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The effect of polyamines on the expression of olfactory related genes in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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Abstract. Polyamines are nitrogenous compounds that alter the odour sensitivity of insects. The objective of this study was to determine the effects of polyamines on the expression levels of olfactory-related genes in male/female adult diamondback moths, *Plutella xylostella*. Results indicate that treatment with polyamines or α-difluoromethyl-ornithine (DFMO), a polyamine synthesis inhibitor, significantly affected the expression of various olfactory related genes. Although DFMO had no significant effect on the expression of *PxylPBP1*, it did significantly decrease the expression of other olfactory related genes (*PxylGOBP1*, *PxylGOBP2* and *PxylCSP1*) in a gender-related fashion that correlated with the mating status of the adults. Polyamines [spermine (spm), putrescine (put) and spermidine (spd)] decreased the expression of *PxylGOBP2* in naive male moths, but increased the expression of the same gene in virgin female moths. In addition, put treatment significantly increased the expression of *PxylGOBP1* in virgin females and unmated male moths, but significantly decreased its expression in mated female and male moths. Based on these results we conclude that polyamines have a regulatory role in the expression of olfactory related genes and that these effects depend on the gene, gender and mating status of diamondback moths. These results indicate that polyamines affect the expression of the genes in insects that determine their olfactory ability.

INTRODUCTION

The polyamines spermidine (spd) and spermine (spm), as well as their precursor amine putrescine (put) are low molecular weight nitrogenous compounds naturally produced by living cells (Morgan & Wallace, 1994). The precise function of these polyamines is currently unknown however, their ubiquity, high cellular concentration and accumulation in rapidly growing tissues indicate an involvement in a broad range of biological processes (Smith, 1985; Evans & Malmberg, 1989). Polyamines not only enhance transcription, processing and incorporation of RNA in ribosomes (Blair, 1985), but also bind to DNA and regulate gene expression (Feuerstein et al., 1991; Childs et al., 2003). Moreover, polyamines are involved in many cellular processes, including chromatin condensation, maintenance of DNA structure, translation, protein activation, affect the formation of compacted chromatin and have a well-established role in DNA aggregation.

Polyamines are also involved in insect growth and metamorphosis, gestation, vitellogenin synthesis and hormone metabolism (Birnbaum et al., 1988; Birnbaum & Gilbert, 1990; Callerts et al., 1992; Cayre et al., 1993; Kogan & Hagedorn, 2000). Insects recognize a wide range of hydrophobic semiochemical signals, such as odours and tastes, which enable them to detect each other, locate suitable food, identify their hosts and control their behaviour. These chemicals are bound by soluble odorant-binding proteins (OBPs) and transported through the sensillar lymph to the olfactory receptors, where signal transduction is initiated (Vogt et al., 1999). With respect to their physiological function, it is now recognized that OBPs solubilize ligands, help transport hydrophobic semiomolecules through the aqueous environment of the sensillar lymph towards the dendritic membrane of olfactory sensory neurons, where the olfactory receptor proteins are located and contribute to the sensitivity of the insect olfactory system, thus initiating the first biochemical step in odour perception (Leal, 2013). Interestingly, polyamines modulate the phosphorylation of several antennary proteins of *Periplaneta Americana* (Tirard et al., 2002). On the other hand, newly emerged males treated with the polyamine synthesis inhibitor α-difluoromethyl-ornithine (DFMO) have an enhanced antennal sensitivity to female pheromones and treatment with polyamines modulates the phosphorylation of several antennary proteins (Tirard et al., 2002). Furthermore, polyamine-depleted female house crickets (*Acheta domesticus*) delay egg-laying (Cayre et al., 1996). Moreover, an earlier study indicates that diamondback moths (*Plutella xylostella* L.) are attracted to polyamines and that their antennae have strong electrophysiological responses to polyamine compounds (Zhang et al., 2009c). We also found that treatment with polyamines and α-difluoromethyl-ornithine (DFMO) significantly affects the sensitivity of the moth's antennae

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Table 1. Oligonucleotide primers used for expression analysis of *Plutella xylostella* olfactory related genes.

Purpose/primer name	Sequence (5′–3′)
PxylGOBP1-forward	CACCATGACAACACGCACAAG
PxylGOBP1-reverse	ACTCTGCCATCAGCATCTCCA
PxylGOBP2-forward	GAAGAATCCGGCCTATCC
PxylGOBP2-reverse	CAGCCTCACCATCGTCTC
PxylPBP1-forward	ACCTGGTGGGCGACTTCT
PxylPBP1-reverse	AGCCTCGTGCTGCTTCTC
PxylCSP1-forward	CACTACACCGACCGCTATGAC
PxylCSP1-reverse	ATTTGCCGCAGTTGTTCTCC
PxylActin-forward	GCGACTTGACCGACTACC
PxylActin-reverse	GGAATGAGGGCTGGAACA

to odours. As such, polyamines may stimulate olfactory reception in insects. However, there are only a few other reports of polyamine-dependent regulation of odour detection. The aim of this study was to determine the effects of polyamines on the gender- and mating-related expression of olfactory related genes and any subsequent changes that occur in the behaviour of diamondback moths.

MATERIAL AND METHODS

Insect rearing and tissue collection

Diamondback moths were reared indoors (16L : 8D cycle; 25 ± 1°C; 60–70% r.h.) and fed a diet of Chinese cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae), until pupation. Sexed pupae were kept inside glass tubes in an environmental chamber (16L : 8L cycle; 25 ± 1°C; 60–80% r.h.) until the moths emerged. A number of pairs newly emerged male and female moths were allowed to mate in glass tubes. Antennae were excised from male and female moths, immediately transferred to Eppendorf tubes, immersed in liquid nitrogen and stored at −75°C until further use.

Polyamines and DFMO treatments

Put, spd, and spm (Sigma Aldrich, MO, USA) were dissolved in 75% acetone to make a 1% solution; DFMO (Sigma Aldrich) was dissolved in 75% acetone to a final concentration of 5 mg ml^{-1}.

Pupae were collected 12–20 h after pupation and 2 µl of polyamines or DFMO applied to the cuticle of the thoracic dorsum of each pupa using a microapplicator. Controls were treated with 2 µl of 75% acetone. Pupae were collected individually in glass tubes and kept in the same conditions as described above.

RNA extraction and cDNA synthesis

Total RNA was extracted from 200 antennae of sexually mature *P. xylostella* males and females using TRIpure LS Reagent (BioTeke, Beijing, China). Single-stranded cDNA was synthesized from 1 µg of RNA with the MBI RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, Glen Burnie, MD, USA) according to the manufacturer's instructions.

Real-time PCR

To determine the mating-related expression profile of adult moths, the antennae of virgin and mated individuals were collected. Virgin male and female moths were sampled at 0, 4, 8 and 16 h after emergence. For mated moths, pairs of newly emerged male and female moths were placed in glass tubes and allowed to mate. After 16 h, mated moths antennae were collected for bioassay. Total RNA from 100 moths was extracted for each time after emergence and group, and cDNA was synthesized according

Table 2. Relative quantification (mean ± SE) of *PxylGOBP1* amplified in *Plutella xylostella* moths after treatment with polyamines and DFMO.

Moth age	Treatment	$10^{\Delta Ct}$	Moth age	Treatment	$10^{\Delta Ct}$
m0h	acetone	4.4438 ± 0.1655Aa	f0h	acetone	0.9397 ± 0.0006Bb
	put	4.5996 ± 0.2046Aa		put	4.6484 ± 0.1443Aa
	spd	3.8231 ± 0.0578Ab		spd	0.0442 ± 0.0015Cc
	spm	0.0251 ± 0.0027Bc		spm	0.1691 ± 0.0056Cc
	DFMO	0.256 ± 0.0036Bc		DFMO	0.1839 ± 0.005Cc
m4h	acetone	0.3552 ± 0.0234Cc	f4h	acetone	1.8096 ± 0.0401Bb
	put	0.5909 ± 0.0064Bb		put	2.4046 ± 0.1852Bb
	spd	1.2056 ± 0.0228Aa		spd	11.322 ± 0.2865Aa
	spm	0.292 ± 0.0049Cc		spm	0.0972 ± 0.0178Cc
	DFMO	0.1134 ± 0.0075Dd		DFMO	0.1822 ± 0.009Cc
m8h	acetone	0.7747 ± 0.1303Bb	f8h	acetone	16.4589 ± 1.3497Aa
	put	2.5002 ± 0.0208Aa		put	1.4353 ± 0.0248Cc
	spd	0.414 ± 0.0143BCc		spd	0.1834 ± 0.0103Cc
	spm	0.1264 ± 0.0152Cd		spm	5.4465 ± 0.3386Bb
	DFMO	0.0679 ± 0.0014Cd		DFMO	1.132 ± 0.0512Cc
m16h	acetone	0.3586 ± 0.0493Bb	f16h	acetone	0.8377 ± 0.0253Cc
	put	2.7011 ± 0.0736Aa		put	3.4056 ± 0.0851Aa
	spd	0.18 ± 0.0073Bbc		spd	1.7495 ± 0.0351Bb
	spm	0.1528 ± 0.0031Bc		spm	0.1722 ± 0.0063Dd
	DFMO	0.1452 ± 0.0052Bc		DFMO	0.2385 ± 0.0099Dd
mMated	acetone	4.6467 ± 0.6978Aa	fMated	acetone	4.8707 ± 0.1937Bb
	put	3.0073 ± 0.1011Ab		put	0.8057 ± 0.0164Cc
	spd	0.9456 ± 0.0422Bc		spd	11.5392 ± 0.202Aa
	spm	0.1839 ± 0.0084Bc		spm	4.2771 ± 0.2718Bb
	DFMO	0.4008 ± 0.0365Bc		DFMO	0.1069 ± 0.0006Cc

Note: The data in this table are means ± *SEs*, different letters indicate significant differences (lower case letters: $P < 0.05$, capital letters: $P < 0.01$, Duncan's multiple comparison test). The same in Tables 3–5.

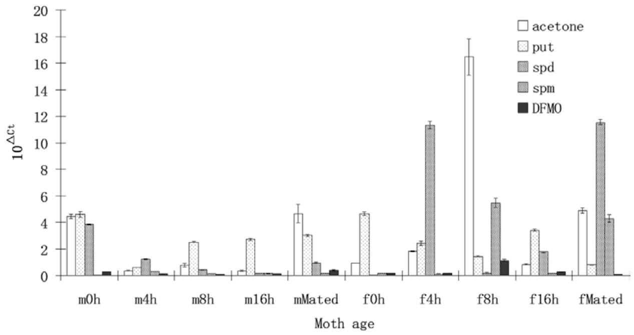

Fig. 1. Relative quantification (mean ± SE) of PxylGOBP1 and PxylActin amplified in *Plutella xylostella* moths of different ages using real-time PCR. m – male antenna; f – female antenna; 0, 4, 8, 16 h, and Mated 0, 4, 8 and 16 h after eclosion, and 16 h-old mated moth, respectively.

to the methods described above. Primer pairs used for RT-PCR quantification (Zhang et al., 2009a, b, d) (listed in Table 1) were expected to amplify the products of 209 bp (*PxylGOBP1*), 219 bp (*PxylGOBP2*), 227 bp (*PxylPBP1*), 227 bp (*PxylCSP1*) and 252 bp (PxylActin). Real-time PCR was performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) via SYBR green dye bound to double stranded DNA (QuantiTect SBGR Green PCR kit; Qiagen, Beijing, China) (15 min at 95°C, followed by 40 cycles of 15 s at 94°C, 30 s at 56°C, and 30 s at 72°C). PCR reactions were performed in triplicate and data processed using the relative absolute quantification method. The given sample was diluted to C0 (1, 4, 16, 64 and 256) and the Ct values of *PxylPBP1* and PxylActin genes were measured. Subsequently, the standard curves were analyzed by Ct and logC0

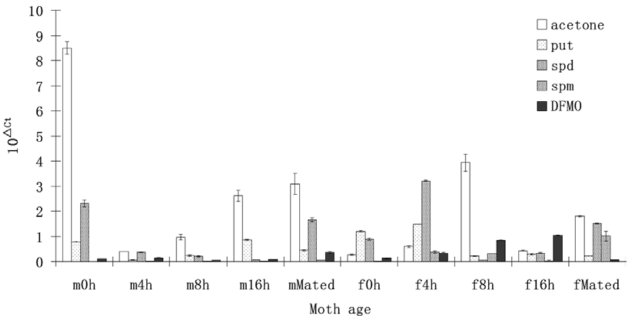

Fig. 2. Relative quantification (mean ± SE) of PxylGOBP2 and PxylActin amplified in *Plutella xylostella* moths of different ages using real-time PCR. For further details see Fig. 1.

(the slope K and intercept B) with a relative value of $10^{\Delta Ct}$ [$\Delta Ct = Ct_{PxylPBP1}\{-(Ct-B)/K\} - Ct_{PxylActin}\{-(Ct-B)/K\}$].

Statistical analysis

Results are reported as means ± standard errors (SEM). Analysis of variance (ANOVA) was conducted on Ct data. Differences in the pairs of mean values between treatments were separated using Duncan's multiple comparisons test. Data analyses were carried out using SPSS 11.0 (SPSS, 2001).

RESULTS

PxylGOBP1 levels

PxylGOBP1 is expressed during the entire adult stage of diamondback moths (Fig. 1, Table 2). The transcription levels of *PxylGOBP1* decreased sharply in males following eclosion and increased sharply in female moths. Mated

TABLE 3. Relative quantification (mean ± SE) of *PxylGOBP2* amplified in *Plutella xylostella* moths after treatment with polyamines and DFMO.

Moth age	Treatment	$10^{\Delta Ct}$	Moth age	Treatment	$10^{\Delta Ct}$
m0h	acetone	8.4938 ± 0.2453Aa	f0h	acetone	0.2724 ± 0.0235Cc
	put	0.7777 ± 0.0118Cc		put	1.1966 ± 0.0244Aa
	spd	2.3123 ± 0.1331Bb		spd	0.8807 ± 0.0502Bb
	spm	0.0052 ± 0.0006Dd		spm	0.0261 ± 0.0019Dd
	DFMO	0.1064 ± 0.0025CDd		DFMO	0.1262 ± 0.006CDd
m4h	acetone	0.3899 ± 0.0031Aa	f4h	acetone	0.5868 ± 0.0388Cc
	put	0.053 ± 0.0116Cc		put	1.4677 ± 0.0057Bb
	spd	0.3648 ± 0.0099Aa		spd	3.2043 ± 0.0282Aa
	spm	0.0253 ± 0.0023Cc		spm	0.3808 ± 0.045Dd
	DFMO	0.1423 ± 0.0096Bb		DFMO	0.3405 ± 0.02Dd
m8h	acetone	0.9769 ± 0.11Aa	f8h	acetone	3.9327 ± 0.3402Aa
	put	0.2321 ± 0.0266Bb		put	0.2109 ± 0.0076Bbc
	spd	0.1958 ± 0.0199Bb		spd	0.0435 ± 0.0015Bc
	spm	0.0151 ± 0.0016Bb		spm	0.3076 ± 0.0029Bbc
	DFMO	0.0463 ± 0.0011Bb		DFMO	0.8411 ± 0.0163Bb
m16h	acetone	2.6137 ± 0.2151Aa	f16h	acetone	0.428 ± 0.0089Bb
	put	0.8561 ± 0.0187Bb		put	0.2894 ± 0.0151Cc
	spd	0.0611 ± 0.0054Cc		spd	0.3318 ± 0.0137Cc
	spm	0.0241 ± 0.002Cc		spm	0.0321 ± 0.0013Dd
	DFMO	0.0853 ± 0.0008Cc		DFMO	1.0429 ± 0.0189Aa
mMated	acetone	3.0849 ± 0.4221Aa	fMated	acetone	1.7986 ± 0.0261Aa
	put	0.4419 ± 0.0249Cc		put	0.2142 ± 0.0043Cc
	spd	1.6584 ± 0.0762Bb		spd	1.5217 ± 0.0197ABa
	spm	0.037 ± 0.0015Cc		spm	1.0057 ± 0.1932Bb
	DFMO	0.3528 ± 0.0496Cc		DFMO	0.0699 ± 0.0064Cc

For further details see Table 2.

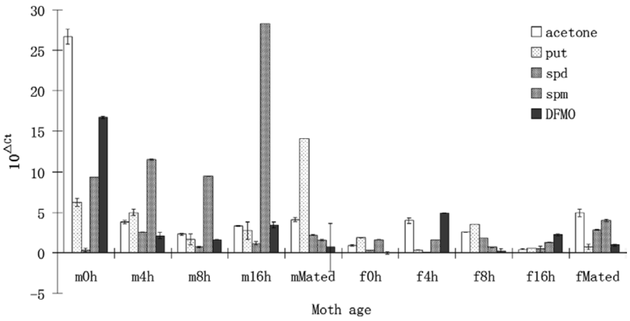

Fig. 3. Relative quantification (mean ± SE) of PxylPBP1 and PxylActin amplified in *Plutella xylostella* moths of different ages using real-time PCR. For further details see Fig. 1.

individuals showed much higher levels of *PxylGOBP1* expression 16 h post emergence than unmated individuals. Interestingly, *PxylGOBP1* levels were highest in the antennae of 8 h-old female moths. Furthermore, transcription levels of *PxylGOBP1* in unmated moths increased significantly following treatment with put. In contrast, *PxylGOBP1* levels decreased sharply in mated moths. Levels of *PxylGOBP1* also decreased in male moths (except 4 h-old) following spd treatment but increased (except 16 h-old) in female moths. However, transcription levels of *PxylGOBP1* decreased in both male and female moths following treatment with both spm and DFMO, with DFMO having the greater effect (Fig. 1, Table 2).

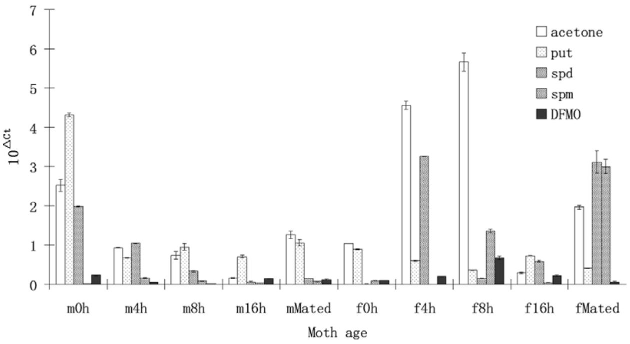

Fig. 4. Relative quantification (mean ± SE) of PxylCSP1 and PxylActin amplified in *Plutella xylostella* moths of different ages using real-time PCR. For further details see Fig. 1.

PxylGOBP2 levels

PxylGOBP2 was expressed throughout the entire adult period of the diamondback moth (Fig. 2, Table 3). Like PxylGOBP1, the levels of *PxylGOBP2* also decreased sharply in males following eclosion but then began to increase over time. The level also increased sharply following eclosion in females (except 16 h-old). Mated moths had much higher levels of *PxylGOBP2* expression 16 h post emergence than unmated moths. Treatment with put and spd both decreased the levels of *PxylGOBP2* in unmated moths (except for 0 h-old and 4 h-old female moths), but put had a greater effect. Furthermore, the transcription levels of *PxylGOBP2* in male moths also decreased after

Table 4. Relative quantification (mean ± SE) of *PxylPBP1* amplified in *Plutella xylostella* moths after treatment with polyamines and DFMO.

Moth age	Treatment	$10^{\Delta Ct}$	Moth age	Treatment	$10^{\Delta Ct}$
m0h	acetone	26.7004 ± 0.8834Aa	f0h	acetone	0.9125 ± 0.0496Bb
	put	9.3386 ± 0.4903Cc		put	1.6404 ± 0.0238Aa
	spd	16.7161 ± 0.2078Bb		spd	0.0172 ± 0.0014Cd
	spm	0.3376 ± 0.0088Ee		spm	0.3617 ± 0.0446Cc
	DFMO	6.2407 ± 0.1684Dd		DFMO	1.8687 ± 0.1481Aa
m4h	acetone	3.7946 ± 0.1931BCb	f4h	acetone	3.9835 ± 0.3085Bb
	put	11.546 ± 0.4048Aa		put	1.6272 ± 0.0334Cc
	spd	2.1124 ± 0.0465Dc		spd	4.9062 ± 0.067Aa
	spm	2.5519 ± 0.0746CDc		spm	0.027 ± 0.0033Dd
	DFMO	5.0138 ± 0.3764B b		DFMO	0.3571 ± 0.0411Dd
m8h	acetone	2.2751 ± 0.0746Bb	f8h	acetone	2.5622 ± 0.0142Bb
	put	9.4457 ± 0.6654Aa		put	0.675 ± 0.015Dd
	spd	1.6286 ± 0.0684Bbc		spd	0.2272 ± 0.0069Dd
	spm	0.6792 ± 0.0151Bc		spm	1.7956 ± 0.022Cc
	DFMO	1.6526 ± 0.0315Bbc		DFMO	3.5211 ± 0.22Aa
m16h	acetone	3.2906 ± 0.0904Bb	f16h	acetone	0.4254 ± 0.0239Bb
	put	28.3344 ± 1.0484Aa		put	1.2692 ± 0.0191ABb
	spd	3.4304 ± 0.203Bb		spd	2.2088 ± 0.3958Aa
	spm	1.1994 ± 0.01Bb		spm	0.4476 ± 0.0277Bb
	DFMO	2.7446 ± 0.3431Bb		DFMO	0.56 ± 0.0829Bb
mMated	acetone	4.0878 ± 0.2433Bb	fMated	acetone	4.9487 ± 0.4408Aa
	put	1.557 ± 0.0193Bb		put	4.0094 ± 0.3133ABa
	spd	0.6863 ± 0.0194Bb		spd	0.9579 ± 0.0635Cc
	spm	2.2023 ± 0.1141Bb		spm	2.7942 ± 0.1595Bb
	DFMO	14.1081 ± 2.9869Aa		DFMO	0.7162 ± 0.0961Cc

For further details see Table 2.

Table 5. Relative quantification (mean ± SE) of *PxylCSP1* amplified in *Plutella xylostella* moths after treatment with polyamines and DFMO.

Moth age	Treatment	$10^{\Delta Ct}$	Moth age	Treatment	$10^{\Delta Ct}$
m0h	acetone	2.5188 ± 0.1561Bb	f0h	acetone	1.0302 ± 0.0011Aa
	put	4.3166 ± 0.0463Aa		put	0.8889 ± 0.0174Bb
	spd	1.9841 ± 0.0118Cc		spd	0.0067 ± 0.0012Dd
	spm	0.0176 ± 0.0007Dd		spm	0.0944 ± 0.0076Cc
	DFMO	0.2311 ± 0.0075Dd		DFMO	0.1021 ± 0.0013Cc
m4h	acetone	0.9243 ± 0.0051Bb	f4h	acetone	4.5546 ± 0.1126Aa
	put	0.6708 ± 0.0083Cc		put	0.5965 ± 0.0182Cc
	spd	1.0429 ± 0.0088Aa		spd	3.2606 ± 0.0059Bb
	spm	0.1601 ± 0.0148Dd		spm	0.0015 ± 0Dd
	DFMO	0.0614 ± 0.0009Ee		DFMO	0.2001 ± 0.0096Dd
m8h	acetone	0.7334 ± 0.0925Aa	f8h	acetone	5.6601 ± 0.2377Aa
	put	0.9483 ± 0.0866Aa		put	0.3594 ± 0.0055Cc
	spd	0.3291 ± 0.0168Bb		spd	0.1524 ± 0.0119Cc
	spm	0.0834 ± 0.0053Bbc		spm	1.3566 ± 0.0494Bb
	DFMO	0.019 ± 0.0001Bc		DFMO	0.6656 ± 0.0441Cc
m16h	acetone	0.1654 ± 0.0165Bb	f16h	acetone	0.2881 ± 0.0177Cc
	put	0.702 ± 0.0381Aa		put	0.7171 ± 0.0083Aa
	spd	0.0636 ± 0.0038Bcd		spd	0.5889 ± 0.017Bb
	spm	0.0346 ± 0.0015Bd		spm	0.0324 ± 0.0051Dd
	DFMO	0.14 ± 0.0032Bbc		DFMO	0.2204 ± 0.0194Cc
mMated	acetone	1.2631 ± 0.0916Aa	fMated	acetone	1.9619 ± 0.0539Bb
	put	1.0562 ± 0.074Aa		put	0.4053 ± 0.0078Cc
	spd	0.1484 ± 0.0027Bb		spd	3.1123 ± 0.2867Aa
	spm	0.072 ± 0.0071Bb		spm	2.9914 ± 0.1828Aa
	DFMO	0.1212 ± 0.0037Bb		DFMO	0.063 ± 0.0052Cc

For further details see Table 2.

treatment with spm. DMFO decreased the levels of *PxylGOBP2* in both male and female moths (except 16 h-old female moths) (Fig. 2, Table 3).

PxylPBP1 levels

PxylPBP1 was also expressed throughout the entire adult period of the diamondback moth (Fig. 3, Table 4). The transcription levels of *PxylPBP1* decreased sharply after eclosion in male moths, but then increased for 16 h. In contrast, *PxylPBP1* increased following eclosion and then decreased as they aged. Mated individuals showed much higher levels of *PxylPBP1* expression 16 h post emergence than unmated individuals; it increased at a higher rate in females than males. However, we did not find an effect of put on the transcription levels of *PxylPBP1* in unmated moths (except for 0 h-old male moths, in which it decreased significantly). On the other hand, put treatment resulted in large increases in *PxylPBP1* in mated male moths and significant decreases in mated females. Spd caused the transcription levels of *PxylPBP1* to decrease in both male and female moths (except for 16 h-old female). Moreover, spm decreased *PxylPBP1* levels in both male and female mated moths, but caused sharp increases in the expression of this gene in unmated males (except 0 h-old) and 0 h-old and 16 h-old female moths. However, spm resulted in decreased levels of *PxylPBP1* in 4 h-old and 8 h-old female moths. Once again, DMFO decreased the transcription levels of *PxylPBP1* in both unmated and mated male and female moths (except for 4 h-old and 16 h-old female moths) (Fig. 3, Table 4).

PxylCSP1 levels

PxylCSP1 is also expressed throughout the entire adult period of the diamondback moth (Fig. 4, Table 5). The transcription levels of *PxylCSP1* decreased sharply after eclosion in male moths, but increased sharply in females. Mated individuals showed much higher levels of *PxylCSP1* 16h post-emergence than unmated individuals. The transcription levels of *PxylCSP1* were the highest in the antennae of 8 h-old females. The transcription levels of *PxylCSP1* decreased in mated males, mated females and unmated females (except 16 h-old) following put treatment and increased sharply in unmated male moths (except 4 h-old). Spd decreased the transcription levels of *PxylCSP1* in all moths (except 4 h-old male and 16 h-old mated female). Finally, transcription levels of *PxylCSP1* decreased sharply to very low levels in both male and female moths following treatment with spm and DFMO (Fig. 4, Table 5).

DISCUSSION

The mechanism by which polyamines and DFMO affect insect olfactory sensitivity is unclear. A possible explanation could involve the influence of polyamines on ion channels. Indeed, endogenous polyamines, in particular, spermine, block or modulate several types of ion channel (Lopatin et al., 1995; Lee et al., 1999; Lu & Ding, 1999). Within the olfactory system, polyamines induce strong inward rectification in the cyclic nucleotide-gated (CNG) channel (Lynch, 1999). However, to the best of our knowledge, all this data was obtained based on studies of mammals and nothing is known about the effects of polyamines on insect CNG channels. Our results indicate the mechanism may involve the expression of olfactory related genes.

This study demonstrates that both polyamines and DFMO significantly affected the expression of olfactory related genes in the diamondback moth, *Plutella xylostella*. Other studies indicate that odorant binding proteins solubilize and carry olfactory signal molecules (Vogt et al., 1991; Du & Prestwich, 1995; Feixas et al., 1995; Maida et al., 2000). In recent years, many studies have confirmed the binding ability to olfactory signal molecules of a number of insects' odorant binding proteins including pheromone binding proteins (Li et al., 2008; Gong et al., 2009; Qiao et al., 2009; Yu et al., 2009; Zhou et al., 2009; Gu et al., 2011; Sun et al., 2013). Similarly, Tirard et al. (2002) found that treatment with DFMO enhanced the sensitivity of the male *Periplaneta americana* antenna to female pheromones, as well as altering the polyamine-modulated phosphorylation of several antennary proteins. Our previous work indicates that polyamines and DFMO significantly affect the sensitivity of diamondback moth antennae (Zhang et al., 2008) and diamondback moth behaviour (Zhang et al., 2009c). Taken together, these findings strongly indicate that polyamines play a regulatory role in olfactory detection in insects, which most likely involves changes in the gene expressions of odorant binding proteins.

In this paper, RT-PCR data confirm that the effect of polyamines and DFMO on the expression of olfactory related genes depends on the gene. For example, DFMO had little effect on the expression of *PxylPBP1*, but significantly decreased the expression of other olfactory related genes. It is well known that different olfactory related genes bind different odours. Previously, the affinity of 12 compounds for three aphid OBPs were measured in competitive binding experiments. Similar behaviour of all three proteins was observed, but (E)-β-farnesene (the alarm pheromone) and farnesol both exhibited a clear selectivity for OBP3 (Qiao et al., 2009). In the silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae), PBP1, PBP2, GOBP2, and ABPx all bind to the sex pheromone component (10E, 12Z)-hexadecadien-1-ol (bombykol). BmorPBP1, BmorPBP2 and BmorABPx also bind the pheromone component (10E,12Z)-hexadecadienal (bombykal) equally well, whereas BmorGOBP2 can discriminate between bombykol and bombykal (Zhou et al., 2009). In addition, in *Chilo suppressalis*, GOBP2 has a significant affinity for cis-11-hexadecenal (Z11-16: Ald) (the main component of the sex pheromone) and laurinaldehyde and benzaldehyde, two general plant volatiles (Gong et al., 2009). In addition, some studies indicate that different PBPs have different affinities for pheromone components (Hooper et al., 2009; Guo et al., 2012; Zhang et al., 2012; Sun et al., 2013).In a previous study, we showed that polyamines and DFMO affect the sensitivity of diamondback moth antennae depending on the olfactory stimulus they receive (Zhang et al., 2008, 2009c). Polyamines and DFMO are both likely to affect the expression of olfactory related genes that regulate the sensitivity of diamondback moths to odours.

The current study indicates that the effect of polyamine treatment on the expression of olfactory related genes relies on the sex of the moth. For example, put and spd decrease the expression of *PxylGOBP2* in 0-h male moths, but significantly increased the expression of the same gene in 0-h female moths. Furthermore, the effect of treatment on the expression of olfactory related genes also depends on the mating status of the moth. Similarly, our previous study also found that the effect of polyamines and DFMO on antennal sensitivity to general odours also depends on the sex and mating status of the moth (Zhang et al., 2008). Furthermore, untreated mated and unmated females both exhibited strong EAG responses to general odours, but treated mated and unmated females showed different EAG responses to the same odours (Zhang et al., 2008). Studies have shown that mating status affects the behavioural responses of female moths to host plant volatiles. For example, Rojas (1999) showed that mated females of *Mamestra brassicae* (Lepidoptera: Noctuidae) flew upwind to allylisothiocyanate and landed on the odour target, whereas virgin females did not. Similar phenomena are recorded for adult female *M. sexta* (Mechaber et al., 2002). Taken together, these results suggest that mating-status can affect the polyamine content of an insect, which affect the expression of olfactory-related genes and subsequently alter EAG responses and the behaviour of diamondback moths.

In insects, five kinds of protein determine their sensitivity to chemical information. These are the odorant-binding proteins (OBPs), the chemosensory proteins (CSPs), olfactory receptors (ORs, which contain nerve membrane protein), odour-degrading enzymes (ODEs) and sensory neuron membrane proteins (SNMPs) (Hu et al., 2013). In the current study only polyamines and DFMO had a significant effect on the expression of some olfactory related genes (GOBP1, GOBP2, PBP1 and CSP1) in the diamondback moth, *Plutella xylostella.* To determine whether polyamines and DFMO regulate insect olfactory detection by affecting the expression of olfactory related genes, more genes need to be studied.

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