific phenotype.

We found that H3K4me1 modifications at the genome-wide level were higher in the workers than in the queens overall and had more up-regulated peaks in the workers. Moreover, the differential H3K4me1 peaks between the queens and workers were primarily distributed in promoter and intron regions. H3K4me1 is found at both transcriptional active promoters and distal regulatory elements, such as enhancers, and the H3K4me1 modification of promoters is often associated with the conditional repression of inducible genes.[62,63] Similarly, we found a negative correlation between methylation of the H3K4me1promoter and gene expression. Our results suggest that the queen–worker differential H3K4me1 peaks could result in worker phenotypes by repressing gene transcription in the workers.

Among the genes that showed difference in all three omics, we used RNAi to confirm that the *Map3k15* (*ask3*) gene of the MAPK signaling pathway has an obvious effect on caste differentiation between the queens and workers. *Map3k15* is a member of the apoptosis signal-regulating kinases and plays an indispensable role in the signal transduction pathway implicated in cell death triggered by various types of cellular stresses, as well as in tumor initiation and progression.[64,65] Our RNAi results suggest that *Map3k15* could regulate cell proliferation and differentiation in honeybees. Moreover, we found that the differentially regulated H3K4me1 peaks of *Map3k15* between the queens and workers was located in the intron region of this gene (Figure 6E), suggesting that H3K4me1 modifications could act on the introns of *Map3k15* to regulate its differential expression between the queens and workers which, in turn, leads to caste differentiation.

In conclusion, we found significant differences in chromatin interactions and the modifications of H3K27ac and H3K4me1 between the queens and workers, and these epigenetic modifications could be important causes of phenotypic dimorphism between the queens and workers. Furthermore, we found that the *Map3k15* gene, which showed differences in three omics, is a potential caste gene in queen–worker differentiation. These results suggest that phenotypic differentiation between the queens and workers is a complex process that is regulated by multiple epigenetic modifications.

Limitations of the study

This study found extensive caste differences in chromosome interaction and H3K27ac and H3K4me1 modifications between queens and workers by Hi-C and ChIP-Seq technologies. However, the queen–worker differences are exhibited in many tissues/organs, our study just considered these epigenetic differences at the whole body level and not at the tissue/organ level. In further studies, we need to explore how epigenetic modifications affect the developmental differentiation of specific tissues/organs between queens and workers.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Insects
- METHOD DETAILS
  - Rearing of queens and workers
  - RNA-seq
  - ChIP-seq
  - Hi-C
  - GO and KEGG analysis
  - RNAi
  - qRT-PCR

- Elisa
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106308.

ACKNOWLEDGMENTS

We thank Ms. Zhen Xiu Zeng for her help on the experimental honeybee colony and Novogene Corporation (Beijing, China) for the sequencing service. This work was supported by the National Natural Science Foundation of China (32172790, 31702193), Jiangxi provincial academic and technical leader project (20204BCJL23041) and the Earmarked Fund for China Agriculture Research System (CARS-44-KXJ15).

AUTHOR CONTRIBUTIONS

Z.J.Z. designed the research. M.J.J., Z.L.W., X.J.H., and Y.Z. performed the research. M.J.J. conducted most experiments. M.J.J., Z.L.W., X.J.H., Y.Z., and Q.H. analyzed the data. M.J.J. and Z.L.W. performed validation experiments. Z.L.W. and L.Z.Z. collected experimental data, X.B.W. and W.Y.Y. conceived of the study, designed the study, coordinated the study, and helped draft the manuscript. Z.L.W., M.J.J., L.C., P. J.H., and Z.J.Z. wrote and revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interest. All authors were involved in the preparation of the final manuscript.

Received: May 23, 2022
Revised: January 12, 2023
Accepted: February 24, 2023
Published: February 28, 2023

REFERENCES

1. Robinson, G.E. (2002). Genomics and integrative analyses of division of labor in honeybee colonies. Am. Nat. *160*, S160–S172. https://doi.org/10.1086/342901.

2. Zhang, H., Zeng, Z.J., Yan, W.Y., Wu, X.B., and Zhang, Y.L. (2010). Effects of three aliphatic esters of brood pheromone on development and foraging behavior of Apis cerana cerana workers. Acta Entomol. Sin. *53*, 55–60. https://doi.org/10.1016/S1002-0721(10)60377-8.

3. I'Anson Price, R., Dulex, N., Vial, N., Vincent, C., and Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. Sci. Adv. *5*, eaat0450. https://doi.org/10.1126/sciadv.aat0450.

4. Kearns, C.A., and Inouye, D.W. (1997). Pollinators, flowering plants, and conservation biology. Bioscience *47*, 297–307. https://doi.org/10.2307/1313191.

5. Delaplane, K.S., Mayer, D.R., and Mayer, D.F. (2000). Crop Pollination by Bees (Cabi). https://doi.org/10.1079/9780851994482.0000.

6. Page, R.E., Jr., and Peng, C.Y. (2001). Aging and development in social insects with emphasis on the honey bee, Apis mellifera L. Exp. Gerontol. *36*, 695–711. https://doi.org/10.1016/S0531-5565(00)00236-9.

7. Sakagami S.F., and Akahira Y. Comparison of Ovarian Size and Number of Ovarioles Between the Workers of Japanese and European Honeybees: Studies on the Japanese Honeybee, Apis indica cerana Fabricius. I. 昆蟲, Japanese Journal of entomology, 26; 1958. p. 103–109. http://dl.ndl.go.jp/info:ndljp/pid/10649688.

8. Grozinger, C.M., Fan, Y., Hoover, S.E.R., and Winston, M.L. (2007). Genome-wide analysis reveals differences in brain gene expression patterns associated with caste and reproductive status in honey bees (Apis mellifera). Mol. Ecol. *16*, 4837–4848. https://doi.org/10.1111/j.1365-294X.2007.03545.x.

9. Mohammedi, A., Paris, A., Crauser, D., and Le Conte, Y. (1998). Effect of aliphatic esters on ovary development of queenless bees (Apis mellifera L.). Naturwissenschaften *85*, 455–458.

10. Hoover, S.E.R., Keeling, C.I., Winston, M.L., and Slessor, K.N. (2003). The effect of queen pheromones on worker honey bee ovary development. Naturwissenschaften *90*, 477–480. https://doi.org/10.1007/s00114-003-0462-z.

11. Fahrbach, S.E., and Robinson, G.E. (1995). Behavioral development in the honey bee: toward the study of learning under natural conditions. Learn. Mem. *2*, 199–224. https://doi.org/10.1101/lm.2.5.199.

12. Robinson, G.E., Fahrbach, S.E., and Winston, M.L. (1997). Insect societies and the molecular biology of social behavior. Bioessays *19*, 1099–1108. https://doi.org/10.1002/bies.950191209.

13. Wilde, J.d., and Beetsma, J. (1982). The physiology of caste development in social insects. Adv. Insect Physiol. *16*, 167–246. https://doi.org/10.1016/S0065-2806(08)60154-X.

14. Usherwood, P.N.R. (1985). Comprehensive insect physiology, biochemistry and pharmacology. In Insect Control, G.A. Kerkut and L.I. Gilbert, eds. (Oxford University Press), pp. 849–863.

15. Haydak, M.H. (1970). Honey bee nutrition. Annu. Rev. Entomol. *15*, 143–156.

16. Sun, G.I., Su, S.K., Chen, S.,L., and Du, H.H. (2008). Biological functions of the royal jelly proteins. Zool. Res. *29*, 459–464. https://doi.org/10.3724/SP.J.1141.2008.00459.

17. Kamakura, M. (2011). Royalactin induces queen differentiation in honeybees. Nature *473*, 478–483. https://doi.org/10.1038/nature10093.

18. Mao, W., Schuler, M.A., and Berenbaum, M.R. (2015). A dietary phytochemical alters caste-associated gene expression in honey bees. Sci. Adv. *1*, e1500795. https://doi.org/10.1126/sciadv.1500795.

19. Rachinsky, A., Strambi, C., Strambi, A., and Hartfelder, K. (1990). Caste and metamorphosis: hemolymph titers of juvenile hormone and ecdysteroids in last instar honeybee larvae. Gen. Comp. Endocrinol. *79*, 31–38. https://doi.org/10.1016/0016-6480(90)90085-Z.

20. Mutti, N.S., Dolezal, A.G., Wolschin, F., Mutti, J.S., Gill, K.S., and Amdam, G.V. (2011). IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. J. Exp. Biol. *214*, 3977–3984. https://doi.org/10.1242/jeb.061499.

21. Evans, J.D., and Wheeler, D.E. (1999). Differential gene expression between developing queens and workers in the honey bee, Apis mellifera. Proc. Natl. Acad. Sci. USA *96*, 5575–5580. https://doi.org/10.1073/pnas.96.10.5575.

22. Shilo, B.-Z. (2005). Regulating the dynamics of EGF receptor signaling in space and time. Development *132*, 4017–4027. https://doi.org/10.1242/dev.02006.

23. Barchuk, A.R., Cristino, A.S., Kucharski, R., Costa, L.F., Simões, Z.L.P., and Maleszka, R. (2007). Molecular determinants of caste differentiation in the highly eusocial honeybee Apis mellifera. BMC Dev. Biol. *7*, 1–19. https://doi.org/10.1186/1471-213X-7-70.

24. Yamanaka, N., and O'Connor, M.B. (2011). Apiology: royal secrets in the queen's fat body. Curr. Biol. *21*, R510–R512. https://doi.org/10.1016/j.cub.2011.05.037.

25. Cameron, R.C., Duncan, E.J., and Dearden, P.K. (2013). Biased gene expression in early honeybee larval development. BMC Genom. *14*, 903–912. https://doi.org/10.1186/1471-2164-14-903.

26. Shao, X.L., He, S.Y., Zhuang, X.Y., Fan, Y., Li, Y.H., and Yao, Y.G. (2014). mRNA expression and DNA methylation in three key genes involved in caste differentiation in female honeybees (Apis mellifera). Dongwuxue Yanjiu *35*, 92–98. https://doi.org/10.11813/j.issn.0254-5853.2014.2.092.

27. Kucharski, R., Maleszka, J., Foret, S., and Maleszka, R. (2008). Nutritional control of reproductive status in honeybees via DNA methylation. Science *319*, 1827–1830. https://doi.org/10.1126/science.1153069.

28. Shi, Y.Y., Huang, Z.Y., Zeng, Z.J., Wang, Z.L., Wu, X.B., and Yan, W.Y. (2011). Diet and cell size both affect queen–worker differentiation through DNA methylation in honey bees (Apis mellifera, Apidae). PLoS One *6*, e18808. https://doi.org/10.1371/journal.pone.0018808.

29. Foret, S., Kucharski, R., Pellegrini, M., Feng, S., Jacobsen, S.E., Robinson, G.E., and Maleszka, R. (2012). DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. Proc. Natl. Acad. Sci. USA *109*, 4968–4973. https://doi.org/10.1073/pnas.1202392109.

30. Barker, S.A., Foster, A.B., Lamb, D.C., and Jackman, L.M. (1959). Biological origin and configuration of 10-Hydroxy-Δ2-decenoic acid. Nature *184*, 634. https://doi.org/10.1038/184634a0.

31. Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., Bui, T.V., Cross, J.R., and Thompson, C.B. (2009). ATP-citrate lyase links cellular metabolism to histone acetylation. Science *324*, 1076–1080. https://doi.org/10.1126/science.1164097.

32. Spannhoff, A., Kim, Y.K., Raynal, N.J.-M., Gharibyan, V., Su, M.-B., Zhou, Y.-Y., Li, J., Castellano, S., Sbardella, G., Issa, J.-P.J., and Bedford, M.T. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. EMBO Rep. *12*, 238–243. https://doi.org/10.1038/embor.2011.9.

33. Guo, X., Su, S., Skogerboe, G., Dai, S., Li, W., Li, Z., Liu, F., Ni, R., Guo, Y., Chen, S., et al. (2013). Recipe for a busy bee: microRNAs in honey bee caste determination. PLoS One *8*, e81661. https://doi.org/10.1371/journal.pone.0081661.

34. Shi, Y.-Y., Zheng, H.-J., Pan, Q.-Z., Wang, Z.-L., and Zeng, Z.-J. (2015). Differentially expressed microRNAs between queen and worker larvae of the honey bee (Apis mellifera). Apidologie *46*, 35–45. https://doi.org/10.1007/s13592-014-0299-9.

35. Zhu, K., Liu, M., Fu, Z., Zhou, Z., Kong, Y., Liang, H., Lin, Z., Luo, J., Zheng, H., Wan, P., et al. (2017). Plant microRNAs in larval food regulate honeybee caste development. PLoS Genet. *13*, e1006946. https://doi.org/10.1371/journal.pgen.1006946.

36. He, X.J., Barron, A.B., Yang, L., Chen, H., He, Y.Z., Zhang, L.Z., Huang, Q., Wang, Z.L., Wu, X.B., Yan, W.Y., and Zeng, Z.J. (2022). Extent and complexity of RNA processing in honey bee queen and worker caste development. iScience *25*, 104301. https://doi.org/10.1016/j.isci.2022.104301.

37. Wheeler, D.E., Buck, N.A., and Evans, J.D. (2014). Expression of insulin/insulin-like signalling and TOR pathway genes in honey bee caste determination. Insect Mol. Biol. *23*, 113–121. https://doi.org/10.1111/imb.12065.

38. Ashby, R., Forêt, S., Searle, I., and Maleszka, R. (2016). MicroRNAs in honey bee caste determination. Sci. Rep. *6*, 18794–18815. https://doi.org/10.1038/srep18794.

39. Duncan, E.J., Hyink, O., and Dearden, P.K. (2016). Notch signalling mediates reproductive constraint in the adult worker honeybee. Nat. Commun. *7*, 12427–12510. https://doi.org/10.1038/ncomms12427.

40. Fernandez-Nicolas, A., and Belles, X. (2016). CREB-binding protein contributes to the regulation of endocrine and developmental pathways in insect hemimetabolan pre-metamorphosis. Biochim. Biophys.ActaGen. Subj. *1860*, 508–515. https://doi.org/10.1016/j.bbagen.2015.12.008.

41. Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature *389*, 349–352. https://doi.org/10.1038/38664.

42. Lachner, M., and Jenuwein, T. (2002). The many faces of histone lysine methylation. Curr. Opin. Cell Biol. *14*, 286–298. https://doi.org/10.1016/S0955-0674(02)00335-6.

43. Wojciechowski, M., Lowe, R., Maleszka, J., Conn, D., Maleszka, R., and Hurd, P.J. (2018). Phenotypically distinct female castes in honey bees are defined by alternative chromatin states during larval development. Genome Res. *28*, 1532–1542. https://doi.org/10.1101/gr.236497.118.

44. Lieberman-Aiden, E., Van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science *326*, 289–293. https://doi.org/10.1126/science.1181369.

45. Jin, F., Li, Y., Dixon, J.R., Selvaraj, S., Ye, Z., Lee, A.Y., Yen, C.-A., Schmitt, A.D., Espinoza, C.A., and Ren, B. (2013). A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature *503*, 290–294. https://doi.org/10.1038/nature12644.

46. Rao, S.S.P., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., and Aiden, E.L. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell *159*, 1665–1680. https://doi.org/10.1016/j.cell.2014.11.021.

47. Bickmore, W.A., and van Steensel, B. (2013). Genome architecture: domain organization of interphase chromosomes. Cell *152*, 1270–1284. https://doi.org/10.1016/j.cell.2013.02.001.

48. Bonev, B., and Cavalli, G. (2016). Organization and function of the 3D genome. Nat. Rev. Genet. *17*, 661–678. https://doi.org/10.1038/nrg.2016.112.

49. Rowley, M.J., and Corces, V.G. (2018). Organizational principles of 3D genome architecture. Nat. Rev. Genet. *19*, 789–800. https://doi.org/10.1038/s41576-018-0060-8.

50. Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature *485*, 376–380. https://doi.org/10.1038/nature11082.

51. Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A., and Cavalli, G. (2012). Three-dimensional folding and functional organization principles of the Drosophila genome. Cell *148*, 458–472. https://doi.org/10.1016/j.cell.2012.01.010.

52. Lyko, F., Foret, S., Kucharski, R., Wolf, S., Falckenhayn, C., and Maleszka, R. (2010). The honey bee epigenomes: differential methylation of brain DNA in queens and workers. PLoS Biol. *8*, e1000506. https://doi.org/10.1371/annotation/2db9ee19-faa4-43f2-af7a-c8aeacca8037.

53. Zeng, J., and Yi, S.V. (2010). DNA methylation and genome evolution in honeybee: gene length, expression, functional enrichment covary with the evolutionary signature of DNA methylation. Genome Biol. Evol. *2*,

770–780. https://doi.org/10.1093/gbe/evq060.

54. Foret, S., Kucharski, R., Pittelkow, Y., Lockett, G.A., and Maleszka, R. (2009). Epigenetic regulation of the honey bee transcriptome: unravelling the nature of methylated genes. BMC Genom. *10*, 472–511. https://doi.org/10.1186/1471-2164-10-472.

55. Elango, N., Hunt, B.G., Goodisman, M.A.D., and Yi, S.V. (2009). DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, Apis mellifera. Proc. Natl. Acad. Sci. USA *106*, 11206–11211. https://doi.org/10.1073/pnas.0900301106.

56. Rosa-Garrido, M., Chapski, D.J., Schmitt, A.D., Kimball, T.H., Karbassi, E., Monte, E., Balderas, E., Pellegrini, M., Shih, T.-T., Soehalim, E., et al. (2017). High-resolution mapping of chromatin conformation in cardiac myocytes reveals structural remodeling of the epigenome in heart failure. Circulation *136*, 1613–1625. https://doi.org/10.1161/CIRCULATIONAHA.117.029430.

57. Duggal, G., Wang, H., and Kingsford, C. (2014). Higher-order chromatin domains link eQTLs with the expression of far-away genes. Nucleic Acids Res. *42*, 87–96. https://doi.org/10.1093/nar/gkt857.

58. Doyle, B., Fudenberg, G., Imakaev, M., and Mirny, L.A. (2014). Chromatin loops as allosteric modulators of enhancer-promoter interactions. PLoS Comput. Biol. *10*, e1003867. https://doi.org/10.1371/journal.pcbi.1003867.

59. Priest, D.G., Kumar, S., Yan, Y., Dunlap, D.D., Dodd, I.B., and Shearwin, K.E. (2014). Quantitation of interactions between two DNA loops demonstrates loop domain insulation in E. coli cells. Proc. Natl. Acad. Sci. USA *111*, E4449–E4457. https://doi.org/10.1073/pnas.1410764111.

60. Grubert, F., Srivas, R., Spacek, D.V., Kasowski, M., Ruiz-Velasco, M., Sinnott-Armstrong, N., Greenside, P., Narasimha, A., Liu, Q., Geller, B., et al. (2020). Landscape of cohesin-mediated chromatin loops in the human genome. Nature *583*, 737–743. https://doi.org/10.1038/s41586-020-2151-x.

61. Creyghton, M.P., Cheng, A.W., Welstead, G.G., Kooistra, T., Carey, B.W., Steine, E.J., Hanna, J., Lodato, M.A., Frampton, G.M., Sharp, P.A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc. Natl. Acad. Sci. USA *107*, 21931–21936. https://doi.org/10.1073/pnas.1016071107.

62. Barski, A., Cuddapah, S., Cui, K., Roh, T.-Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. Cell *129*, 823–837. https://doi.org/10.1016/j.cell.2007.05.009.

63. Cheng, J., Blum, R., Bowman, C., Hu, D., Shilatifard, A., Shen, S., and Dynlacht, B.D. (2014). A role for H3K4 monomethylation in gene repression and partitioning of chromatin readers. Mol. Cell *53*, 979–992. https://doi.org/10.1016/j.molcel.2014.02.032.

64. Kaji, T., Yoshida, S., Kawai, K., Fuchigami, Y., Watanabe, W., Kubodera, H., and Kishimoto, T. (2010). ASK3, a novel member of the apoptosis signal-regulating kinase family, is essential for stress-induced cell death in HeLa cells. Biochem. Biophys. Res. Commun. *395*, 213–218. https://doi.org/10.1016/j.bbrc.2010.03.164.

65. Morishita, K., Watanabe, K., and Ichijo, H. (2019). Cell volume regulation in cancer cell migration driven by osmotic water flow. Cancer Sci. *110*, 2337–2347. https://doi.org/10.1111/cas.14079.

66. Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. *37*, 907–915. https://doi.org/10.1038/s41587-019-0201-4.

67. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics *30*, 923–930. https://doi.org/10.1093/bioinformatics/btt656.

68. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. *15*, 550. https://doi.org/10.1186/s13059-014-0550-8.

69. Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140. https://doi.org/10.1093/bioinformatics/btp616.

70. Yu, G., Wang, L.G., Han, Y., and He, Q.Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS *16*, 284–287. https://doi.org/10.1089/omi.2011.0118.

71. Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics *34*, i884–i890. https://doi.org/10.1089/omi.2011.0118.

72. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinforma; 2010.

73. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics *25*, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

74. Ramírez, F., Dündar, F., Diehl, S., Grüning, B.A., and Manke, T. (2014). deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res. *42*, W187–W191. https://doi.org/10.1093/nar/gku365.

75. Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nusbaum, C., Myers, R.M., Brown, M., Li, W., and Liu, X.S. (2008). Model-based analysis of ChIP-seq (MACS). Genome Biol. *9*, R137–R139. https://doi.org/10.1186/gb-2008-9-9-r137.

76. Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. *11*, R14. https://doi.org/10.1186/gb-2010-11-2-r14.

77. Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods *9*, 357–359. https://doi.org/10.1038/nmeth.1923.

78. Servant, N., Varoquaux, N., Lajoie, B.R., Viara, E., Chen, C.-J., Vert, J.-P., Heard, E., Dekker, J., and Barillot, E. (2015). HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. Genome Biol. *16*, 259–311. https://doi.org/10.1186/s13059-015-0831-x.

79. Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell *38*, 576–589. https://doi.org/10.1016/j.molcel.2010.05.004.

80. Xie, C., Mao, X., Huang, J., Ding, Y., Wu, J., Dong, S., Kong, L., Gao, G., Li, C.Y., and Wei, L. (2011). Kobas 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. *39*, W316–W322. https://doi.org/10.1093/nar/gkr483.

81. Pan, Q.-Z., Wu, X.-B., Guan, C., and Zeng, Z.-J. (2013). A new method of queen rearing without grafting larvae. Am. Bee J. *153*, 1279–1280.

82. Wolff, J., Bhardwaj, V., Nothjunge, S., Richard, G., Renschler, G., Gilsbach, R., Manke, T., Backofen, R., Ramírez, F., and Grüning, B.A. (2018). Galaxy HiCExplorer: a web server for reproducible Hi-C data analysis, quality control and visualization. Nucleic Acids Res. *46*, W11–W16. https://doi.org/10.1093/nar/gky504.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Honeybee (*Apis mellifera*) queen and worker	This paper	N/A
Critical commercial assays		
400 U MboIrestriction enzyme (NEB)	NEB (USA)	Cat#:R0147L
T4 DNA ligase (NEB)	Enzymatics (USA)	Cat#:L6030-HC-L
SDS solution	Amresco (USA)	Cat#:0227
proteinase K	Tiangen (China)	Cat#:RT403
Total RNA Kit	Ambin (USA)	Trizol:15596-018
dNTP solution	New England Biolabs (USA)	Cat#:NEB N0447
AMPure XP beads	Beckman Coulter (USA)	Cat#:A63882
Deposited data		
RNA-Seqdata	NCBI database	BioProject: PRJNA812631
ChIP-seqdata	NCBI database	BioProject:PRJNA816613
Hi-Cdata	NCBI database	BioProject: PRJNA816706
Experimental models: Organisms/strains		
Honeybees (*Apis mellifera*)	Honey bee Research institute, Jiangxi Agricultural university (China)	N/A
Software and algorithms		
hisat2	Kim et al.[66]	version 2.0.5
featureCounts	Liao et al.[67]	version 1.5.0-p3
DESeq2	Love et al.[68]	version 1.16.1
edgeR	Robinson et al.[69]	version 3.18.1
clusterProfiler	Yu et al.[70]	version 3.4.4
Fastp	Chen et al.[71]	version 0.19.11
Fastqc	Andrews et al.[72]	version 0.11.5
BWA	Li et al.[73]	version 0.7.12-r1039
deepTools(bamCorrelate bins)	Ramirez et al.[74]	version 1.5.9
MACS2	Zhang et al.[75]	version 2.1.0
Goseq, topGO, Bioconductor (2.13)	Young et al.[76]	version 4.10.2
Bowtie2	Langmead et al.[77]	version 2.23
HiC-Pro	Servant et al.[78]	version 2.11.1
DomainCaller	Dixon et al.[50]	
HOMER	Heinz et al.[79]	version 4.11
KOBAS	Xie et al.[80]	version 2.0

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Prof. Zhi Jiang Zeng (bees1965@sina.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The sequence data for all the fastq sequences of RNA-Seq, ChIP-seqdata, Hi-Cdata have been deposited in the NCBI database under the accession: NCBI BioProject: PRJNA812631, PRJNA816613, PRJNA816706. All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Insects

Three healthy honeybee colonies (*Apis mellifera*) each with a mated queen were used for this study. Each colony had ten frames with approximately 35,000 bees. These colonies were maintained at the Honey bee Research Institute, Jiangxi Agricultural University, Nanchang, China, according to the standard beekeeping techniques.

METHOD DETAILS

Rearing of queens and workers

The bees were from colonies of *A. mellifera* in the spring of 2020 that were maintained at the Honey bee Research Institute, Jiangxi Agricultural University, Nanchang, China (28.46°N, 115.49°E).

A naturally mated laying queen was caged for 6 h on a comb of plastic worker cells to lay eggs.[81] On the second day, some eggs were moved to the queen cells to breed queens, and the rest were continued to be cultivated as workers. The queen and worker larvae were reared in the same colony and removed from the colony two days before eclosion and placed in a 34°C incubator 80% relative humidity to emerge. The newly emerged queens and workers were collected with frozen storage tubes and maintained in liquid nitrogen. For all three omics, three biological replicates of newly emerging queens and workers were sampled.

RNA-seq

The total RNA was extracted from the queen and worker samples using standard methods. RNA integrity was measured using an RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). For each sample, 1 μg RNA was used for library construction. The sequencing libraries were constructed using an NEB Net Ultra RNA Library Preparation Kit for Illumina (NEB, Ipswich, MA, USA). Preliminary quantification was performed using a Qubit2.0 fluorimeter (Invitrogen, Carlsbad, CA, USA), and the insert size of libraries was assessed on an Agilent Bioanalyzer 2100 system (Agilent Technologies). The effective concentration of the libraries (above 2 nM) was accurately quantified by real-time quantitative reverse transcription PCR (qRT-PCR). The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, CA, USA). After clustering, the libraries were sequenced on an Illumina NovaSeq platform, and 150 bp paired-end reads were generated. By removing reads that contain adapters, poly-N, and low-quality reads from the raw data, clean data were obtained and used for subsequent analyses. The reference genome and gene model annotation files of *A. mellifera* were downloaded from the GenBank database in NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/003/254/395/GCA_003254395.2_Amel_HAv3.1/). The paired-end clean reads were mapped to the reference genome using Hisat2 v2.0.5. DEGs were identified using the DESeq2 R package (1.20.0) with the criteria $|\log2(\text{fold change})| \geqq 1$ and $p < 0.05$.

ChIP-seq

The levels of histone H3K27 acetylation and H3K4 monomethylation on chromatin were evaluated by chromatin immunoprecipitation. Briefly, newly emerged queens and workers were cross-linked in 1% formaldehyde for 10 min. After the extraction of chromatin and sonication, the genomic DNA was immunoprecipitated with anti-histone H3 acetylation antibody or anti-histone H3 methylation antibody. ChIP DNA contamination and degradation was monitored on agarose gels. The DNA purity was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, USA). The DNA concentration was measured using a Qubit DNA Assay Kit in a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). ChIP-seq libraries were prepared from purified DNA. The libraries were constructed by Novogene Corporation (Beijing, China). The library quality was assessed on an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

The ChIP-seq libraries were sequenced on an Illumina HiSeq 3000 platform to produce 150 bp single-end reads. The clean reads were then aligned to the *A. mellifera* genome version Amel_HAv3.1by BWA version 0.7.12-r1039. MACS2 version 2.1.0 software was used to estimate the fragment size and calculate the level of the reads enrichment. After mapping the reads to the reference genome, the MACS2 version 2.1.0 peak-seeking algorithm was used to identify IP-enriched regions on the background. The enrichment q-value threshold for all the datasets was 0.05.

Hi-C

Newly emerged queens and workers were treated with 1% formaldehyde to crosslink DNA and proteins. The cell nuclei were then isolated from these samples, and the chromatin was cleaved with 400 U Mbo I restriction enzyme (NEB) at 37°C. The DNA fragments that were obtained were end-repaired, labeled with biotin, and ligated using T4 DNA ligase (NEB) to form loops. After ligation, the proteins that linked the DNA fragments were digested with proteinase K at 65 °C. The DNA fragments were then purified, and the unlinked ends were removed. The purified DNA fragments were fragmented to a size range of 300-500bp using ultrasonic waves and subsequently end-repaired. Biotin-labeled DNA fragments were isolated on Dynabeads M−280 Streptavidin (Life Technologies). The Hi-C libraries were then quality controlled and sequenced on an Illumina platform.

The clean reads were aligned to the *A. mellifera* genome version Amel_HAv3.1 using HIC-pro version 2.11.1. The genome-wide contact maps were produced with HOMER version 4.11. The eigenvector values of the matrix were calculated by a principal component analysis. The chromosomes were divided into A/B compartments based on the first principal component (E1). The signs of the eigenvalues were inverted according to the histone (H3K27ac) content.[82]

GO and KEGG analysis

The GO functional enrichment analysis on differential gene sets was performed using clusterProfiler software. The statistical enrichment of peak-related genes in the KEGG pathways was tested using KOBAS version 2.0 software.

RNAi

A naturally mated laying queen was caged on worker cell comb to lay eggs for 6 h. After hatching, some of the larvae were transferred into 24-well culture plates that contained 350 μL of larval food (6% fructose, 6% glucose, 1% yeast powder, 37% distilled water, and 50% royal jelly). After that, the larvae were fed 300 μL of larval food three times per day until they pupated.

Six genes, including loc408533, loc410649, loc725117, loc100577697, loc726280, loc411985, were chosen for RNAi. Small interfering RNAs (siRNAs) of these genes were designed based on their mRNA sequences and synthesized by GenePharma (Shanghai, China). The 3-day-old larvae were used for RNAi experiments. For each gene, two experimental groups with the same number of randomly assigned larvae were established, one group was injected with 2 ng (1 μg/μL) of siRNA into the abdomen. The second group was injected with the same volume and concentration of random siRNA as a negative control. A total of 24 h after injection, some of the larvae were sampled to test the efficiency of RNAi through quantitative PCR (qPCR). Every two larvae were mixed as a sample, and five biological replicates were sampled.

When the larvae had developed into adults, the weights of the bees in the RNAi and control groups were weighed after emergence with an electronic balance. The data were analyzed by a *t*-test using SPSS17.0 (SPSS, Inc., Chicago, IL, USA).

qRT-PCR

The total RNAs were extracted from the samples described above using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. A total of 1 μg of total RNA in every sample was reverse transcribed into cDNA using reverse transcriptase in a PrimeScript RT reagent Kit (Perfect Real Time, TaKaRa, Dalian, China). Real-time PCR amplification was conducted using TB Green Premix Ex Taq II (Tli RNase H Plus). Primers (Table S15) were used for the qRT-PCR detection of six honey bee genes, and RP49-F and RP49-R were used to amplify the endogenous control gene *rp49*. Gene-specific quantitative real-time PCR analyses were executed on a QuantStudioTM5 Real-Time PCR Instrument (Applied Biosystems).

The reaction system of final volume was 10 μL, and contained 5 μL of TB Green Premix Ex Taq II (Tli RNase H Plus), 0.4 μL of PCR forward Primer, 0.4 μL of PCR reverse Primer, 0.2 μL of ROX Reference Dye or Dye II, 3 μL of sterile water and 1 μL of first strand cDNA. The PCR amplification specificity of all the samples was assessed by a melting curve analysis. The cDNA was diluted 10-fold to correct the differences in amplification efficiency, and three biological replicates were performed for each sample. The mean Ct values were converted to the relative levels of expression using the comparative Ct.

Elisa

A total of 36 h after injection of the siRNA, some of the larvae were sampled to test the content of H3K27ac and H3K4me1 using Elisa. Each larva was weighed, and then a PBS solution of nine times its weight was added for homogenization, followed by centrifugation and collection of the supernatant. The concentrations of H3K27ac and H3K4me1 were measured by the H3K27ac ELISA kit (Elisa, mlbio, Shanghai, China) and H3K4me1 ELISA kit (Elisa, mlbio, Shanghai, China) following the manufacturers'instructions.

QUANTIFICATION AND STATISTICAL ANALYSIS

The data of numbers of *cis* and *trans* interactions in queen and worker were analyzed by a *t*-test using SPSS17.0. The p value <0.05 was considered as significantly different. The expression fold changes (queen/worker) of genes associated with A/B compartment switches analyzed by Unpaired *t*-test with Welch's correction using SPSS17.0. The p value <0.05 was considered as significantly different. The data of birth weight and length of bees between siRNA group and control group were analyzed by a *t*-test using SPSS17.0. The p value <0.05 was considered as significantly different. For the data analysis of qRT-PCR, relative expression of the group RNAi and the group Control for six genes were calculated using $2^{-\Delta\Delta Ct}$ comparative Ct method and were transformed by taking their square root to be normally distributed. The data were analyzed by a *t*-test using SPSS17.0. The p value <0.05 was considered as significantly different. All groups had three biological replicates from three different honeybee colonies, and each biological replicate had four technical replicates.