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2种杀虫剂对舞蹈蜜蜂大脑转录组中 可变剪切的影响

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摘要:【目的】蜜蜂是一种重要的经济性社会昆虫,也是一种重要的传粉昆虫,对农业生态系统平衡与植物多样性具有重要贡献。为防治农作物的病虫害,生产中杀虫剂的大量使用,引起蜜蜂中毒事件逐年增加,给养蜂业带来重大的经济损失,同时农作物因为得不到充分授粉,导致产量下降和品质降低,进而影响农业的收益。可变剪切(alternative splicing, AS)是调控基因表达和产生蛋白质多样性的重要机制,也是造成物种之间、器官之间、生理与病理之间差异的重要因素。为了揭示可变剪切基因在蜜蜂应对杀虫剂过程中的作用,对2种亚致死剂量杀虫剂诱导舞蹈蜜蜂基因的可变剪切进行深入分析。【方法】基于前期蜜蜂舞蹈行为学试验和已获得杀虫剂诱导的舞蹈蜜蜂大脑转录组数据,应用tophat分别比对吡虫啉处理组与对照组,溴氰菊酯处理组与对照组包含的所有大脑转录组中剪切位点信息。【结果】在吡虫啉处理组间和溴氰菊酯处理组间中,外显子跳跃(skipped exon, SE)是主要的可变剪切类型,检测到各组之间事件数目占总事件数达60%以上,而SE类型的差异事件数占差异AS事件总数的50%以上。在不同距离(300, 500, 1 000 m)的试验地点,2种杀虫剂处理的舞蹈蜜蜂脑部转录组中发生5种可变剪接的事件总数之间存在显著差异($P < 0.001$),且发生5种可变剪接的基因数目之间存在显著差异($P < 0.001$)。根据这些发生可变剪切的基因做了KEGG(kyoto encyclopedia of genes and genomes)富集分析,主要涉及新陈代谢、细胞修复、氨基酸代谢、脂肪酸代谢和信号传导等生物学活动,与重要的信号通路和免疫应答通路密切相关。【结论】2种亚致死剂量杀虫剂能够使蜜蜂体内的基因发生可变剪切,可能改变配体受体结合、转录因子、生理节律、内吞作用以及神经信号传导。可变剪切基因参与蜜蜂响应杀虫剂胁迫过程中,会对细胞功能产生影响,包括免疫应答、学习记忆和飞行定位等信号通路,从而影响蜜蜂的舞蹈行为学。研究2种亚致死剂量杀虫剂诱导的舞蹈蜜蜂内在基因表达和调控情况,不但对吡虫啉和溴氰菊酯的环境毒性风险评价有参考价值,并进一步制定相应的防护措施,对于保护这种农业重要的授粉昆虫具有重要的价值。

关键词: 蜜蜂; 舞蹈; 杀虫剂; 亚致死剂量; 可变剪切

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The Effects of Two Insecticides in the Transcriptome of the Brains of Dancing Bees on the Alternative Splicing

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Abstract: [Objective] Honeybee is an important economic and social insect, as well as an important pollinator, which contributes to the balance of agricultural ecosystem and plant diversity. In order to prevent the diseases and pests of the crops, the usage of pesticides is increasing yearly, which inevitably threaten the safety of beneficial insects such as honeybees. As a result of the extensive use of pesticides, bee poisoning cases increase every year, which leads up to substantial economic losses to the apiculture industry. Due to insufficient pollination, the yield and quality of the crops are reduced, which in turn affects the agricultural income. Alternative splicing (AS) is an important mechanism to regulate gene expression and protein diversity, and it is also an important factor to differentiate species, organs, physiology and pathology. In order to reveal the role of alternatively splicing genes in response to sub-lethal doses of insecticides in honeybees, the responses of alternatively splicing towards two insecticides were analyzed. [Method] Based on previous studies of dance behavior in honeybees and data from the transcriptome of the brain of the insecticide-induced dance honeybees, tophat was used to compare the splice sites between imidacloprid treated group and the control group, as well as between deltamethrin treated group and the control group. [Result] Between the imidacloprid treated group and deltamethrin treated group, skipped exon (SE) was the main alternatively spliced type, and the number of events detected between each group accounted for more than 60% of the total number of events, while the number of different events of SE type accounted for more than 50% of the total number of different AS events. There were significant differences in the total number of five alternatively splicing events in the brain transcriptome of two insecticide-treated dancing bees at different trial distances at 300, 500, 1 000 m from hive ($P < 0.001$). And the number of genes with five alternatively splicing events were significant different between the two insecticide-treated groups at different distances ($P < 0.001$). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out according to these variable splicing genes, which mainly involved in metabolism, cell repair, amino acid metabolism, fatty acid metabolism, signal transduction and other biological activities, closely related to important signal pathways and immune response pathways. [Conclusion] Both sub-lethal doses of insecticides cause genes in honeybees to undergo alternatively splicing, potentially altering ligand receptor binding, transcription factors, circadian rhythms, endocytosis, and nerve signal transduction. In response to insecticide stress, alternatively splicing events affect cellular functions, including immune responses, learning, memory and navigation ect, thus influencing honeybee dance behavior. The study on the gene expression and regulation of dance bees induced by the two sub-lethal doses of insecticides not only has reference value for the environmental toxicity risk assessment of imidacloprid and deltamethrin, but also makes corresponding protective measures, it is of great value for the conservation of the agriculture-important pollinating insect.

Keywords: honeybees; waggle dance; insecticides; sublethal dose; alternative splicing

【研究意义】蜜蜂是一种重要的经济性社会昆虫,为人类提供蜂蜜、蜂王浆、蜂花粉等蜂产品。而且也是一种重要的传粉昆虫,为许多虫媒植物授粉,对农业生态系统平衡与植物多样性具有重要贡献^[1]。随着蜜蜂基因组测序工作的完成,蜜蜂基因功能、转基因技术和调控机制的研究将成为新的热点^[1-2],人们进行有关农药诱导的蜜蜂基因功能和调控机制的研究意义深远。【前人研究进展】Aufauvre等^[3]发现蜜蜂体内编码羧酸酯酶、细胞色素 P450 和谷胱甘肽转移酶的基因被认为主要负责杀虫剂代谢;通过转录组分析蜜蜂对微孢子和杀虫剂应答反应,免疫相关基因显著下调,并影响屏障防御和海藻糖代谢的显著改变。Wu等^[4]在蜜蜂幼虫期暴露于亚致死剂量吡虫啉蔗糖溶液,羽化出房后蜜蜂进行转录组测序分析,

得出免疫相关基因和王浆蛋白相关基因显著下调,而且解毒基因和感官相关基因特异表达;此外,多巴胺转运体编码基因和谷氨酸受体编码基因显著下调,而 γ -氨基丁酸(γ -aminobutyric acid, GABA)神经递质编码基因显著上调,这些转运体和受体基因可能扰乱蜜蜂的神经系统,进而影响学习记忆能力,损害蜜蜂的大脑功能。李志国等^[5]通过蜜蜂脑部转录组分析亚致死剂量吡虫啉对嗅觉学习记忆的影响,表明吡虫啉诱导的氧化应激可能导致蜜蜂大脑氧化还原失衡,参与光传导的基因显著下调,扰乱蜜蜂大脑钙离子平衡和长期记忆和嗅觉敏感性,同时参与免疫应答、解毒和化学感应的基因显著下调,进而降低蜜蜂的学习记忆能力。

【本研究切入点】可变剪切(alternative splicing, AS)是真核生物基因表达的一种常见现象,基因转录成前体 mRNA(pre-mRNA)后,可通过不同的剪接方式产生不同的 mRNA 剪接异构体,这些不同的 mRNA 可能被翻译成不同的蛋白质。因此,一个基因可能编码多个蛋白质,可变剪切是调节基因表达和产生蛋白质多样性的重要机制^[6]。此外,可变剪切可能是造成物种之间、器官之间、生理与病理之间差异的更重要因素,如表现在剪切调控的物种、组织和发育特异性方面^[7-9]。目前许多研究表明西方蜜蜂体内存在大量可变剪切^[10-11],而且可变剪切在海角蜂(*A. mellifera capensis*)的群居社会可塑性的控制上起重要作用^[10]。石元元等^[12]研究发现,蜂王浆对雌性蜜蜂发育与基因可变剪切密切相关,参与生长发育、物质代谢的基因都发生了可变剪切。蜜蜂的免疫系统包括细胞免疫和体液免疫。郭睿等^[13]研究发现,许多 AS 基因全方位参与宿主响应球囊菌胁迫的免疫应答。这些共有 AS 基因富集在多个细胞免疫通路,如黑化作用,泛素介导的蛋白水解、内吞作用、溶酶体、吞噬作用和细胞凋亡等多个体液免疫通路和信号通路。【拟解决的关键问题】前人不仅做了大量有关杀虫剂对蜜蜂生长发育、繁殖力和生理免疫能力影响方面的研究,还做了杀虫剂对蜜蜂采集行为和学习行为的影响研究。本课题组前期已进行了杀虫剂对蜜蜂舞蹈行为及内在分子机理的研究,通过采食添加杀虫剂糖水后,舞蹈蜜蜂大脑转录组水平存在差异。本研究结合前期行为学试验和跳舞蜜蜂转录组测序数据,进一步对2种杀虫剂诱导的舞蹈蜜蜂的可变剪切基因进行深入分析,旨在揭示可变剪切基因在蜜蜂应对杀虫剂过程中的作用,为可变剪切基因功能研究提供依据。

1 材料与方法

1.1 转录组数据来源

本研究前期先进行蜜蜂舞蹈行为学试验,分别用24 $\mu\text{g/kg}$ 吡虫啉蔗糖溶液,235 $\mu\text{g/kg}$ 溴氰菊酯蔗糖溶液和50%蔗糖溶液(对照组)各饲喂3群采集蜂,并在距蜂箱不同距离(300,500,1 000 m)的3个试验地点进行相关的行为学试验,然后将27个跳舞蜜蜂脑部样本(25个蜜蜂大脑)送检。通过本课题组获得的跳舞蜜蜂脑部转录组全部数据,本研究基于上述转录组数据对跳舞蜜蜂脑部的AS基因进行分析。

1.2 可变剪切分析

以每个进行差异可变剪切分析的比较组为单位,先统计发生的可变剪切事件的种类及数量,再分别计算每类可变剪切事件表达量,最后对每类可变剪切事件进行差异分析。使用跨越内含子的reads来鉴定2个外显子的边界,从而判定 junction 的位置,tophat 比对结果中包含了所有剪切位点信息,rMATS 软件可以将 AS 事件分成5类,并且可以对有生物学重复的样品进行差异 AS 分析。5类可变剪切事件分别是外显子跳跃(skipped exon, SE),可变5'端剪切(alternative 5' splice site, A5SS),可变3'端剪切(alternative 3' splice site, A3SS),外显子选择性跳跃(mutually exclusive exon, MXE)和内含子保留(retained intron, RI)。

1.3 共有与特有 AS 基因的 KEGG pathway 富集分析

KEGG pathway 显著性富集分析以 KEGG pathway 为单位,应用超几何检验,找出与整个基因组背景相比,在共有及特有 AS 基因中显著性富集的 pathway。

2 结果与分析

2.1 吡虫啉诱导舞蹈蜜蜂脑部 AS 基因类型及数量

由表1可知,吡虫啉处理组检测出5种可变剪切:外显子跳跃(SE),可变5'端剪切(A5SS),可变3'端

剪切(A3SS),外显子选择性跳跃(MXE),内含子保留(RI)等。SE是主要的可变剪切类型,检测到各组之间事件数目占总事件数达60%以上。而SE类型的差异事件数占差异AS事件总数的50%以上。在300,500,1 000 m发生5种可变剪接的事件总数之间存在显著差异($\chi^2=88.872, df=8, P<0.001$),而发生5种可变剪接的差异事件数目之间不存在显著差异($\chi^2=13.705, df=8, P=0.09$)。

表1 吡虫啉处理组间差异AS分类和数量统计

Tab.1 AS classification and quantity statistics between imidacloprid treatment group and control group

AS事件类型 Event type	AS事件总数 NumEvent. JC. only			差异AS事件总数 SigEvents. JC. only		
	300 m	500 m	1 000 m	300 m	500 m	1 000 m
SE	5 607	8 296	8 724	281	126	176
MXE	972	1 492	1 591	64	13	35
A5SS	1 012	1 245	1 235	80	34	46
A3SS	1 334	1 551	1 562	91	55	49
RI	411	515	504	38	18	30

由图1可知,各种类型可变剪切在3个距离组间的基因数目,其中SE类型的基因数依次分别为2 567,3 237和3 395;A3SS类型的基因数分别为935,1 047和1 049;A5SS类型的基因数分别为704,786和781;MXE类型的基因数分别为332,518和561;RI类型的基因数分别为311,388和380。在300,500,1 000 m发生5种可变剪接的基因数目之间存在显著差异($\chi^2=36.501, df=8, P<0.001$)。

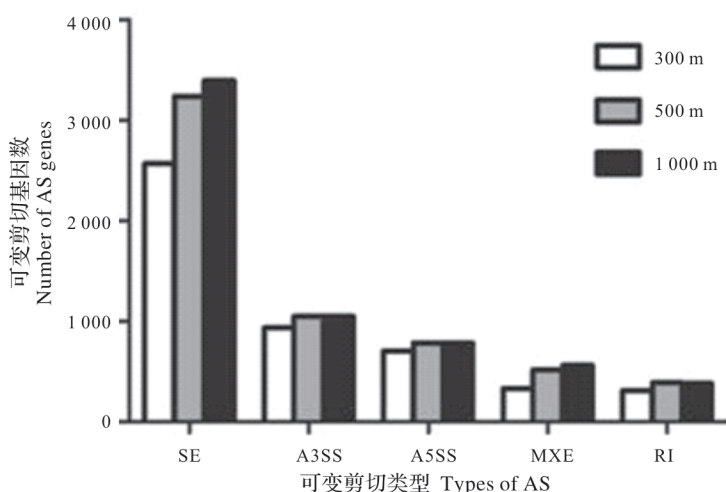


图1 吡虫啉处理组间可变剪切基因数

Fig.1 Number of AS genes between imidacloprid treatment group and control group

2.2 吡虫啉诱导舞蹈蜜蜂脑部AS基因KEGG pathway富集分析

根据这些发生可变剪切的基因做了KEGG富集分析,这些显著富集的通路可以影响机体的新陈代谢、细胞修复、氨基酸代谢和信号传导等生物学活动,与重要的信号通路(wnt signaling pathway, MAPK signaling pathway-fly, phototransduction-fly, hippo signaling pathway-fly, neuroactive ligand-receptor interaction)和免疫应答通路(ubiquitin mediated proteolysis, endocytosis, lysosome)密切相关(表2)。

2.3 溴氰菊酯诱导舞蹈蜜蜂脑部AS基因类型及数量

由表3可知,本试验中检测出5种可变剪切:外显子跳跃(SE),可变5'端剪切(A5SS),可变3'端剪切(A3SS),外显子选择性跳跃(MXE),内含子保留(RI)等。SE是主要的可变剪切类型,检测到各组之间事件数目占总事件数达60%以上。而SE类型的差异事件数占差异AS事件总数的55%以上。在300,500,1 000 m发生5种可变剪接的事件总数之间存在显著差异($\chi^2=101.225, df=8, P<0.001$),且发生5种可变剪接的差异事件数目之间存在显著差异($\chi^2=23.844, df=8, P=0.002$)。

表 2 可变剪切基因主要显著富集通路分析(吡虫啉)
Tab.2 Main Pathway Enrichment of AS Genes(Imidacloprid)

通路号 Pathway ID	通路名称 Pathway	P 值 P-value	基因 Genes
ko04745	Phototransduction-fly	0.000	LOC406106;LOC410823;LOC551691;LOC413197; LOC410489;LOC550818
ko04310	Wnt signaling pathway	0.009	LOC408691;LOC408976;LOC411046;LOC551691; LOC726113;LOC726671;LOC406106;LOC409321
ko04120	Ubiquitin mediated proteolysis	0.046	LOC408749;LOC411182;LOC552700
ko04013	MAPK signaling pathway-fly	0.041	LOC412003;LOC412529
ko04391	Hippo signaling pathway-fly	0.014	LOC409348;LOC409904
ko04144	Endocytosis	0.042	LOC409274;LOC413197;LOC413680
ko04080	Neuroactive ligand-receptor interaction	0.018	LOC411050;LOC726953
ko04142	Lysosome	0.015	LOC724550;LOC725688

表 3 溴氰菊酯处理组间差异 AS 分类和数量统计
Tab.3 AS classification and quantity statistics between deltamethrin
treatment group and control group

AS 事件类型 Event Type	AS 事件总数 NumEvent. JC. only			差异 AS 事件总数 SigEvents. JC. only		
	300 m	500 m	1 000 m	300 m	500 m	1 000 m
SE	5763	8195	8928	317	144	216
MXE	1010	1556	1647	42	18	42
A5SS	1050	1222	1245	67	39	65
A3SS	1355	1534	1562	104	37	37
RI	424	503	515	31	21	18

由图 2 可知,各种类型可变剪切在 3 个距离组间的基因数目,其中 SE 类型的基因数依次为分别为 2 590,3 221 和 3 438;A3SS 类型的基因数分别为 945,1 037 和 1 053;A5SS 类型的基因数分别为 711,788 和 791;MXE 类型的基因数分别为 344,531 和 558;RI 类型的基因数分别为 316,380 和 385。在 300,500,1 000 m 发生 5 种可变剪接的基因数目之间存在显著差异($\chi^2=34.724,df=8,P<0.001$)。

2.4 溴氰菊酯诱导舞蹈蜜蜂脑部 AS 基因 KEGG pathway 富集分析

根据这些发生可变剪切的基因做了 KEGG 富集分析,这些显著富集的通路可以影响机体的新陈代谢、糖类代谢、脂肪酸代谢、氨基酸代谢和信号传导等生物学活动,与重要的信号通路(wnt signaling pathway, TGF-beta signaling pathway, notch signaling pathway, MAPK signaling pathway-fly, phototransduction-fly, mTOR signaling pathway, phosphatidylinositol signaling system, neuroactive ligand-receptor interaction)和免疫应答通路(regulation of autophagy, microbial metabolism in diverse environments, nicotinate and nicotinamide metabolism, caffeine metabolism)密切相关(表 4)。

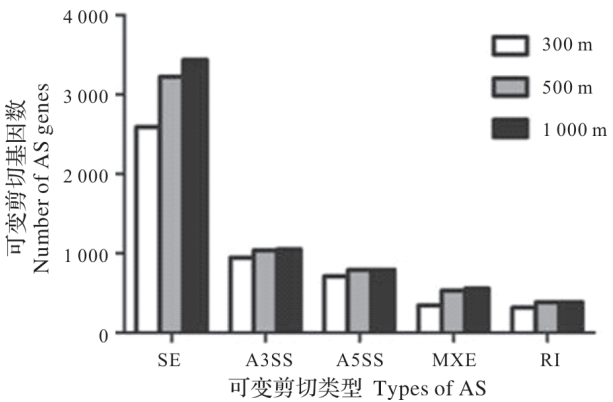


图 2 溴氰菊酯处理组间可变剪切基因数
Fig.2 Number of AS genes between deltamethrin
treatment group and control group

表 4 可变剪切基因主要显著富集通路分析(溴氰菊酯)
Tab.4 Main Pathway Enrichment of AS Gene(Deltamethrin)

通路号 Pathway ID	通路名称 Pathway	P 值 P-value	基因 Genes
ko04745	Phototransduction-fly	0.027	LOC406106;LOC410823;LOC551691; LOC408791;LOC410489
ko04310	Wnt signaling pathway	0.004	LOC406106;LOC408691;LOC408976;LOC409050; LOC551691;LOC726280;LOC408894
ko04150	mTOR signaling pathway	0.042	LOC406106;LOC551173;LOC551668; LOC413467;LOC551198
ko04140	Regulation of autophagy	0.007	LOC413467;LOC726781
ko01120	Microbial metabolism in diverse environments	0.030	LOC413924;LOC727153
ko00760	Nicotinate and nicotinamide metabolism	0.022	LOC408474
ko04350	TGF-beta signaling pathway	0.045	LOC408691;LOC725711;LOC726280
ko00232	Caffeine metabolism	0.048	LOC724718
ko04330	Notch signaling pathway	0.035	LOC408894
ko04013	MAPK signaling pathway-fly	0.032	LOC100576369
ko04070	Phosphatidylinositol signaling system	0.037	LOC406096;LOC406106;LOC408791
ko04080	Neuroactive ligand-receptor interaction	0.045	LOC410435;LOC411760;LOC412993

3 讨 论

本试验中,笔者通过饲喂 2 种亚致死剂量的杀虫剂吡虫啉和溴氰菊酯,蜜蜂转录组测序发现 5 种类型的可变剪切,发生可变剪切的事件和基因主要集中在 SE。由于 1 个基因可能通过不同类型可变剪切产生不同转录本^[14],使 1 个基因由于时间环境的不同翻译出不同的蛋白质。由于杀虫剂的刺激,蜜蜂基因组发生一定可变剪切,可能改变配体受体结合、转录因子、生理节律、内吞作用以及神经信号传导,会对细胞功能产生影响,包括免疫应答、学习记忆和飞行定位等。

在蜜蜂采食混有杀虫剂的糖水后,体内某些参与信号通路的基因发生了可变剪切,如参与 MAPK signaling pathway-fly 的调节基因(LOC412003, LOC412529, LOC100576369),参与 Wnt signaling pathway 的调节基因(LOC406106, LOC411046, LOC551691, LOC726671, LOC408691, LOC408976)和参与 Neuroactive ligand-receptor interaction 的调节基因(LOC411050, LOC726953, LOC410435, LOC411760)。MAPK 信号通路是由小 GTP 酶中的卍家族控制的信号级联放大反应^[15],调节细胞生理活动。大量行为学试验证明 MAPK 信号通路能影响长期恐惧记忆^[16-17]、空间记忆^[18-19]和嗅觉气味记忆的激活^[20]。参与 Wnt signaling 路径的基因与学习记忆有关^[21]。而 Neuroactive ligand-receptor interaction 通路是一批位于原生质膜上参与神经可塑性及学习记忆的受体^[22]。说明 2 种杀虫剂都能够影响基因的可变剪切,从而影响蜜蜂的学习记忆能力,但其具体的功能还需要进行蛋白互作、RNA 干扰等试验进一步研究。另外,发生可变剪切基因还参与细胞间的信号传导、新陈代谢和正常的生理活动,但可变剪切基因对农药的免疫应答机制还尚不清楚,有待进一步的研究。

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