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中华蜜蜂和意大利蜜蜂感染囊状幼虫病毒后免疫及发育相关基因表达比较

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摘　要：【目的】本文旨在探究囊状幼虫病毒(sacbrood virus, SBV)对中华蜜蜂(*Apis cerana cerana*, 简称中蜂)和意大利蜜蜂(*Apis mellifera ligustica*, 简称意蜂)工蜂幼虫发育和免疫基因、营养代谢基因、抗病毒基因、细胞发育及代谢相关基因表达的影响。【方法】从蜂群中移取 2 日龄的中蜂和意蜂幼虫，在培养箱(34 ℃, RH 85%)进行人工饲养，3 日龄时接种 SBV 病毒，每天观察记录死亡情况，并通过实时定量 PCR (real-time quantitative polymerase chain reaction, RT-qPCR)检测 4 日龄和 7 日龄幼虫体内 *SBV* 基因相对表达量，及免疫基因(*Apidaecin*、*Abaecin*、*Hymenoptaecin*、*Denfensin*、*Lys-1*、*Pgrp-lc*、*Kenny*、*Domeless*)、营养代谢基因(*Ilp1*、*Hex110*、*Vg*)、抗病毒基因(*Dis3*、*Dicer*、*Ago1*)、细胞组成及发育调控基因(*Vhdl*、*Co-1-iv*)以及细胞代谢和调控基因(*Mta1*)的表达水平。【结果】通过分析比较发现，感染相同剂量的 SBV 后，中蜂幼虫在 8 日龄时全部死亡，意蜂幼虫则有部分羽化出房，并且同日龄中蜂幼虫体内 *SBV* 基因表达水平显著高于意蜂。与对照组相比，4 日龄中蜂幼虫体内 *Abaecin*、*Apidaecin*、*Hex110*、*Dicer*、*Vhdl* 基因表达水平显著上调，而意蜂幼虫体内仅有 *Hymenoptaecin*、*Ago1* 基因表达显著上调，同时 *Abaecin*、*Apidaecin*、*Vg*、*Vhdl*

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基因表达量显著下降。在 7 日龄时，中蜂幼虫体内的 *Hex110*、*Dis3*、*Ago1* 基因表达量显著下降，而意蜂幼虫体内的 *Ilp1*、*Dicer*、*Co-1-iv* 基因表达水平显著下降。【结论】中蜂和意蜂在幼虫期都能受到 SBV 侵染，但中蜂的敏感度显著高于意蜂；中蜂与意蜂感染 SBV 后在免疫及生长发育相关基因的表达水平存在显著差异，可能与不同蜂种对病虫害的防御机制、机体的调控营养代谢及病毒引起的 siRNA 反应差异有关。

关键词：中华蜜蜂；意大利蜜蜂；囊状幼虫病毒；基因表达；免疫应答

Comparison of expression of the genes associated with immunity and development between *Apis cerana cerana* and *Apis mellifera ligustica* infected with sacbrood virus

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Abstract: [Objective] To investigate the effects of sacbrood virus (SBV) infection on the genes associated with the immunity, metabolism, resistance to virus, and cell growth and metabolism of *Apis cerana cerana* and *Apis mellifera ligustica*. **[Methods]** The 2-day-old larvae were collected from the colonies of *A. c. cerana* and *A. m. ligustica* and reared in an incubator at 34 ℃ and RH 85%. The larvae were infected with SBV at 3 days old, and the dead larvae were then recorded every day. Real-time quantitative polymerase chain reaction (RT-qPCR) was employed to measure the expression levels of gene *SBV*, as well as the genes associated with immunity (*Apidaecin*, *Abaecin*, *Hymenoptaecin*, *Defensin*, *Lys-1*, *Pgrp-lc*, *Kenny*, and *Domeless*), metabolism (*Ilp1*, *Hex110*, and *Vg*), resistance to viruses (*Dis3*, *Dicer*, and *Ago1*), and cell development and metabolism (*Vhdl*, *Co-1-iv*, and *Mta1*), in the 4- and 7-day-old larvae. **[Results]** After infection with SBV at the same dose, all the larvae of *A. c. cerana* died at 8 days old, while some larvae of *A. m. ligustica* emerged. The relative expression of *SBV* in *A. c. cerana* was significantly higher than that in *A. m. ligustica* of the same days old. Compared with the control group, SBV infection significantly up-regulated the expression of *Abaecin*, *Apidaecin*, *Hex110*, *Dicer*, and *Vhdl* in the 4-day old larvae of *A. c. cerana*, and it up-regulated the expression of *Hymenoptaecin* and *Ago1* and down-regulated that of *Apidaecin*, *Abaecin*, *Vg*, and *Vhdl* in the 4-day-old larvae of *A. m. ligustica*. In addition, SBV infection down-regulated the expression levels of *Hex110*, *Dis3*, and *Ago1* in the 7-day-old larvae of *A. c. cerana* and *Ilp1*, *Dicer*, and *Co-1-iv* in the 7-day-old larvae of *A. m. ligustica*. **[Conclusion]** The larvae of *A. c. cerana* were more susceptible to SBV infection than

those of *A. m. ligustica*. The significant differences in the expression levels of the genes involved in immunity and development between *A. c. cerana* and *A. m. ligustica* may be associated with the differences in the defense mechanism against diseases and pests, the regulation of nutrient metabolism, and the virus-caused siRNA response.
Keywords: *Apis cerana cerana*; *Apis mellifera ligustica*; sacbrood virus; gene expression; immune response

中华蜜蜂(简称中蜂；*Apis cerana cerana*)和意大利蜜蜂(简称意蜂；*Apis mellifera ligustica*)是我国目前饲养的 2 个主要蜂种，都是农作物和野生植物的重要授粉者。意蜂属于西方蜜蜂，原产于意大利，腹部细长，腹板几丁质黄色，抗巢虫能力强，对蜂螨抵抗力弱，以繁殖力强、产蜜和产浆量高等优点深受广大养蜂者欢迎[1]。中蜂是东方蜜蜂亚种，具有耐低温、对寄生螨(*Varroa destructor*)具有抗性、善于采集零星蜜粉源等特点，对保持山地和森林的生物多样性发挥着重要的作用[2-3]。

蜜蜂囊状幼虫病是除残翅病毒(deformed wing virus, DWV)外对蜜蜂幼虫存活影响较大的一类病毒病，病原体为蜜蜂囊状幼虫病毒(sacbrood virus, SBV)，为单股正链 RNA 病毒[4]。该病毒为软腐病病毒属(*Iflavirus*)，基因组全长约 8 800 bp，呈 20 面体对称球状结构，具有一个大的开放阅读框，共编码 2 860 个氨基酸[5]。SBV 由内到外分为 3 层结构，其中病毒衣壳蛋白包括 VP1、VP2、VP3。VP4 与衣壳蛋白松散连接，处于中间，属于膜状物[6]。SBV 主要感染蜜蜂幼虫阶段，患病幼虫躯体变软，颜色由白色晶亮变为黄褐色，从巢房中取出时呈囊袋状，死亡幼虫最后脱水成鳞片状并在巢脾上成片分布[7]。在蜂群中，SBV 可能通过食物源和交尾进行传播。工蜂清除感染 SBV 幼虫时也可能受到 SBV 侵染成为病毒携带者，并在蜂群内饲喂时传播 SBV。感染 SBV 的雄蜂与蜂王交尾，会造成蜂王感染 SBV，因此，蜂王所产的卵中也能检测出病毒[8-9]。已有研究证明，SBV 能够影响工蜂肠道微生物的组成，造成核心菌数量减少[10]，对 SBV 有抗性的中蜂体内的肠道微生物多样性高于对 SBV 敏感的工蜂[11]。尽管 SBV 最早是在意蜂体内发现并报道的，但意蜂具有较强的识别和清除受病毒感染幼虫的卫生行为，对 SBV 具有抗性，因此 SBV 感染不会对意蜂群体造成严重的威胁[12]。

近年来，中蜂不断受到 SBV 的威胁。从中蜂囊状幼虫病毒分离出来的病毒株又称为中蜂囊状幼虫病毒(Chinese sacbrood virus, CSBV)[13-14]。CSBV 毒株根据其地理来源和宿主物种表现出相关性[15]。在之前的研究中，CSBV 与 SBV 相比，在 *VP1* 基因中有 52 bp 的缺失[16]。Choe 等将从韩国中蜂分离出的 CSBV 毒株与 SBV-UK 进行比对，AcSBV-kor3 和 SBV-UK 的同源性为 90.15%[17]。CSBV-LN 与 CSBV-GZ 和 SBV-UK 的开放阅读框序列相似性分别为 93.7% 和 90.5%[18]。总而言之，从不同地区的中华蜜蜂分离出的 CSBV 毒株与 SBV-UK 基因组以及中国其他地区 CSBV 毒株都有差异，并且 CSBV 能够跨物种感染意蜂[19]。CSBV 对依赖中蜂授粉的农业和自然生态系统造成了严重威胁。

蜜蜂的免疫系统由物理屏障、体液和细胞介导的免疫反应组成，使它们能够抵抗某些病原体和寄生虫[20,51]。细胞免疫包括细胞吞噬和凝集作用，体液免疫是指昆虫机体能够产生一些包括抗菌肽(antimicrobial peptides, AMPs)在内的免疫因子以响应外源物质的入侵[21]。在病

毒侵染机体的过程中，抗菌肽(AMPs)作为宿主防御反应的一部分，它能快速产生并有效促进病毒的清除和防止病毒在宿主内传播[22]。蜜蜂体内的 AMPs 主要有 4 种，分别是 apidaecin[23]、abaecin[24]、hymenoptaecin[25]和 defensin[26]。通常情况下，受细菌、真菌、寄生虫、杀螨剂、病毒感染的蜜蜂其体内的抗菌肽表达水平都会上调[27-28,43]。蜜蜂体内已报道与免疫相关的信号通道包括 Toll、Imd、JNK 和 JAK/STAT 通路，Toll 和 Imd 通路主要调控抗菌肽和其他先天性免疫病毒基因，*Apidaecin* 和 *Defensin* 基因主要受 Toll 通路调控，*Hymenoptaecin* 基因则仅受 Imd 通路调控，而 *Abaecin* 基因受 Toll 和 Imd 共同调控[29-30]。Imd 通路在蜜蜂体内高度保守，在信号传导同时能够激活 JNK 通路[43]，JAK/STAT 已经被证明能够参与编码果蝇体内由严重应激诱导的体液因子[31]。

蜜蜂囊状幼虫病毒(SBV)侵染范围广，在多个国家的蜜蜂病毒检测试验中均被检出。在法国，SBV 在蜜蜂最流行病毒中排第 2 位，在各蜂场收集的感染蜜蜂样本中检出率高达 86%[32]。SBV 对奥地利和丹麦的蜜蜂也有较大影响，其中奥地利的 SBV 检出率为 49%[33]，丹麦的 96 个蜂场的 SBV 检出率达到 81.25%[34]。通过对哥斯达黎加的蜜蜂样本进行检测，试验发现 SBV 流行率比 2011 年高 10 倍[35]。在中国，该病毒在中蜂中的流行率为 86%，远高于意蜂 21%的流行率[36]，并且对中蜂的危害也明显大于意蜂[37]。意蜂对 SBV 有一定的抗性，在应对病毒侵染时可能有不同防御机制，但具体的机制尚不明确。已有试验证明长链非编码 RNA (lincRNA)在蜜蜂病毒感染中进行特异性调控，并且在中蜂、意蜂不同组织中各 lincRNAs 表达水平不同[38]。Vung 等在被 SBV 侵染的中蜂蜂群中加入意蜂封盖子能够显著降低中蜂幼虫死亡率，提高中蜂封盖率[39]。

SBV 防治是中蜂养殖目前面临的主要难题[40]，鉴于中蜂和意蜂在感染 SBV 后免疫相关基因的表达及转录水平差异方面的研究较少，本研究通过对中蜂和意蜂的幼虫饲喂 SBV 病毒液并进行人工饲养，检测蜜蜂的寿命、免疫基因、营养代谢基因、抗病毒基因、细胞发育及代谢相关基因的表达，以探究中蜂和意蜂对 SBV 感染的免疫应答差异，为进一步了解蜜蜂免疫途径、防御机制及 SBV 的防治提供依据。

1 材料与方法

1.1 蜜蜂幼虫样品

本研究中的中蜂 2 日龄幼虫和意蜂 2 日龄幼虫均来自江西农业大学蜜蜂研究所饲养的健康蜂群。

1.2 SBV 病毒液制备

从江西省安义县的养蜂场采集具有典型 SBV 感染症状的中蜂幼虫，提取 RNA 后通过 PCR (T100^{TM}Thermal Cycler，伯乐公司)扩增确定其感染了 SBV，PCR 步骤：95 ℃ 10 min；95 ℃ 15 s，54 ℃ 30 s，72 ℃ 30 s，40 个循环。取感染 SBV 的 200 只幼虫，用无菌研磨机在 10 mL 无菌磷酸盐缓冲液(phosphate-buffered saline, PBS)中匀浆，将混合物在 12 000 r/min、4 ℃下离心 30 min，取上清液，再用 0.45 μm 的细胞过滤器过滤，最后用 0.22 μm 的细胞过滤器过滤[41]，得到病毒液，将收集到的病毒液置于 –80 ℃保存，用于后续接种试验。采用绝对定量仪(QX200^{TM}Droplet Reader，伯乐公司)定量病毒液浓度，步骤为：将病毒液稀释为 10、10^2、10^3、10^4、10^5、10^6、10^7 倍数，配制探针法定量反应体系：TB Green Premix Ex *Taq* 10.0 μL，ROX Reference 0.4 μL，正反向引物各 0.8 μL，cDNA 模板 2 μL，ddH_2O 6 μL。反应条件：95 ℃

10 min；94 ℃ 30 s，59 ℃ 60 s，98 ℃ 10 min，40 个循环。最后得到的拷贝数作为横坐标，C_t 值作为纵坐标，绘制标准曲线，得到的 SBV 滴度为 7.8×10^7 copies/μL。通过梯度试验，将病毒液稀释 2 倍、4 倍、6 倍，每组 3 个重复，记录每日死亡数量，在 4 日龄和 7 日龄取样，抽提 RNA 并且进行实时定量 PCR (real-time quantitative polymerase chain reaction, RT-qPCR) (QuantStudio™ 5 Real-Time PCR Instrument，赛默飞世尔科技公司)，得到 C_t 值计算 SBV 相对表达量。根据不同浓度组间死亡率无显著差异和 SBV 表达量差异，最终选择稀释 50% (幼虫食物稀释)作为本研究饲喂病毒浓度。

1.3 幼虫体外培养及病毒接种

1.3.1 幼虫食物

饲喂幼虫的食物配方为：50%新鲜蜂王浆(石城康皇蜂业有限公司)、37%无菌水、6%葡萄糖(AR，西陇科学股份有限公司)、6% D-果糖(北京索莱宝生物科技有限公司)、1%酵母粉(金客隆生物技术公司)[42]。

1.3.2 病毒接种

试验前先对试验巢脾进行冷冻处理，确保其不含任何虫卵或幼虫。为了采集到相同日龄的蜜蜂幼虫，提前将蜂王用隔王栅隔离到巢脾上让其产卵 6 h，96 h 后用移虫笔将 2 日龄蜜蜂幼虫移至已加入 20 μL 幼虫食物的 48 孔板中。将 48 孔板置于 34 ℃、相对湿度 85%的黑暗的培养箱(GZ-250-GI，广西科技设备发展公司)中培养。中蜂和意蜂的试验组和对照组均各设 3 个重复，每个重复 30 只幼虫。3 日龄时，试验组饲喂用幼虫食物稀释 2 倍的病毒液 20 μL，对照组则饲喂用幼虫食物稀释 2 倍的 PBS 缓冲液 20 μL；4 日龄幼虫食物量为 40 μL；5 日龄幼虫食物量为 50 μL；6 日龄幼虫食物量为 60 μL，幼虫对照组和试验组饲喂的食物量保持一致，稀释的方式与 3 日龄保持一致。每日统计对照组与试验组蜜蜂幼虫的死亡数，并在幼虫取食期 4 日龄和封盖期 7 日龄取样，置于–80 ℃冰箱中保存，用于后续试验。

1.4 RNA 的提取及 cDNA 的合成

根据 RNA 提取试剂盒(TransZol Up Plus RNA Kit，北京全式金生物科技有限公司)的操作说明分别提取中蜂幼虫和意蜂幼虫的总 RNA，并用分光光度计(NanoDrop One C，赛默飞世尔科技有限公司)测定所有样品的浓度，按照反转录试剂盒(PrimeScript™ RT reagent Kit with gDNA Eraser，TaKaRa 公司)说明，取 1 μg RNA 进行反转录，反转录反应条件：42 ℃ 2 min，4 ℃暂存，37 ℃ 15 min，85 ℃ 5 s，4 ℃保温，将反转录后的 cDNA 放置于–20 ℃冰箱中保存。

1.5 RT-qPCR 扩增

根据昆虫免疫通路(Toll、Imd、JNK 和 JAK/STAT)的相关文献，选取了 17 个基因进行定量分析，其中包括 8 个免疫基因、3 个抗病毒基因、3 个营养代谢基因、2 个细胞组成及发育相关基因以及 1 个细胞代谢和调控相关基因，同样引用了已报道文献中的引物检测蜜蜂幼虫样本体内 *SBV* 表达量，以 *β-actin* 作为内参基因(表 1)[43-51]。所有引物由生工生物工程(上海)股份有限公司合成。PCR 反应体系为：TB Green Premix Ex *Taq* 5.0 μL，ROX Reference 0.2 μL，正反向引物各 0.4 μL，cDNA 模板 1 μL，ddH_2O 3 μL。反应条件为：95 ℃预变性 2 min；95 ℃变性 15 s，55 ℃/59 ℃退火 15 s，72 ℃延伸 60 s，40 个循环；溶解曲线阶段：95 ℃ 15 s，60 ℃ 60 s，95 ℃ 15 s，80 个循环。

1.6 数据分析

RT-qPCR (QuantStudio™ 5 Real-Time PCR Instrument，赛默飞世尔科技公司)获得 C_t 值，以 *β-actin* 为内参基因进行样本间的标准化处

表 1　RT-qPCR 引物

Table 1　Primers used for RT-qPCR

Gene	Gene ID	Primer sequence (5′→3′)	T_m (℃)	References
Immunogene				
Apidaecin	GB17782	F: TAGTCGCGGTATTTGGGAAT	59	[43]
		R: TTTCACGTGCTTCATATTCTTCA		
Lys-1	GB10231	F: GAACACACGGTTGGTCACTG	55	[43]
		R: ATTTCCAACCATCGTTTTCG		
Defensin	GB10036	F: GCAACTACCGCCTTTACGTC	55	[43]
		R: GGGTAACGTGCGACGTTTTA		
Kenny	GB17106	F: GCTGAACCAGAAAGCCACTT	55	[43]
		R: TGCAAGTGATGATTGTTGGA		
Abaecin	GI:58585177	F: CAGCATTCGCATACGTACCA	59	[44]
		R: GACCAGGAAACGTTGGAAAC		
Hymenoptaecin	GB51223	F: CTCTTCTGTGCCGTTGCATA	59	[44]
		R: GCGTCTCCTGTCATTCCATT		
Pgrp-lc	GB17188	F: TCCGTCAGCCGTAGTTTTTC	55	[45]
		R: CGTTTGTGCAAATCGAACAT		
Domeless	GB16422	F: TTGTGCTCCTGAAAATGCTG	55	[45]
		R: AACCTCCAAATCGCTCTGTG		
Metabolic gene				
Ilp1	GI:1477757163	F: CGATAGTCCTGGTCGGTTTG	59	[46]
		R: CAAGCTGAGCATAGCTGCAC		
Hex110	GB44996	F: ACGGACAATACCCGAACACC	59	[47]
		R: AGCATGCTGATGCCTCTGTT		
Vg	GB49544	F: TCGACAACTGCGATCAAAGGA	59	[48]
		R: TGGTCACCGACGATTGGATG		
Antiviral gene				
Dis3	GB41692	F: TGTCCAAGCAGTTACGAAGACA	59	[49]
		R: AAGTCAACAGCAGCATCAGGA		
Dicer	GB15170	F: AGCAGTAGCTGATTGTGTGGA	59	[49]
		R: ATTTCAGAAGCGCAAGGCAT		
Ago1	GB122654	F: TGGCCCAGATCAAGTAGAGC	55	[49]
		R: AATTTGATAGCGTTTGTGGTGAT		
Cell development and metabolism				
Vhdl	GB726182	F: GCATCACCTTCTGACCAACC	55	[50]
		R: ACCTCGTCCAACATCCTTCT		
Co-1-iv	GB14564	F: GGTTACGTTCGTTCCCGGTT	55	[46]
		R: TACCTTGCTCGCCCCTGTAA		
Mta1	GI:110756803	F: CATCTCTGTGCTTCTCCTC	55	[46]
		R: ACTCGATCTGGTTGTTTTC		
Virogene				
SBV	HM237361.6	F: TATTCAGGGGGACGCTACAC	59	[51]
		R: GCGTGAGTTGACAGAAAATC		
Reference genes				
β-actin	NM_001185145.1	F: TTGTATGCCAACACTGTCCTTT	59	[44]
		R: TGGCGCGATGATCTTAATTT		

理，基因相对表达量用 $2^{-\Delta\Delta C_t}$ 来表示。采用 SPSS 软件(17.0 版)进行统计分析，用单因素方差分析(one-way analysis of variance, one-way ANOVA)的方法检验不同日龄以及不同蜂种间 *SBV* 的表达量，用独立样本 *t* 检验的方法检验试验组与对照组间基因表达量的差异显著性，以 $P<0.05$ 为差异显著性水平。利用 Kaplan-Meier Method 来分析试验组和对照组蜜蜂幼虫的存活率，用 Log-Rank 方法分析其存活率的差异，差异显著水平为 $P<0.05$。

2 结果与分析

2.1 SBV 侵染对中蜂和意蜂幼虫发育的影响

中蜂 3 日龄幼虫接种 SBV 后，从第 3 天开始存活率显著降低($x^2=26.42$, $P=0.000\ 1$)，第 8 天全部死亡($x^2=40.97$, $P=0.000\ 1$) (图 1A)，没有幼虫能够完成化蛹。意蜂 3 日龄幼虫接种 SBV 后，存活率也在不断下降，但有部分幼虫能够完成化蛹，最终羽化出房。与对照组相比，意蜂幼虫存活率无显著差异($x^2=3.477$, $P=0.062\ 2$) (图 1B)。中蜂和意蜂的对照组幼虫大部分能正常化蛹，出现复眼等蛹期特征，并正常出房。

2.2 SBV 侵染的蜜蜂幼虫体内 *SBV* 相对表达量

SBV 侵染后的中蜂和意蜂幼虫在 4 日龄和 7 日龄时与对照组相比，体内 *SBV* 表达量均显著高于对照组幼虫 (Ac4d: $P=0.015$, Ac7d: $P=0.018$; Am4d: $P=0.021$, Am7d: $P=0.027$)。4 日龄中蜂幼虫体内 *SBV* 表达量显著高于 7 日龄幼虫($P=0.001$)。4 日龄意蜂幼虫体内 *SBV* 表达量也显著高于 7 日龄幼虫($P=0.000$)。同日龄条件下，中蜂幼虫体内 *SBV* 表达量均高于意蜂幼虫(4 d: $P=0.006$, 7 d: $P=0.000$)。综上所述，中蜂和意蜂幼虫期均能受到 SBV 的侵染，*SBV* 在中蜂幼虫体内表达水平更高(图 2)。

2.3 SBV 侵染的中蜂 4 日龄幼虫免疫及发育相关基因表达

SBV 侵染后的中蜂 4 日龄幼虫与对照组比较，检测的 17 个基因中有 2 个免疫基因(*Abaecin*:

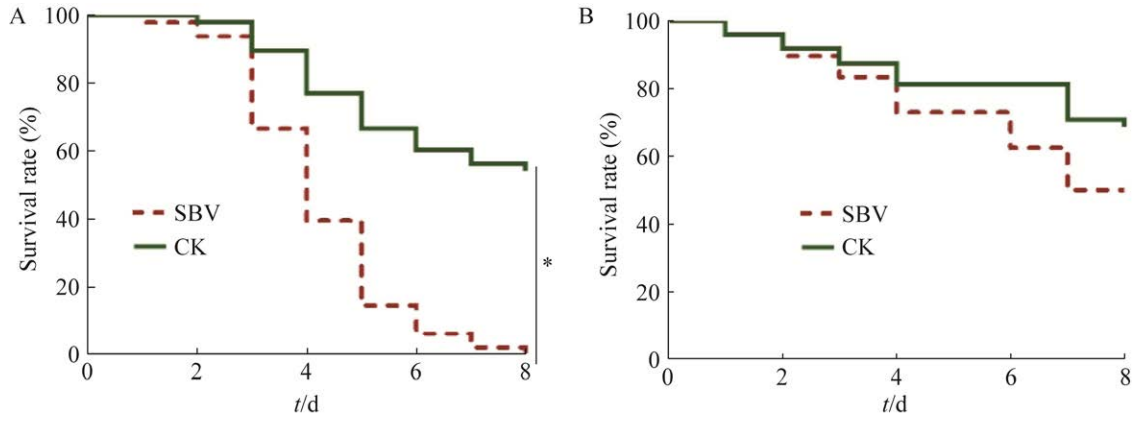

图 1 幼虫生存曲线 A：中蜂. B：意蜂

Figure 1 Survival curves of larvae. A: *Apis cerana*. B: *Apis mellifera*. SBV: Larvae fed with the diet inoculated with SBV; CK: Larvae fed with normal diet with PBS. The survival curve was prepared using the Kaplan-Meier method in SPSS Statistics26.0; The Log Rank method was used to analyze the difference of survival rate between the SBV group and the control group; The asterisk indicates significant difference ($P<0.05$).

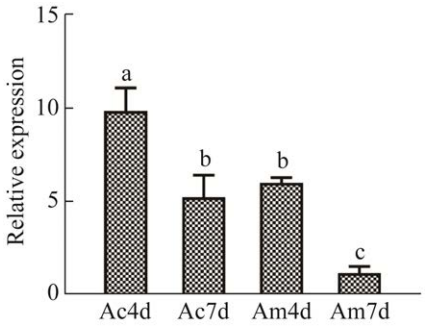

图 2　中蜂和意蜂不同发育时期体内的 *SBV* 相对表达量

Figure 2　Relative expression level of *SBV* in different developmental stages of *Apis cerana* and *Apis mellifera*. Data in the figure are mean±SE. Ac4d means 4-day old larvae of *Apis cerana*; Ac7d means 7-day old larvae of *Apis cerana*; Am4d means 4-day old larvae of *Apis mellifera*; Am7d means 7-day old larvae of *Apis mellifera*. The same below. The asterisk indicates significant difference in SBV expression between the SBV group and the control group (CK) (P<0.05, t-test); Different letters indicated that SBV expression levels of Ac and Am were significantly different at different ages (P<0.05, one-way ANOVA).

t=1.205, P=0.003; *Apidaecin*: t=1.610, P=0.046)、1 个营养代谢相关基因(*Hex110*: t=0.800, P=0.032)、1 个抗病毒基因(*Dicer*: t=1.763, P=0.001)和 1 个细胞组成及发育相关基因(*Vhdl*: t=1.267, P=0.042)表达均显著上调(图 3)。

2.4　SBV 侵染的中蜂 7 日龄幼虫免疫及发育相关基因表达

SBV 侵染后的中蜂 7 日龄幼虫与对照组相比，有 2 个免疫基因表达量显著上升(*Abaecin*: t=0.899, P=0.027; *Hymenoptaecin*: t=2.083, P=0.000)，1 个营养代谢相关基因(*Hex110*: t=–1.517, P=0.000)、2 个抗病毒基因(*Dis3*: t=–1.526, P=0.019; *Ago1*: t=–1.515, P=0.003)、2 个细胞组成及发育相关基因(*Co-1-iv*: t=–2.461, P=0.001; *Vhdl*: t=–1.730, P=0.000)表达量显著下降。其他营养代谢相关基因以及免疫基因的表达无显著差异(图 4)。

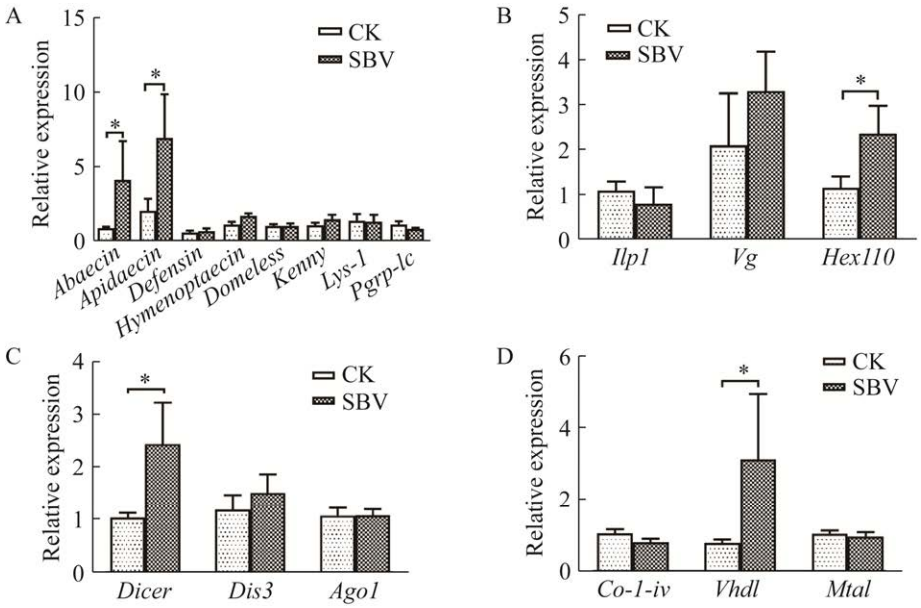

图 3　SBV 侵染的中蜂 4 日龄幼虫体内基因的相对表达水平

Figure 3　Relative gene expression levels in 4-day-old larvae of *Apis cerana* after SBV infection. A: The relative expression of genes associated with immune. B: The relative expression of genes associated with metabolism. C: The relative expression of genes associated with anti-virus. D: The relative expression of genes associated with cell development and metabolism. Asterisk indicates a significant difference between SBV group and control group (P<0.05, t-test). The same below.

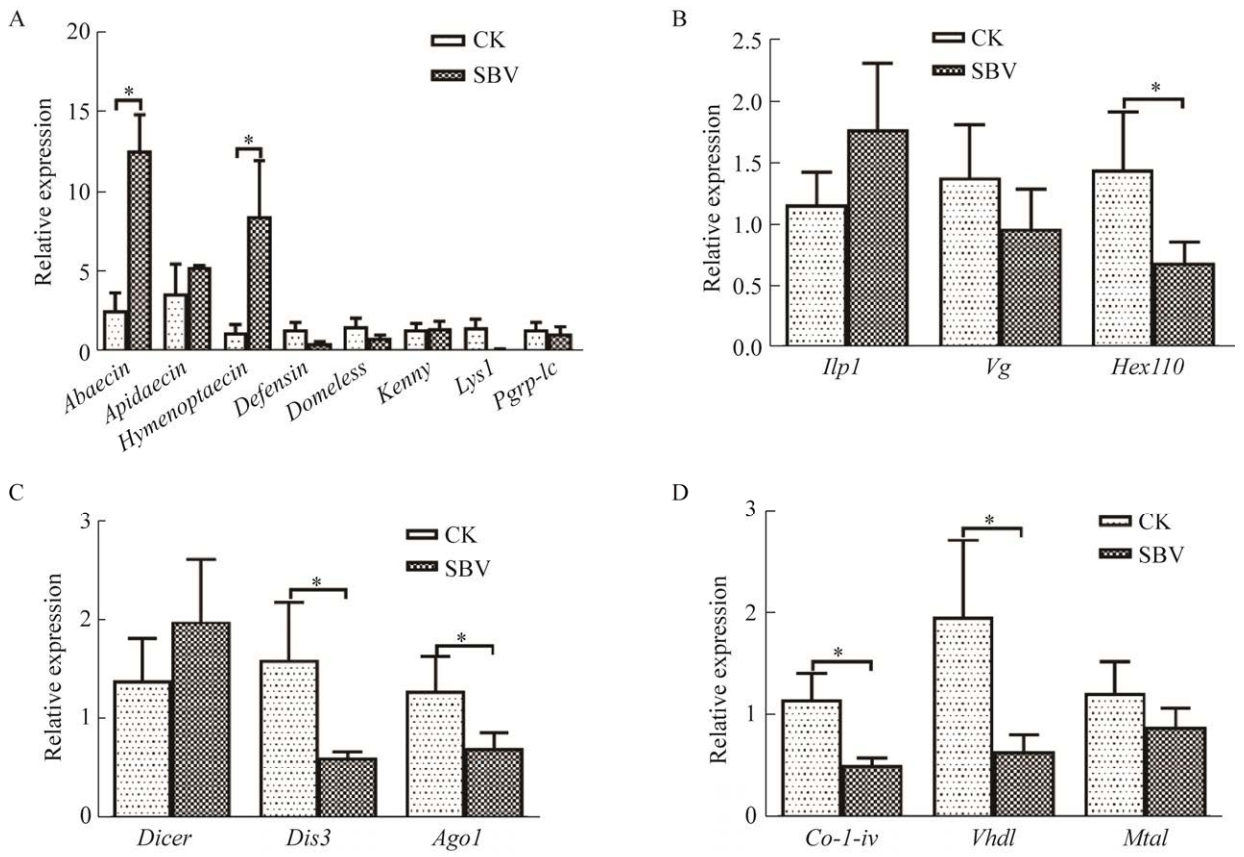

图 4　SBV 侵染的中蜂 7 日龄幼虫体内基因的相对表达水平

Figure 4　Relative gene expression levels in 7-day-old larvae of *Apis cerana* after SBV infection. A: The relative expression of genes associated with immune. B: The relative expression of genes associated with metabolism. C: The relative expression of genes associated anti-virus. D: The relative expression of genes associated with cell development and metabolism.

2.5　SBV 侵染的意蜂 4 日龄幼虫免疫及发育相关基因表达

SBV 侵染后的意蜂 4 日龄幼虫与对照组相比，有 3 个免疫基因表达量差异显著，其中 2 个基因 (*Abaecin*: t=–0.558, P=0.009; *Apidaecin*: t=–1.440, P=0.017)表达量显著下降，1 个基因 (*Hymenoptaecin*: t=1.814, P=0.037)表达量显著上升。此外，1 个营养代谢基因(*Vg*: t=–3.305, P=0.043)表达量显著下降。1 个抗病毒基因 (*Ago1*: t=3.519, P=0.037)表达量则显著上升。1 个细胞组成及发育相关基因(*Vhdl*: t=–9.342, P=0.015)表达量显著下降。细胞代谢和调控相关基因的表达量与对照组相比无显著差异(图 5)。

2.6　SBV 侵染的意蜂 7 日龄幼虫免疫及发育相关基因表达

SBV 侵染后的意蜂 7 日龄幼虫与对照组相比，有 1 个营养代谢基因(*Ilp1*: t=–6.987, P=0.014)表达量显著下降，1 个抗病毒基因 (*Dicer*: t=–13.653, P=0.023)表达量显著下降，1 个细胞组成及发育相关基因(*Co-1-iv*: t=–5.345, P=0.025)表达量显著下降。所有的免疫基因表达量与对照组相比都没有表现出显著差异(图 6)。

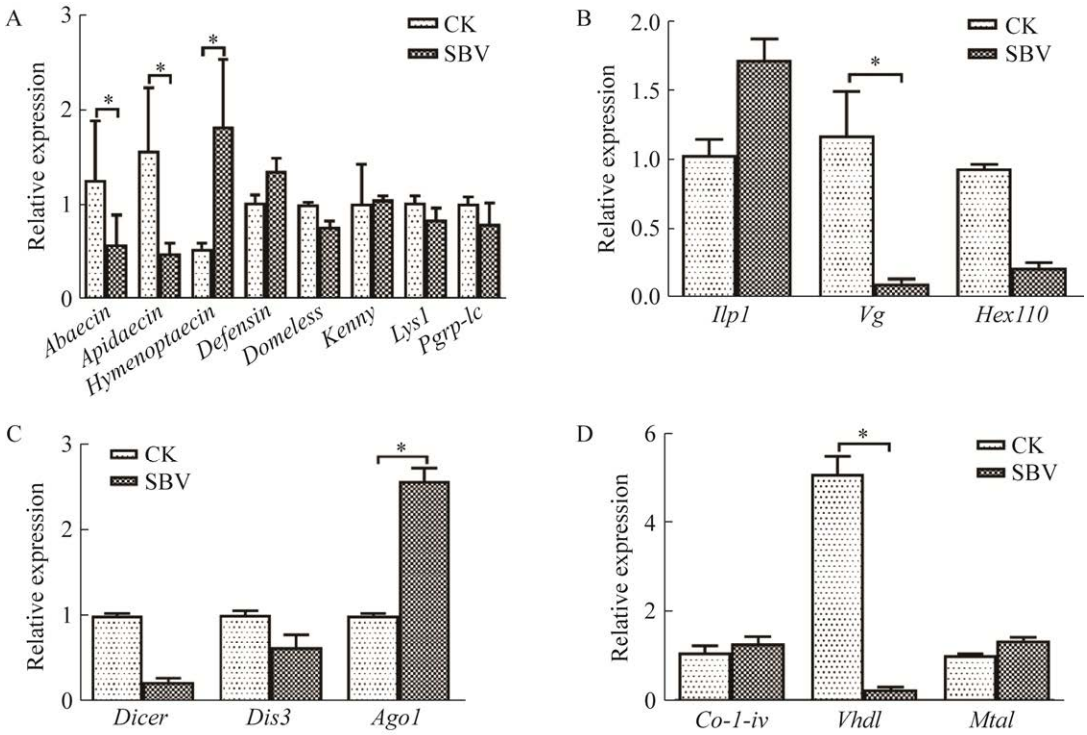

图 5　SBV 侵染的意蜂 4 日龄幼虫体内基因的相对表达水平

Figure 5　Relative gene expression levels in 4-day-old larvae of *Apis mellifera* after SBV infection. A: The relative expression of genes associated with immune. B: The relative expression of genes associated with metabolism. C: The relative expression of genes associated with the anti-virus. D: The relative expression of genes associated with cell development and metabolism.

3　讨论与结论

本研究通过室内人工饲养幼虫，在 3 日龄接种 SBV，记录幼虫的死亡率，检测 4 日龄和 7 日龄幼虫体内 *SBV* 基因、免疫及发育相关基因表达水平，比较中蜂和意蜂对 SBV 病毒的免疫应答差异。结果表明，中蜂幼虫体内 SBV 感染水平显著高于意蜂，免疫及发育相关基因表达也存在显著差异，病毒感染水平可能与机体相关免疫反应和营养水平存在一定相关性[52]。

中蜂幼虫感染 SBV 后，免疫通道 Toll、Imd 相关的免疫基因 *Toll*、*Apidaecin*、*Abaecin*、*Hymenoptaecin* 等表达显著上调[51]。中蜂在被微孢子虫感染后 4 种 AMPs 也都有增加的趋势[53]。在本研究中，4 日龄时中蜂幼虫 AMPs 基因 *Apidaecin*、*Abaecin*、*Hymenoptaecin* 显著上调，反映出中蜂宿主先天免疫在病毒侵染后迅速起作用，Toll 和 Imd 通路被激活。意蜂幼虫在应对外界刺激的表现与中蜂存在差异，在被微孢子虫感染的试验中，意蜂的 AMPs 水平表现出下调趋势[54]。有研究表明，感染急性蜜蜂麻痹病毒(acute bee paralysis virus, ABPV)的意蜂幼虫和成虫体内抗菌肽水平都并未升高[55]，而受 DWV 感染的意蜂蛹体内 AMPs 的水平在 24 h 内显著上调[56]。在 Ryabov 等的试验中，意蜂幼虫感染相同滴度的 DWV 和 SBV，DWV 感染后的幼虫与对照组相比 AMPs 水平差异不显著，但 SBV 能够诱导 *Hymenoptaecin* 和 *Defensin* 的表达上调[57]。

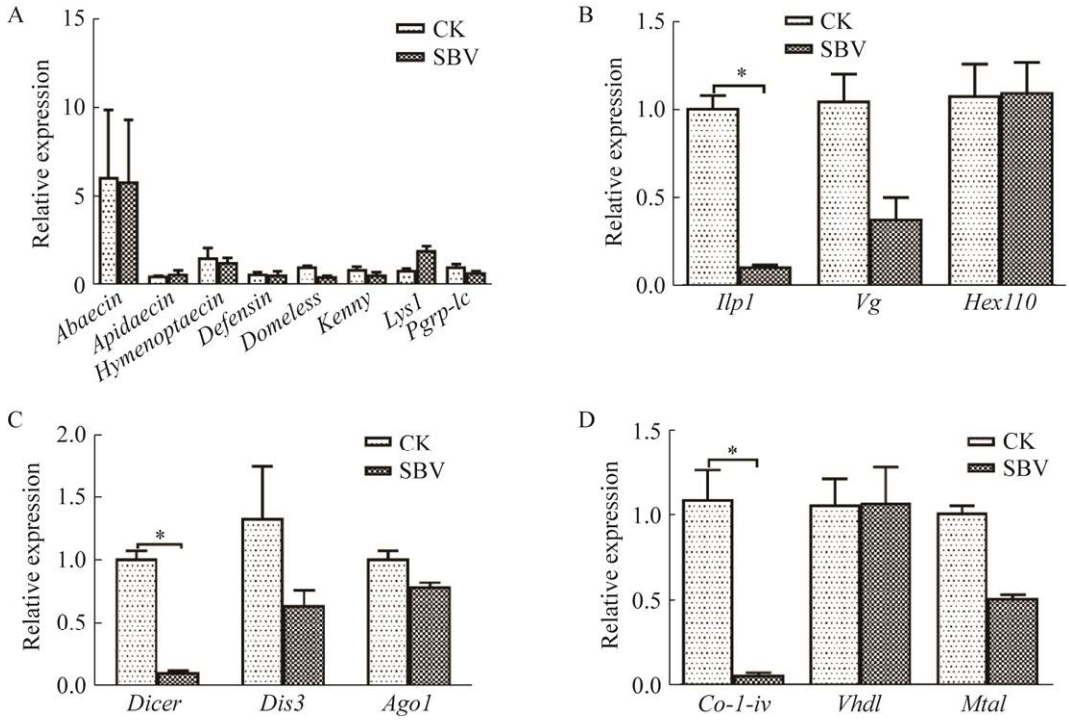

图 6　SBV 侵染的意蜂 7 日龄幼虫体内基因的相对表达水平

Figure 6　Relative gene expression levels in 7-day-old larvae of *Apis cerana* after SBV infection. A: The relative expression of genes associated with immune. B: The relative expression of genes associated with metabolism. C: The relative expression of genes associated with the anti-virus. D: The relative expression of genes associated with cell development and metabolism.

从调控模式上看，意蜂在受 SBV 和 DWV 侵染时产生不同的免疫反应。Toll 通路受到病原体和宿主种类的影响，SBV 侵染可能抑制意蜂的 Toll 通路[49]。目前仍不清楚具体是什么因素导致意蜂幼虫在 SBV 侵染后免疫基因表达水平下降。

蜜蜂的病毒感染情况与机体的营养供给和病毒介导的 siRNA 反应也相关[58]。脂肪体是蜜蜂幼虫能量储存组织，能通过主动感知营养状况并相应地以脂质、糖原和蛋白质的形式储存或释放能量来调节新陈代谢[59]。瓦螨主要通过吸食脂肪体来达到损害宿主蜜蜂的目的[60]，已有试验证实，能量供应会影响机体免疫力[61]。Hex110、Ilp1、Vg、Vhdl 都是幼虫脂肪体内重要的蛋白，参与了主要代谢过程。Hex110 蛋白是幼虫向成虫发育阶段所需的氨基酸储备[62]，并且随变态发育进行会逐渐耗尽[63]。*Ilp1* 是主要胰岛素代谢通道基因，依据蜜蜂个体营养水平变化进行调控和代谢[62,64]，在果蝇体内 *Ilp1* 负责脂质的储存与代谢[65]。已有研究表明，低营养储备能够增加胰岛素信号[66]，并且 *Ilp1* 和 *Hex110* 的表达水平能够反映幼虫的营养状况[65]。感染 SBV 后机体 *Ilp1* 和 *Hex110* 显著降低[51]。*Vg* 是昆虫体内卵黄发生过程的关键基因，在微生物感染的情况下脂肪体内 *Vg* 转录水平上调[67]，*Vg* 与 *Ilp1* 共同调控脂肪体代谢，且 *Ilp1* 与 *Vg* 丰度正相关[59]。*Vhdl* 是一种幼虫特异性血淋巴蛋白，在变态前的迅速上升可能是为了增加脂质的运输[68]。*Hex110*、*Vhdl* 基因在中蜂幼虫体内显著上升可能意味着感染初期的幼虫试图通过加速自身代谢强化对病毒的抵抗力，后期的

显著下降可能是营养物质消耗极大导致无法在蛹期提供足够的能量[69], 并且 SBV 加速了中蜂幼虫细胞凋亡进程[51]。意蜂幼虫在感染时期营养代谢水平与中蜂不同，可能是由于意蜂抵抗 SBV 侵染的防御机制不同。

蜜蜂通过 RNA 干涉(RNA interference, RNAi)介导抗病毒免疫[58,70], 可通过饲喂和注射 dsRNA 来诱导，还可利用病毒特异性 dsRNA 激活 siRNA 应答[58]。研究表明，给蜜蜂喂食针对病毒的特异性 dsRNA 后，可显著降低病毒的感染水平[70]。*Dicer* 和 *Ago1* 是 RNAi 的核心组成，*Dicer* 能够将 dsRNA 切割成 siRNA[71]，*Ago1* 是一种能够与 siRNA 结合的特异性基因沉默效应分子[49]，两者相继在降解病毒 RNA 中发挥作用[47,72]。熊蜂感染 IAPV 后，*Dicer* 和 *Ago1* 显著升高[73]。本研究中 4 日龄中蜂和意蜂的 *Dicer* 和 *Ago1* 的基因表达趋势相反，可能是由 SBV 侵染触发 RNAi 后病毒感染水平不同引起的，这可能也是防御机制产生差异的原因。siRNA 反应被证明能够抑制 DWV 复制，但是 DWV 可能会表达 siRNAs 抑制剂，从而增加 DWV 丰度[74]。以色列急性麻痹病毒(Israeli acute paralysis virus, IAPV)同源的 dsRNA 病毒也能成功防止 IAPV 感染蜂群[75]。余肖给感染 SBV 的中蜂幼虫饲喂 4 种 dsRNA，发现 4 种目的 dsRNA 都能达到抑制病毒复制的效果[76]。此外，摄入 SBV 特异性 dsRNA 可以显著降低蜜蜂感染病毒滴度[58], 这为中蜂防治 SBV 感染提供了新思路。

综上所述，SBV 感染诱导中蜂幼虫迅速产生显著的免疫应答，抗菌肽的表达水平迅速升高，营养代谢和 RNAi 方面也会做出反应和调整以应对 SBV 的侵染。意蜂幼虫在受到 SBV 感染后，部分抗菌肽表达水平显著下降，营养代谢、细胞组成及发育等方面也都广泛下调，表现出与中蜂幼虫完全不同的基因表达趋势。中蜂与意蜂不同的基因表达趋势，可能与两者在应对 SBV 侵染时不同的防御机制、机体调控营养代谢方式以及病毒引起的 siRNA 反应相关，深入了解意蜂应对 SBV 时的防御机制及 RNAi 介导的免疫机制对中蜂防治 CSBV 感染有重要意义。本研究在实验室条件下基于蜜蜂幼虫个体研究蜜蜂对 SBV 病毒的免疫应答反应，对于蜂群的抗病毒防御机制以及与社会性、免疫功能之间的潜在关系仍需更深入的研究。

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