

# 欧洲型舞毒蛾气味受体基因 OrCo 的克隆及序列分析\*

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**摘要** 【目的】 克隆欧洲型舞毒蛾 *Lymantria dispar* Linnaeus 的 OrCo 气味受体基因，并分析其序列特征，为进一步研究舞毒蛾嗅觉机制提供有益参考。【方法】 本研究利用 RT-PCR 和 RACE 方法，克隆获得欧洲型舞毒蛾 OrCo 受体基因 cDNA 全长序列，将该基因命名为 *LdisOrCo*，在 GenBank 中的登录号为 KF482409。【结果】 序列分析结果显示，*LdisOrCo* 开放阅读框全长为 1 311 bp，编码 436 个氨基酸，序列中有 7 个跨膜区和高度保守的 C 端区域。序列联配分析表明，*LdisOrCo* 基因的氨基酸序列与近缘种灰翅夜蛾 (*Spodoptera littoralis* Or83b) 的同源性高达 89%，与鞘翅目台湾黑金龟 (*Holotrichia plumbea* Or83b) 同源性高达 64%，与已经报道的其他昆虫的嗅觉受体同源性都在 60% 以上，特别是在 C 端几乎完全一致。【结论】 嗅觉受体 OrCo (Olfactory receptor coreceptor) 在不同昆虫体内高度保守，克隆欧洲型舞毒蛾 OrCo 基因可以为进一步研究舞毒蛾气味受体的功能，OrCo 基因的进化以及揭秘嗅觉机制奠定基础。

**关键词** 欧洲型舞毒蛾，嗅觉受体，OrCo，基因克隆，序列分析

## Molecular cloning and sequence analysis of the odorant receptor gene OrCo in *Lymantria dispar* (European gypsy moth)

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**Abstract** [Objectives] Exploring the mechanism of olfaction in *Lymantria dispar* (European gypsy moth) by cloning and analyzing the olfactory receptor coreceptor (OrCo) gene. [Methods] The full-length cDNA sequence of *LdisOrCo* was cloned using reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) (GenBank accession number KF482409). [Results] Sequence analysis revealed that the open reading frame (ORF) consisted of 1 311 nucleotides, encoding 436 amino acids with 7 putative transmembrane domains, and has a highly conserved C-terminus. Alignment analysis indicated that the nucleotide similarity of *LdisOrCo* with Or83b of *Spodoptera littoralis* and *Holotrichia plumbea* was 89%, 64%, respectively, and that it shares high homologies (>60%) with the corresponding receptor from other insects; the sequence of the C-terminus is almost the same. [Conclusion] The highly conserved olfactory receptor gene of *Lymantria dispar* (European gypsy moth) was sequenced in order to provide a basis for exploring the functions of the olfactory receptor, the evolution of the OrCo gene and the mechanism of insect olfaction.

**Key words** *Lymantria dispar* (European gypsy moth), olfactory receptor, sequence analysis

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昆虫灵敏的嗅觉在昆虫的多种行为中发挥了重要作用,为其适应复杂多变的环境,生存繁衍提供了有力的保障。昆虫嗅觉感受器感知空气中气味分子的过程很复杂,即进入嗅觉感受器表皮的气味分子进入淋巴液后要与气味分子结合蛋白(Odorant binding proteins,OBPs)结合成复合体,穿过淋巴液(Sensillum lymph)后与嗅觉神经元树突膜上的嗅觉气味受体蛋白(Olfactory receptors,Ors)或共同受体(Olfactory receptor coreceptor,OrCo)结合,然后再在其它多种蛋白的参与下进行信号传导(Subchev and Jurenka, 2001; Matousková et al., 2007; Leal, 2013)。昆虫气味分子结合蛋白在1981年首次被发现(Vogt and Riddifoed, 1981),从1999年报道鉴定出黑腹果蝇*Drosophila melanogaster*的第一个气味受体以来(Pennisi, 1999),通过多种技术手段来寻找昆虫气味受体基因在近几年一直是热点。昆虫传统的气味受体Ors在不同昆虫间同源性较低(Fox, et al., 2001; Hill et al., 2002; Krieger et al., 2002; Krieger et al., 2004; Sakurai et al., 2004; Jones et al., 2005; Nakagawa et al., 2005),而另一类非典型气味受体OrCo则在不同昆虫体内高度保守(Dobritsa et al., 2003)。有研究表明,气味受体OrCo对传统气味受体正常功能的行使起着不可或缺的作用(Miller and Tu, 2008)。

舞毒蛾 *Lymantria dispar* Linnaeus 具有食性杂、分布广、危害大、幼虫顺风迁移等特点,是世界性大害虫,依据其地理分布和生活特性被分为亚洲种群、欧洲种群及北美种群。对于舞毒蛾的研究国外起步较早,国内在舞毒蛾的生物学及生态习性(兰星平和万志民, 1996)、天敌与防治(王昌贵等, 1996)等方面也做了广泛研究。舞毒蛾在林区猖獗可以造成巨大经济损失,因此,有效控制舞毒蛾种群密度,使林木免受危害,是一项长期而艰巨的任务。舞毒蛾在寻找寄主、配偶以及取食等过程中,嗅觉在虫体同外界化学信息交流过程中起着重要的作用,并且在监测物质研发方面也具有一定的发展潜力(于艳雪等, 2011),因此,对其成虫嗅觉系统的深入研究就

是控制其为害的一个突破点。研究气味受体的特性、变异及专一性对于阐明嗅觉识别的分子机理是非常重要的(Clyne et al., 1999; Vosshall et al., 1999)。本研究采用RT-PCR(Reverse transcription polymerase chain reaction)和RACE(Rapid-amplification of cDNA ends)技术获得了舞毒蛾非典型受体蛋白OrCo基因,并对其进行序列分析,为深入研究舞毒蛾嗅觉机制打下基础。

## 1 材料与方法

### 1.1 材料

欧洲型舞毒蛾雄虫触角来自意大利卡利亚里大学Paolo Solari博士,触角保存在RNAlater® RNA Stabilization Solution(Invitrogen)中,-80°C保存备用。

### 1.2 试剂和仪器

总RNA提取试剂盒RNA simple Total RAN Kit购自Invitrogen公司;Quant cDNA第一链合成试剂盒(KR103),Taq Plus MasterMix(KT205),PMD19-T Vector均为TIANGEN公司产品;PCR引物由生工生物工程(上海)股份有限公司北京合成部合成;测序也由上海生工公司完成;PCR扩增仪为Applied Biosystems公司;其余试剂均为国产或进口分析纯试剂。

### 1.3 实验方法

**1.3.1 舞毒蛾触角总RNA提取** 取欧洲型舞毒蛾10头雄蛾触角液氮研磨后,用试剂盒RNAsimple Total RAN Kit进行总RNA提取。所有操作按试剂盒说明书进行,最后溶于40μL无RNase的水中,-80°C保存备用。

**1.3.2 cDNA第一链的合成** 以总RNA为模板,按照Quant cDNA第一链合成试剂盒的说明书进行反转录,合成cDNA第1链。将下列物质混合:

Oligo(dT)<sub>15</sub> 2 μL(终浓度1 μmol/L)10×RT Mix 2 μL, Super pure dNTP(2.5 mmol/L each) 2 μL(终浓度0.25 mmol/L each dNTP), Quant

Reverse Transcriptase 1 μL , RNase—Free ddH<sub>2</sub>O 3 μL , 总 RNA 10 μL ( 1 μg )。

之后在 PCR 仪上 37℃ 孵育 60 min。将合成的 cDNA 第一链于-20℃ 保存备用。

**1.3.3 3' RACE 扩增和 5' RACE 扩增** 根据 GenBank 中已经登录的鳞翅目昆虫 OrCo 基因的保守区域 , 利用引物设计软件 Primer Premier 5.0 设计相应的简并引物 ( 见表 1 ) , 用于扩增舞毒蛾 OrCo 受体基因的 cDNA 片段。3' RACE 和 5' RACE 引物是根据 cDNA 扩增片段获得的序列而进一步设计的。 RACE 反应参照 5'-Full RACE Kit 和 3'-Full RACE Core Set Ver.2.0 说明书。扩增的产物用 1.0% 琼脂糖凝胶电泳检测并对目的片段进行回收。

**1.3.4 PCR 产物的克隆和测序** 将 PCR 产物经胶回收后克隆于 PMD19-T Vector 载体上 ( 按试剂盒说明操作 ) , 经蓝白斑筛选 , 选取阳性克隆样品送公司进行测序。

**1.3.5 PCR 扩增** 根据 3' RACE 和 5' RACE 的测序结果 , 拼接获得一个编码欧洲型舞毒蛾 OrCo 的 cDNA 序列 , 该序列全长为 1 532 bp。同时 , 通过分析 , 我们设计了 1 对特异性引物用于扩增欧洲型舞毒蛾气味受体基因 OrCo 的全序列 :

上游引物为 5'-ATGTCTACCCTCCTCCGT AAGATCTACGC-3' ;

下游引物为 5'-CTTCAGTTGTACCAAGTAC CATG-3'。

PCR 反应体系为 25 μL :1 μL 第一链 cDNA 产物、12.5 μL Taq Plus MasterMix、上游引物和下游引物各 1 μL ( 10 μmol/l ) 、ddH<sub>2</sub>O 9.5 μL。扩增欧洲型舞毒蛾 OrCo cDNA 片段的 PCR 反应条件为 :94℃ 5 min ;94℃ 30 s ,55℃ 1 min , 72℃ 1.5 min , 循环 35 次 ; 72℃ 延伸 10 min , 4℃ 保持。

获得该序列的 PCR 产物后 , 重复上一步骤 1.3.4 , 选取阳性克隆样品送公司测序。引物序列见表 1。

表 1 扩增舞毒蛾 OrCo 基因所用引物  
Table 1 Primers for amplifying *Lymantria dispar* OrCo gene

引物名称 The name of the primer	引物序列 ( 5' -3' ) Primer sequences 5' -3'
OR1- Forward	YTHATYGARGAGAGYTCATC
OR1- Reverse	GYTGBAYCAAYACCATGAAG
OR2- Forward	GVWCHGCBATMAARTAYTGGG
OR2- Reverse	TCCATHACBGATGARCTCTC
OR3- Forward	GAVGTCAAygadctvAcvgc
OR3- Reverse	ACGACATGCTTATGCCTCTC
OrCo 5' RACE-Inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
OrCo 5' RACE-Outer	CATGGCTACATGCTGACAGCCTA
OrCo 3' RACE-Inner	CGCGGATCCTCCACTAGTGATTCACTATAGG
OrCo 3' RACE-Outer	TACCGTCGTTCCACTAGTGATT
OrCo Full-Forward	ATGTCTACCCTCCTCCGTAAGATCTACGC
OrCo Full-Reverse	CTTCAGTTGTACCAAGTACCATG

**1.3.6 序列分析** Blast 同源性搜索在 NCBI 网站进行 , 序列分析由 DNAMAN 软件完成 , 跨膜域预测由 TMHMM 程序 (<http://www.cbs.dtu.dk/services/TMHMM/>) 完成。多序列同源性比对和结果输出应用 CLC Sequence Viewer 6 软件进行。昆虫气味受体进化树的构建使用 Mega5.1 软件 (Kumar *et al.*, 2008) 进行 , 采用的是邻接法建树 , 用的是 Bootstrap 检验。

## 2 结果与分析

### 2.1 舞毒蛾 OrCo 基因的克隆

以反转录获得的 cDNA 为模板 , 利用简并引物对其进行 PCR 扩增 , 获得 200 bp 左右的片段 , 与 pGEM-T easy vector 连接 , 并转化大肠杆菌 DH5 $\alpha$  细胞 , 对其进行菌液 PCR , 检测片段大小正确后测序。获得片段序列在 NCBI 网站上进行 BLAST 同源搜索 , 比对结果显示该片段与鳞翅目嗅觉受体基因高度同源。根据获得的序列分别设计 3' RACE 和 5' RACE 所需的特异性引物 (表 1) , 并根据说明书进行扩增 , 拼接结果得到了两个特异性条带 , 同样将这个片段进行连接 , 转化与测序。根据 3' RACE 和 5' RACE 测序结果 , 拼接获得一个编码舞毒蛾 OrCo 的 cDNA 序列 , 该序列全长为 1 532 bp。同时 , 我们设计了 1 对特异性引物 (表 1) 扩增获取全基因 , PCR 电泳结果如图 1 , 目的片段进行克隆测序 , 测序结果与序列拼接结果一致 (图 1)。

### 2.2 序列分析

将克隆获得的欧洲舞毒蛾气味受体基因 OrCo cDNA 全长序列命名为 *LdisOrCo* , 并在 GenBank 注册 , 登录号为 KF482409。如图 2 所示 , *LdisOrCo* 基因的核苷酸序列全长为 1 311 bp 的开放阅读框 , 编码 436 个氨基酸。根据网站 <http://web.expasy.org/protparam/> 预测 *LdisOrCo* 基因编码的氨基酸分子量为 49.59 ku , 等电点为 8.51。跨膜结构分析 (<http://www.cbs.dtu.dk/services/TMHMM/>) 表明 , *LdisOrCo* 基因推断的氨基酸序列具有 7 个  $\alpha$  螺旋跨膜区 , 跨膜位置分

别为 7~29、39~58、99~121、151~173、297~319、339~361、410~432 (图 3) , 类似于一个典型的 G 蛋白偶联受体。

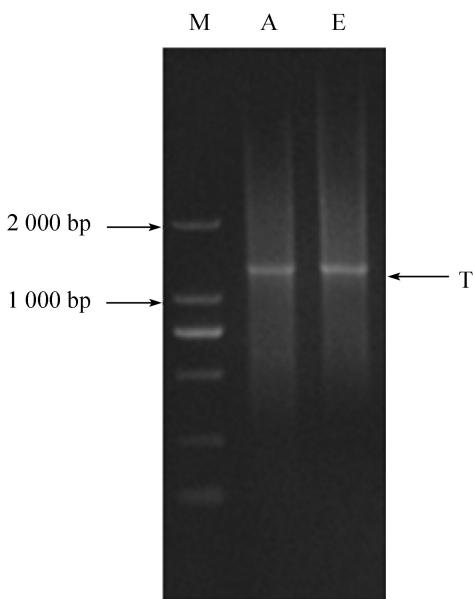


图 1 欧洲型舞毒蛾 OrCo 基因 PCR 扩增结果

Fig. 1 Result of PCR amplification of the OrCo gene in *Lymantria disparia* (European gypsy moth)

M : DNA marker ; A : 亚洲型舞毒蛾 Asian gypsy moth ; E : 欧洲型舞毒蛾 European gypsy moth ; T : 目的基因 Target gene ; 1 : 欧洲型舞毒蛾 OrCo 全长序列 PCR 扩增产物 PCR amplification product of the gene OrCo in *Lymantria disparia* (European gypsy moth).

以往的研究表明 OrCo 受体在昆虫进化过程中高度保守 , 因此选取已知近缘种鳞翅目的棉铃虫 (*Helicoverpa armigera* Or83b) 、烟夜蛾 (*Helicoverpa assulta* Or83b) 和斜纹夜蛾 (*Spodoptera litura* Or83b) 等 , 以及鞘翅目、双翅目和膜翅目具有代表性的昆虫与其进行序列联配。结果显示 , *LdisOrCo* 基因的氨基酸序列与近缘种灰翅夜蛾 (*Spodoptera littoralis* Or83b) 的同源性高达 89% , 与鞘翅目台湾黑金龟 (*Holotrichia plumbea* Or83b) 同源性高达 64% ,

与其它昆虫的嗅觉受体同源性都在 60% 以上 , 尤其在 C 端保守性非常高 , 这与以往的研究相吻合 (图 4)。

翅目和膜翅目具有代表性的昆虫与其进行序列联配。结果显示, *LdisOrCo* 基因的氨基酸序列与近缘种灰翅夜蛾 (*Spodoptera littoralis* Or83b) 的同源性高达 89%, 与鞘翅目台湾黑金龟 (*Holotrichia plumbea* Or83b) 同源性高达 64%, 与其它昆虫的嗅觉受体同源性都在 60% 以上, 尤其在 C 端保守性非常高, 这与以往的研究相吻合(图 4)。

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1      ATGCTCAACCTCCTCGTAAGATCTACGCCGGTATTCTCATACGTTCTCATTCACACTTATTGTTGGAAATAATGCGGCCAATATGCTGATGAAGTCATGAGTTAACGTGCT
1      M S T L L R K I Y A G I H T F L I L L N F I C L G I N A A Q Y A D E V N E L T A
121     AACACTATCAGTGCTTATTTTACACATACACTTATAAGACTCTTTTTCAGTCAATGAACTCCAAAAGCTTACAGGACCTGGGGTATGGAAACAGTCTAACAGTCACCACTT
41      N T I T V L F F T H T L I R L L F F A M N S K N F Y R T L A V W N Q S N S H P L
241     TTAACTGAACTGACGCAAGGATCACCAGATACTTACTAAATGAGGAGGCTCTTATACTTCATTGTTGGAAATGACTGTAATCTCAGTTATAAGCTGGTAACCTAAACGTTCTC
81      F T E S D A R Y H Q I S L T K M R R L L Y F I C G M T V L S V I S W V T I T F F
361     GGTGAATCAGTACGCTTAATTACAGCAAGGAGACAAACGAGACTTGCAGTCAAGCTGGAGATTACCTCTGAAGGCCCTGGTACCCCTTAAACGAAATGAGCCGCACTATGCTATGTC
121     G E S V R L I T S K E T N E T L T E P A P R L P L K A W Y P F F N A M S G T M Y V
481     CTCGCAATTACAGATAATTGGTTCTATTCTCAATGGCTATCCGAACTTGATGGACGTTATCTCTGTTCTGGTTATTTCATGTCAGCAGCTGCAACACTTAAAGCT
161     L A F I F Q I Y W L L F S M A I P N L M D V M F C S W L I F A C E Q L Q H L K A
601     ATTATGAAACCTTGATGGAGTTAAGTGCCTCTCTGAGCACATACAGGCCAAATACTGCAGAGCTTACGAGCTTCTACAGAAAAATCAGAAAGGGTCCAAGATCAGACTGATTTG
201     I M K P L M E L S A S L D T Y R P N T A E L F R A S S T E K S E R V Q D P T D L
721     GATATTCTGGGATATACTCGACTCAGCAAGACTTGGCATGACGATCAGGGTACTGGAGGTAGACTACAGAAATTAACTCAAGGTGGAAATAACCCCTAAATGGACTTTCAAAAGAACCAA
241     D I R G I Y S T Q Q D F G M T I R G T G G R L Q N F N Q G G N N P N G L S K N Q
841     GAGATGCTTGGCAGATCAGCGATCAGAAATATTGGGTAGAACGGCATAAACATGTTGTCAGATTAGTAGCATCCATTGGAGACACATACGGTACTACTCTGCTATTTCAATGTTGGTGTCA
281     E M L A R S A I K Y W V E R H K H V V R L V A S I G D T Y G T T L L F H M L V S
961     ACCATCACACTTACACTGCGTACCCAGCTACTAAGATAAACTGATCGATGTATATGCGTTCACTACAGTGGCTATCTGGTTAACATCGGGACAAGTGGTOCACTCTGCAATT
321     T I T L T L L A Y Q A T K I N R I D V Y A F S T V G Y L G Y T L G Q V F H F C I
1081    TTTGGTAATCGGCTTATTGAAAGAGCTCATCGTGATGGAGCGCCTATTCTGTCAATGGTACGGATCTGAGAAGAAGCGAAGACTTGGTCCAGATTGTCTGTCAGACTGTCAG
361     F G N R L I E E S S S V M E A A Y S C Q W Y D G S E E A K T F V Q I V C Q Q C Q
1201    AAGGGCTATGAGTATCTCAGGGCTAAATTCTCACTGATCCCTTGATTGTTGCTCGGTACTGGGTCTGTAGTAACCTATTTCATGGTACTGGTACAAGTGAATTTCAAA
401     K A M S I S G A K F T V S L D L F A S V L G A V V V T Y F M V L V Q L K *
1321    TGGAACTCTATAATCATTTCTATCTACTATTAGAACTTTTATAATTACATGTTTTTTTATATAAGCGGTAAATTTCAGTCATAAAATTTAGTTTAGTATTAGAATTTAGT
1441    GTATTGATTACATAGTAGTCGTGATTAAATTCTCATCAATTATCTTGTAAAAAAACCTATAGTGAAATCACTAGTGGAGGATCGCG

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图 2 欧洲型舞毒蛾 *OrCo* 核苷酸序列及推导的氨基酸序列

Fig. 2 Nucleotide and amino acid sequence of the *OrCo* gene in *Lymantria dispar* (European gypsy moth)

红色下划线表示预测的 7 个跨膜区; \* 表示终止密码子; 浅红色下划线表示多聚腺苷酸信号序列。

The red underlined part shows seven transmembrane regions; \* shows termination codon; The light red part underlined shows poly A signal sequence.

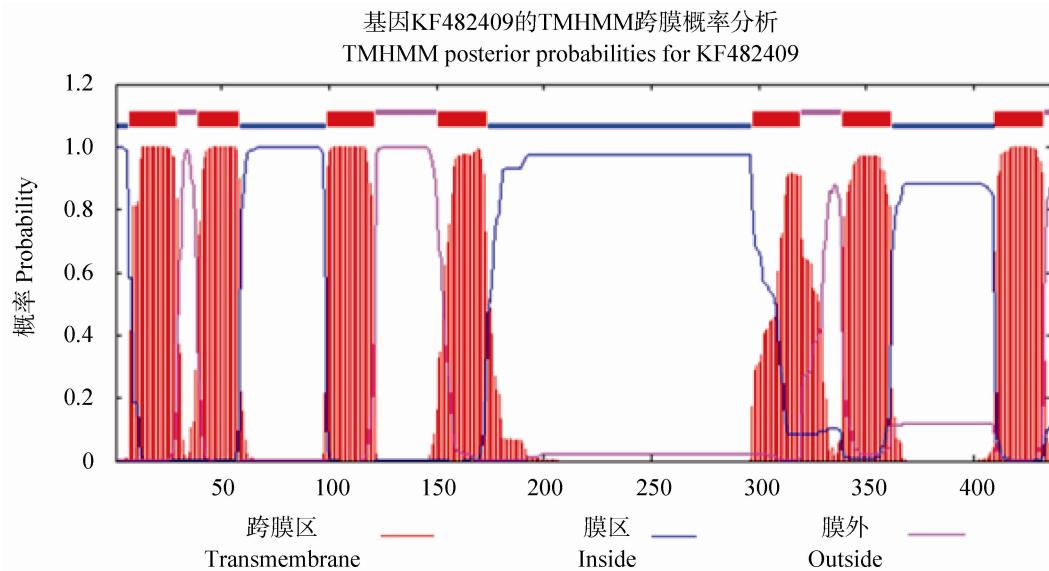


图 3 欧洲型舞毒蛾 OrCo 跨膜区分析

Fig. 3 Analysis of transmembrane region of the OrCo gene in *Lymantria dispar* (European gypsy moth)

跨膜区 1 ( 7 ~ 29 ) ; 跨膜区 2 ( 39 ~ 58 ) ; 跨膜区 3 ( 99 ~ 121 ) ; 跨膜区 4 ( 151 ~ 173 ) ; 跨膜区 5 ( 297 ~ 319 ) ;  
跨膜区 6 ( 339 ~ 361 ) ; 跨膜区 7 ( 410 ~ 432 ) 。

Transmembrane region 1(7-29); Transmembrane region 2(39-58); Transmembrane region 3(99-121); Transmembrane region 4(151-173); Transmembrane region 5(297-319); Transmembrane region6(339-361); Transmembrane region 7(410-432).

Ldis	<b>L</b> SKNQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINRIDVYAFSTVGYLGYTTLGQVFHF	358
Harm	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Hass1	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Hass2	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Hzea	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	394
Hvir	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Slit	<b>L</b> TPKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKINGINVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Pxyl	<b>L</b> TQKQEMLARSAIKYWVERHKHVVRLVASIGDTYGTTLLFHMVLSTITLTLLAYQATKIDGLNVYAFSTI <b>G</b> YLSYTLGQVFHF	395
Bdor	<b>L</b> TRKQEMMVRSAIKYWVERHKHVVRLVAAIGDTYGGALLHMLTSTIMLTLLAYQATKITGVNAYAFTTIGYLGYALAQVFHF	395
Bcuc	<b>L</b> TRKQEMMVRSAIKYWVERHKHVVRLVAAIGDTYGGALLHMLTSTIMLTLLAYQATKITGVNVAFTTIGYLGYALAQVFHF	395
Ccap	<b>L</b> TRKQEMMVRSAIKYWVERHKHVVRLVAAIGDTYGGALLHMLTSTIMLTLLAYQATKITGVNVYAFFTVGYLCYALAQVFHF	395
Hirr	<b>L</b> TKKQEMLVRSAIKYWVERHKHVVRLVVAIGDTYGAALLHMLTSTIKLTLLAYQATKITGVDVYAFTVIGYLGYALAQVFHF	399
Scal	<b>L</b> TKKQEMMVRSAIKYWVERHKHVVRLVAAIGDTYGAALLHMLTSTIKLTLLAYQATKITGVNVYAFTVIGYLGYALAQVFHF	400
Dmel	<b>L</b> TKKQEMMVRSAIKYWVERHKHVVRLVAAIGDTYGAALLHMLTSTIKLTLLAYQATKINGNVYAFTVVGLYGYALAQVFHF	408
Agam	<b>L</b> TRKQEMMVRSAIKYWVERHKHVVRLVSAIGDTYGPALLHMLTSTIKLTLLAYQATKIDGVNVYGLTVIGLYCYALAQVFLF	400
Aaeg	<b>L</b> TKKQELMVRSAIKYWVERHKHVVRLVSAIGETYGAALLHMLTSTIKLTLLAYQATKIDALNVYGLTVIGLYVYALAQVFLF	400
Hplu	<b>L</b> TKKQELMVRSAIKYWVERHKHVVRLVTAIGDAYGIALLHMLTSTILTLAYQATKIDGVNKYALTIVGYLFYALAQVFHF	399
Hobl	<b>L</b> TKKQELMVRSAIKYWVERHKHVVRLVTAIGDAYGIALLHMLTSTILTLAYQATKIDGVNKYALTIVGYLFYALAQVFHF	399
Hpar	<b>L</b> TKKQELMVRSAIKYWVERHKHVVRLVTAIGDAYGIALLHTSASTITLTLLAYQATKIDGVNKYALTIVGYLFYALTQVFHF	398
Consensus	<b>L***QE***RSAIKYWVERHKHVVRLV**IG**YG**LL*H***STI*LTLLAYQA*KI*****Y****GYL*Y*L*QVF*F</b>	
Ldis	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVLVQLK 436	
Harm	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL IQLK 473	
Hass1	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL IQLK 473	
Hass2	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Hzea	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 472	
Hvir	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Slit	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Pxyl	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK - - 471	
Bdor	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Bcuc	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Ccap	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 473	
Hirr	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMWIWGA KFFTVDSDLFASVLA VVTYFMGLVQLK 477	
Scal	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 478	
Dmel	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTVDSDLFASVLA VVTYFMVL VQLK 486	
Agam	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMTI SGA KFFTVDSDLFASVLA VVTYFMVL VQLK 478	
Aaeg	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMTI SGA KFFTVDSDLFASVLA VVTYFMVL VQLK 478	
Hplu	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTISDLFASVLA TVTYFMVL VQLK 477	
Hobl	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTISDLFASVLA TVTYFMVL VQLK 477	
Hpar	<b>C</b> IFGNRLIEESSSVMEAAYSCQWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTISLVL FASVLA TVTYFMVL VQLK 476	
Consensus	<b>C</b> IFGNRLIEESSSVMEAAYSCWYDGSEEAKTFVQIVCQQCQKAM* I *GAKFFT*SL*LF*SVLGA VT*FM*L*QLK	

图 4 欧洲型舞毒蛾 OrCo 与其它昆虫气味受体蛋白的序列比对

Fig. 4 Sequence alignment of the OrCo in *Lymantria dispar*(European gypsy moth) with olfactory receptors in other insects

气味受体 GenBank 登录号 The accession number of olfactory receptors in Genbank : 亚洲型舞毒蛾 *Lymantria dispar*(European gypsy moth) (Ldis,OrCo,mRNA,KF482409); 棉铃虫 *Helicoverpa armigera*(Harm,Or83b,mRNA,HQ186284); 烟夜蛾 *Helicoverpa assulta* (Hass1, Or83b, mRNA, HQ186285), 烟夜蛾 *Helicoverpa assulta*(Hass2,Or83b,mRNA,EU057178); 玉米夜蛾 *Helicoverpa zea* (Hzea, Or83b, mR-NA, AY843204); 苜蓿夜蛾 *Heliothis virescens*(Hvir,Or83b,mRNA,JQ394904); 斜纹夜蛾 *Spodoptera litura* (Slit, Or83b, mRNA, JQ811935); 小菜蛾 *Plutella xylosteana*(Pxyl,Or83b,mRNA,GQ923610); 橘小实蝇 *Bactrocera dorsalis* (Bdor, Or83b, mRNA, EU621792); 瓜实蝇 *Bactrocera cucurbitae* (Bcuc, Or83b, mRNA, HM745934); 地中海实蝇 *Ceratitis capitata* (Ccap, Or83b, mRNA, AY843206); 角蝇 *Haematobia irritans* (Hirr, Or83b, mRNA, EU622915); 厥螯蝇 *Stomoxys calcitrans* (Scal, Or83b, mRNA, EU622914); 果蝇 *Drosophila melanogaster* (Dmel,Or83b,mRNA,AY567998); 冈比亚按蚊 *Anopheles gambiae* (Agam, Or83b, mRNA, AY843205); 埃及伊蚊 *Aedes aegypti* (Aaeg, Or83b, mRNA, XM\_001651376); 台湾黑金龟 *Holotrichia plumbea* (Hplu, Or83b, mRNA, HQ110087); 华北大黑鳃金龟 *Holotrichia obliqua* (Hobl, Or83b, mRNA, JF718662); 暗黑鳃金龟 *Holotrichia parallela* (Hpar, Or83b, mRNA, JF826514).

共有部分表示最保守的氨基酸残基。The most conservative amino acid residues were shown in consensus line.

利用 Mega5.1 软件构建了鳞翅目、鞘翅目、膜翅目、双翅目部分昆虫非典型气味受体的系统进化树(图 5)。经 1 000 次重复后,该进化树非常直观地呈现出 4 个目昆虫之间的进化关系。结果显示,26 个气味受体基因分成 3 个大的分支,其中双翅目和鞘翅目昆虫处于一个分支上,而膜翅目和鳞翅目昆虫各处于一个大分支上,说

明相对于膜翅目和鳞翅目而言,双翅目与鞘翅目在 OrCo 上的亲缘关系较近。这 4 个目的气味受体序列均表现得相对较为保守,未出现较大的分化。在鳞翅目中,欧洲型舞毒蛾 (LdisOrCo) 与二化螟 (*Chilo suppressalis* OrCo) 的非典型气味受体亲缘关系很近,与草地螟 (*Loxostege sticticalis* Or83b) 则相对远些。

Ldis	<b>L</b> SKNQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYGTT <b>L</b> FHMLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NR1DVYAFSTV <b>G</b> YLGY <b>T</b> L <b>Q</b> VF <b>H</b>	358
Harm	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Hass1	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Hass2	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Hzea	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	394
Hvir	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Slit	<b>L</b> TPKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>T</b> ALLF <b>H</b> MLV <b>S</b> T <b>I</b> T <b>L</b> TLLAYQATK <b>I</b> NGIN <b>V</b> YAFSTI <b>G</b> YLSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Pxyl	<b>L</b> TQKQEMLARSAI <b>K</b> YWVERHKHVVRL <b>V</b> AS <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> M <b>L</b> TLLAYQATK <b>I</b> DGLNV <b>T</b> IG <b>Y</b> LSY <b>T</b> L <b>Q</b> VF <b>H</b>	395
Bdor	<b>L</b> TRKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> AA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> M <b>L</b> TLLAYQATK <b>I</b> TGVNA <b>A</b> FTT <b>I</b> <b>G</b> YLG <b>Y</b> ALA <b>Q</b> VF <b>H</b>	395
Bcuc	<b>L</b> TRKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> AA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> M <b>L</b> TLLAYQATK <b>I</b> TGVNV <b>Y</b> AFTT <b>I</b> <b>G</b> YLG <b>Y</b> ALA <b>Q</b> VF <b>H</b>	395
Ccap	<b>L</b> TRKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> AA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> M <b>L</b> TLLAYQATK <b>I</b> TGVNV <b>Y</b> AFTT <b>V</b> <b>G</b> YLCY <b>A</b> LA <b>Q</b> VF <b>H</b>	395
Hirr	<b>L</b> TKKQEMLVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> VA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> K <b>L</b> TLLAYQATK <b>I</b> ITGVDV <b>V</b> AFTV <b>I</b> <b>G</b> YLG <b>Y</b> ALA <b>Q</b> VF <b>H</b>	399
Scal	<b>L</b> TKKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> AA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> K <b>L</b> TLLAYQATK <b>I</b> TGVNV <b>Y</b> AFTV <b>I</b> <b>G</b> YLG <b>Y</b> ALA <b>Q</b> VF <b>H</b>	400
Dmel	<b>L</b> TKKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> AA <b>I</b> GDTYG <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> K <b>L</b> TLLAYQATK <b>I</b> INGVN <b>V</b> YAFTV <b>V</b> <b>G</b> YLG <b>Y</b> ALA <b>Q</b> VF <b>H</b>	408
Agam	<b>L</b> TRKQEMMVRSAI <b>K</b> YWVERHKHVVRL <b>V</b> SA <b>I</b> GDTYG <b>P</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> K <b>L</b> TLLAYQATK <b>I</b> DGVNV <b>V</b> GLTV <b>I</b> <b>G</b> YLCY <b>A</b> LA <b>Q</b> VF <b>L</b>	400
Aaeg	<b>L</b> TKKQELMVRS <b>A</b> I <b>K</b> YWVERHKHVVRL <b>V</b> SA <b>I</b> GETY <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> K <b>L</b> TLLAYQATK <b>I</b> DALNV <b>Y</b> GLTV <b>I</b> <b>G</b> LYV <b>Y</b> ALA <b>Q</b> VF <b>L</b>	400
Hopl	<b>L</b> TKKQELMVRS <b>A</b> I <b>K</b> YWVERHKHVVRL <b>V</b> TA <b>I</b> GDAY <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> L <b>T</b> L <b>L</b> AYQATK <b>I</b> DGVNV <b>K</b> ALT <b>V</b> L <b>G</b> YLF <b>Y</b> ALA <b>Q</b> VF <b>H</b>	399
Hobl	<b>L</b> TKKQELMVRS <b>A</b> I <b>K</b> YWVERHKHVVRL <b>V</b> TA <b>I</b> GDAY <b>G</b> ALLL <b>H</b> MLT <b>S</b> T <b>I</b> L <b>T</b> L <b>L</b> AYQATK <b>I</b> DGVNV <b>K</b> ALT <b>V</b> L <b>G</b> YLF <b>Y</b> ALA <b>Q</b> VF <b>H</b>	399
Hpar	<b>L</b> TKKQELMVRS <b>A</b> I <b>K</b> YWVERHKHVVRL <b>V</b> TA <b>I</b> GDAY <b>G</b> ALLL <b>H</b> TS <b>A</b> ST <b>I</b> L <b>T</b> L <b>L</b> AYQATK <b>I</b> DGVNV <b>K</b> ALT <b>V</b> L <b>G</b> YLF <b>Y</b> ALT <b>Q</b> VF <b>H</b>	398
Consensus	<b>L</b> ***QE***RSA <b>I</b> KYWVERHKHVVRL** <b>I</b> G**YG**LL*H***STI*L <b>T</b> L <b>L</b> AYQA*KI*****Y*****G <b>Y</b> L*Y*L*QVF*F	
Ldis	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	436
Harm	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>I</b> QL <b>K</b>	473
Hass1	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Hass2	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Hzea	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	472
Hvir	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Slit	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Pxyl	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b> --471	471
Bdor	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Bcuc	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Ccap	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	473
Hirr	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	477
Scal	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	478
Dmel	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	486
Agam	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	478
Aaeg	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	478
Hopl	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	477
Hpar	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSCQ <b>W</b> YDGSEE <b>A</b> KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM <b>S</b> <b>I</b> <b>S</b> GAKFFT <b>V</b> SLDL <b>F</b> ASV <b>L</b> GA <b>V</b> VT <b>T</b> FMV <b>L</b> <b>V</b> QL <b>K</b>	476
Consensus	<b>C</b> IFGNRL <b>I</b> EESSSVMEAAYSC*WYDGSEE*KTFV <b>Q</b> IV <b>C</b> QQC <b>Q</b> KAM*I*GAKFFT*SL*LF*SVLGA*VT*FM*L*QL <b>K</b>	

图 4 欧洲型舞毒蛾 OrCo 与其它昆虫气味受体蛋白的序列比对

Fig. 4 Sequence alignment of the OrCo in *Lymantria dispar*(European gypsy moth) with olfactory receptors in other insects

气味受体 GenBank 登录号 The accession number of olfactory receptors in Genbank : 亚洲型舞毒蛾 *Lymantria dispar*(European gypsy moth) (Ldis,OrCo,mRNA,KF482409); 棉铃虫 *Helicoverpa armigera*(Harm,Or83b,mRNA,HQ186284); 烟夜蛾 *Helicoverpa assulta* (Hass1, Or83b, mRNA, HQ186285), 烟夜蛾 *Helicoverpa assulta*(Hass2,Or83b,mRNA,EU057178); 玉米夜蛾 *Helicoverpa zea* (Hzea, Or83b, mR-NA, AY843204); 苜蓿夜蛾 *Heliothis viresplaca*(Hvir,Or83b,mRNA,JQ394904); 斜纹夜蛾 *Spodoptera litura* (Slit, Or83b, mRNA, JQ811935); 小菜蛾 *Plutella xylostella*(Pxyl,Or83b,mRNA,GQ923610); 橘小实蝇 *Bactrocera dorsalis* (Bdor, Or83b, mRNA, EU621792); 瓜实蝇 *Bactrocera cucurbitae* (Beuc, Or83b, mRNA, HM745934); 地中海实蝇 *Ceratitis capitata* (Ccap, Or83b, mRNA, AY843206); 角蝇 *Haematobia irritans* (Hirr, Or83b, mRNA, EU622915); 厥螯蝇 *Stomoxys calcitrans* (Scal, Or83b, mRNA, EU622914); 果蝇 *Drosophila melanogaster* (Dmel,Or83b,mRNA,AY567998); 冈比亚按蚊 *Anopheles gambiae* (Agam, Or83b, mRNA, AY843205); 埃及伊蚊 *Aedes aegypti* (Aaeg, Or83b, mRNA, XM\_001651376); 台湾黑金龟 *Holotrichia plumbea* (Hopl, Or83b, mRNA, HQ110087); 华北大黑鳃金龟 *Holotrichia obliqua* (Hobl, Or83b, mRNA, JF718662); 暗黑鳃金龟 *Holotrichia parallela* (Hpar, Or83b, mRNA, JF826514)。

共有部分表示最保守的氨基酸残基。The most conservative amino acid residues were shown in consensus line.

利用 Mega5.1 软件构建了鳞翅目、鞘翅目、膜翅目、双翅目部分昆虫非典型气味受体的系统进化树(图 5)。经 1 000 次重复后,该进化树非常直观地呈现出 4 个目昆虫之间的进化关系。结果显示,26 个气味受体基因分成 3 个大的分支,其中双翅目和鞘翅目昆虫处于一个分支上,而膜翅目和鳞翅目昆虫各处于一个大分支上,说

明相对于膜翅目和鳞翅目而言,双翅目与鞘翅目在 OrCo 上的亲缘关系较近。这 4 个目的气味受体序列均表现得相对较为保守,未出现较大的分化。在鳞翅目中,欧洲型舞毒蛾 (LdisOrCo) 与二化螟 (*Chilo suppressalis* OrCo) 的非典型气味受体亲缘关系很近,与草地螟 (*Loxostege sticticalis* Or83b) 则相对远些。

### 3 讨 论

目前, OrCo 已经在鳞翅目、鞘翅目、膜翅目、双翅目 4 个目的多种昆虫中被发现 (Krieger *et al.*, 2003; Pitts *et al.*, 2004)。本研究利用基因克隆技术成功获得了欧洲型舞毒蛾 OrCo 基因的 cDNA 全长序列, 并进行了序列分析, 获得的氨基酸序列与近缘种二化螟 *Chilo suppressalis*、灰翅夜蛾 *Spodoptera littoralis* 等嗅觉受体的同源性高达 89%, 与鞘翅目台湾黑金龟 *Holotrichia plumbea* 的同源性高达 64%, 这些都与 OrCo 基因在不同昆虫体内的高度保守性相吻合。对于 OrCo 的功能, 有研究表明 OrCo 能够调节或者协助传统气味受体对气味的识别 (Larsson *et al.*, 2004), 并且有助于传统嗅觉受体在嗅觉神经树突膜上的正确定位 (Benton *et al.*, 2006)。但是, 对于完整的嗅觉受体信号传导机制目前尚不清楚 (Ha and Smith, 2008)。从应用角度上看, 这类在不同昆虫间高度保守的气味受体 OrCo 的发现无疑给害虫防治带来了新机遇, 如果能够干扰或者破坏 OrCo 的功能, 就能够同时影响多个嗅觉受体的正常功能, 并且这种技术一旦成功就能够同时对多种近缘害虫起到控制作用。欧洲型舞毒蛾 OrCo 受体的成功鉴定, 为进一步研究舞毒蛾气味受体的功能, OrCo 基因的进化以及揭秘嗅觉机制奠定了基础。

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