Supporting Information

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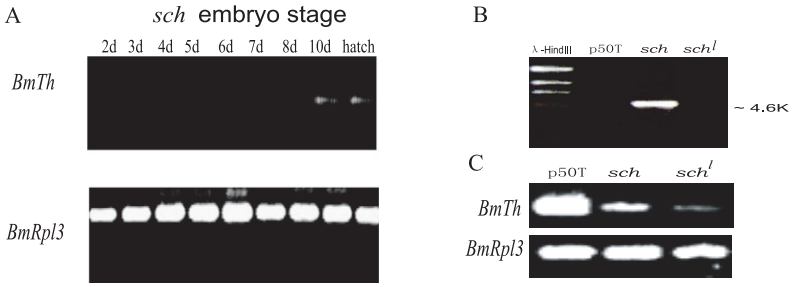

Fig. S1. (*A*) Expression profile of *BmTh* in *sch* embryos with semiquantitative RT-PCR; *BmRpl3* was used for normalization. (*B*) PCR amplification of the ~4.6-kb mariner-like transposon which replaced the ~70-kb sequence upstream of the *BmTh* gene in *sch* with the primer sets sch_d_10k-1F and sch_d_70k-0R (Table S4). PCR condition was 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 5 min, with a final extension at 72 °C for 10 min. (*C*) Expression level of *BmTh* in p50T, *sch*, and *sch*^{l} using semiquantitative RT-PCR.

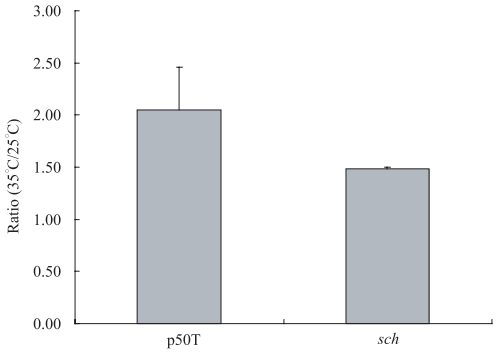

Fig. S2. Ratios of the relative expression level of *BmTh* under the condition of 35 and 25 °C in p50T and *sch* mutant, respectively. The cDNAs were from the p50T and *sch* embryo, which were incubated at 35 and 25 °C for 50 h from the sixth day after ending diapause, respectively. The real-time PCR condition was 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The relative expression level of *BmTh* was compared with *BmRpl3* in p50T and *sch* mutant. The ratios were compared with the relative expression quantity of *BmTh* at 35 and 25 °C (n = 3).

Table S1. Primers for the SNP markers used for constructing the *sch* linkage map

Primer name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
01030	ACATACGTTCTACCTCGTAACGC	TACAGAACATTTTGACACATTACACA
01031	GGTCAATAACGCTGTTTCCTG	TTAATCTCACGTGGCTGCAC
01035	AGACAGGTATGTAGGTACGCCA	CATCCACTTGACAAACGTCG
01017	TGCACACTCAATGAGCGACT	TGTGTGTGATCGTAATGTGAATTT
01022	TGCTGCAATTATAAAGTAGACAATGA	CAGCTTGAGAATTGTTTTCAAAT
01023	CGACAATCTTAAAGTTATCATTTCATT	CGGCCATTCTCTGAATTTCT
01053	CCGACGCTCATCATGACTAT	GGCAGTACTCACCTATCTCAAACA
01020	GGACGATCGTTCTTCGATTT	CGCACGGATCACTTGTATTT
01013	TTGGACACGTAGCTTGACCC	CAAAATCAGAAGTTTCCGCA
R6F1	TACACGATCCTTCACCTTGC	ACTAGTCCCACATACCACGC
R9F1	TCTTGACAGTTCAGTAATAGTTGG	TGTGTTTCGGTAATCGTCC
R9F20	AAGAAAACATTACACACACTACATACC	TCGCTCATTCTACTTTAGTGC
R12F1	AACGATTTATGAAACATGCG	TCATCGTCTATCGGTCTTACC
R12F16	ATGCAAAACACAACTCACG	TGTGCTCAAGCAAATAGTCG
R13F20	AATTGCCGACTCTAATTTGC	CGTAGCTTAAAAGATAGGGCG
R16F1	TACCAAGAGCTAATACCGCC	TAAACGCTTCACTCTCCTCC
R18F6	TGGCATCATACTCAATTTGC	CGAAGATACTAGGTTTCACGC
sch-R1-F21	TAGTGTAGCTTTGGCAACCG	ACGATACCAACTATCCCTGG
sch-R2_F1	CGGAACATTTTATTTATGTTTGC	TGCTTCTCTTTCTATCTTCGC
sch-R8-F17	CCATCAGCGGTAGTTATACG	CAGTGAAATCCTAAGTCGCC
sch-2-R11_F1	AGACTTTCATTTTTCGGTGG	ATAATTTGTTGTTGGCACCC
sch-2-R18_F22	AGTTATCCCATGACGTAGCC	ACAAATGCCTGATCTTTGC
sch-R14-F19	ACGGACCCTTGTATTAGACG	CAAACATTCATTCAGCAAACC

Table S2. Linkage analysis of BC1 segregants

		SNP marker primer sets (F/R)										
Sample ID	Phenotype	R9F20	*sch*-R1_F21	*sch*-R2_F1	*sch*-R8_F17	*sch*-2_R11_F1	*sch*-2_R18_F22	*sch*-R14_F19	R12F1	R12F16	R13F20	R16F1
sch-080124–18-151	*sch*/+	A	A	B	—	B	B	B	B	B	B	B
sch-BF1-07_C17	*sch*/+	A	A	H	—	H	H	H	—	H	H	H
sch-BF1-07_L09	*sch*/+	A	A	H	H	H	H	H	H	H	H	—
sch-BF1-04_L18	*sch*/+	A	A	H	H	H	H	H	H	H	H	—
sch-080124–18-180	*sch*/+	A	A	H	H	H	H	H	H	H	H	—
sch-080124–19-175	*sch*/+	B	—	B	B	B	B	B	B	B	B	A
sch-080124–20-148	*sch*/+	B	—	B	B	B	B	B	B	B	B	A
sch-BF1-02_J15	*sch*	H	H	H	A	A	A	A	A	A	A	—
sch-BF1-08_E17	*sch*	H	H	H	A	A	A	A	A	A	A	—
sch-BF1-02_P11	*sch*	H	H	H	A	A	A	A	A	A	A	—
sch-BF1-03_I01	*sch*	H	H	H	A	A	A	A	A	A	A	—
sch-080124–20-22	*sch*	A	—	A	A	A	A	A	A	A	A	H
sch-080124–19-121	*sch*	A	A	A	A	A	A	A	A	A	H	H

A, *sch* homozygous; B, p50T homozygous; H, heterozygous genotype of p50T/sch.

Table S3. Primers used to sequence *BmTh* cDNA and genes in p50T, *sch*, and *sch^{l}*

Primer name	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
*BmTh*_cDNA_Prime_1	TTTCCACACACCTGAACCTGACTG	GGAGCCCCAGGCAAATGAGC
*BmTh*_cDNA_Prime_2	CGCCCGAGCCAATGACTCTG	GGGCATCAAATCAAGCACGGT
*BmTh*_cDNA_Prime_3	GACCAGGAGTACCAGCCCATCTATT	GTCAGCGTATAATCCTCGTCACCC
*BmTh*_cDNA_Prime_4	TCCTACAGTATCGAGAACGGTTACCC	CGTGAATACAGTCAGGTTCAGGTG
*BmTh*_cDNA_Prime_5	AATGGTCACCTCGCTGATGCTG	TTGACACCCAGCGTCTGAATTTG
*BmTh*_cDNA_Prime_6	ATGCGAAGGGTGACGAGGATTA	GTGAGCCCTTGAGCTTCTTAACG
*BmTh*_gene_Prime_1	CATGAATGGTGACGTGGGTTAA	CGATTATGGAATGTAACGGTCTCAA
*BmTh*_gene_Prime_2	GCATTTGAGACCGTTACATTCCA	CGACCACCAGCAACCCTCAA
*BmTh*_gene_Prime_3	TGGTAAGGGTTGAGGGTTGCT	CAACTGGATTCATCTCCTGCTTTC
*BmTh*_gene_Prime_4	GGTCCTTCCCGTTGCCTGTC	CCCGAGGTTTGATTGATGGACTT
*BmTh*_gene_Prime_5	TCATAATGCGCTTTGCGATAACTAG	CCAAGGAACGACGGCGAGAT
*BmTh*_gene_Prime_6	ACGCAGGTCGGGACAAACTCT	CAAGCGAGGCGAGGAAGTCA
*BmTh*_gene_Prime_7	CCATCTCGCCGTCGTTCCT	ACGTTCCACGCACGCCAAC

Table S4. Primers used to detect the deletion region in *sch* mutant

Primer name	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
*sch*_2_R11_F1	TGTCCATTCGAAAAGAATGC	TACTTAACACCGGTTGAGCC
*sch*_2_R11_F25	ACAGAATATGGTCGAAATAAAGG	GTTTTATTGAAAAATATGTCTTATTGG
*sch*_2_R12_F20	TATCTCAATCTGTTGGTGCG	CCTGAAGTCGATCACTAGTCC
*sch*_2_R13_F1	ATGCACCATGTATATGCACC	TGTTAATATAATAAAATCCCATTTCG
*sch*_2_R13_F11	CGTATCTGTCCAGTTTTGAAGG	TGCACATAACACTTTCTGGC
*sch*_2_R14_F1	AAAGCTACATGTTGGACTCG	CGCATTAAGCACATAGAAGC
*sch*_2_R14_F20	TTAATTTTGACAAACCTGCG	GTGTTTGCTTCAAAATACGC
*sch*_2_R15_F1	TTCAAAATTATTATGGGGGC	GATCGTAATGGTTCCGATAGG
*sch*_2_R15_F22	TCACAATCTGTTCTTTTCTGC	ATGAGACTACAAAATTAAATGATGG
*sch*_2_R16_F1	TTTCGGCTATGAATATCTGC	AGACCTTAGCCTGAATGTGC
*sch*_2_R16_F26	ATTTTTAACAAAAAGAGTTACCTCAGC	CCGTAAATACAATTTTCGTCC
*sch*_2_R17_F1	AAAAAGGTCGTCTCTTCCG	TAACAAGCTTTCGAGGAACC
*sch*_2_R17_F15	CAGTTATGACGGCATCTAGG	TATGTTCTGGAAATTCGTGG
*sch*_2_R18_F1	ATCTTATAACGACTTATCAATCTTAGG	GCTGTTGTCATAAGCTCGG
*sch*_2_R18_F22	AGTTATCCCATGACGTAGCC	ACAAATGCCTGATCTTTGC
*sch*_R8_F1	AATCGATTATTAAGATCGCGTGG	TGTTTTACATTTGTGTCGGG
*sch*_R8_F17	CCATCAGCGGTAGTTATACG	CAGTGAAATCCTAAGTCGCC
*sch*_R9_F1	GATGAAGTCATTCGTTGTTCC	AAAAAGCTCGAATCTTGTGG
*sch*_R10_F1	TGCGCAGGATATACTAAACG	TGTACGAAAGTAAATAAAAGACGC
*sch*_R11_F1	AGACTTTCATTTTTCGGTGG	ATAATTTGTTGTTGGCACCC
*sch*_R11_F18	AAAAGATAAATTCACTATAACTGCG	GGCGTGAGGTTGTTTACC
*sch*_R12_F1	AAAACAATGCCAGGGAGG	TATTCGAAATTAGCTGTGCC
*sch*_R13_F1	GACCATACTTAATCATTTTTCGC	TGTTGTTCGATTCGTTCTCC
*sch*_R13_F25	TTGTTGGACCAGAATACCG	GCGTCCATAGAATAATAGCG
*sch*_R14_F1	TAAATCGCAACATAAATGCC	ATTACGACAGAAACACTGCG
*sch*_d_10k-1	TTCTACTATGTGGAAAACTATCAAAG	TCCGGTTGGGGATCTAGAATTT
*sch*_d_10k-2	TCTGGTAATCTTTCTACGTATGTTT	TTTCATCAATCTGTATCTTTGTGTT
*sch*_d_20k-0	TTCATTAGAGTCCGCAACAGC	TTGAAAGGGCGGTGGATT
*sch*_d_20k-1	ACGCCAGAAAGTGTTATGTGC	TGGTAAAAGAAGCGGTAGAGC
*sch*_d_20k-2	GTGTTCTTGCTGGGGTTAGTG	GGATGCTGAAGGAAGGTAAGA
*sch*_d_30k-0	TAGTTTGAGCCATTGGGTGTTT	TTCGGCGTGACTGAAACCT
*sch*_d_30k-1	TCCACATCCTACCACAACATT	TCAGCGCAACAAAGTTAAATC
*sch*_d_30k-2	TCTCATATGTCGCTAAGCCAG	AGTCATCCGTGAGCACTTATTT
*sch*_d_30k-3	TACCATATACGGAACAACAGGC	GACCTTCCTATTAGATGAGAGCA
*sch*_d_40k-1	CGAGTGAAGCAACTACTGGATAA	CATCAGGTAACCGCACATTGT
*sch*_d_40k-2	GCCTGAAATATGAACTCGGAC	GCATTAAACGCATCGAAAACT
*sch*_d_40k-3	TTACCAACCCCTGCTCATACA	AAAGTTTGTTCAATGTTAATCCG
*sch*_d_50k-0	TGACTCGGTGAGGGCAAAC	GAGCATCAGGTAACCGCACA
*sch*_d_50k-1	AATTTAGACGTATTTTACCGCC	TATCACAATAATCGATTTACGTCC
*sch*_d_60k-0	TCCTGTCAAGCGGATGCC	GGCTGCGTCTCATCGCTTAC
*sch*_d_60k-1	CTATCAGCATTGGTAGCGGG	GAAAAATACGGGAAAATGGC
*sch*_d_70k-0	AATCCCAGAACAGAGCCAAGC	CTTTGGGTGGCTGCTCATC

Table S5. Primers used in reverse PCR to identify both ends of the insertion sequence in *sch^{I}*

Primer name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
sch^{I}-081210–5-1	ACTATAGCTAACTAAACTTTGGATTGCA	GCAGACAGACAGGCACAAGC
sch^{I}-081210–5-2	GCAAACAGACCAATGCATGATG	CGAGACCAAAAGCTCTCGCA
sch^{I}-081210–3-1	GATGATTTTTTGTTGTTTTAG	AAAAATTTGTAGTGCTAAGGACCTC
sch^{I}-081210–3-2	TGCAGTTACTTTTTTTAGAAATTGG	TGGTAAGGGTTGAGGGTTGCT

Table S6. RNAi results of *BmTh* dsRNA injection into the early egg of *pnd^{+}*

dsRNA concentration	*BmTh*-1-dsRNA (1,000 ng/μL)		*BmTh*-1-dsRNA (100 ng/μL)		*BmTh*-2-dsRNA (1,000 ng/μL)		*BmTh*-2-dsRNA (100 ng/μL)		EGFP dsRNA (100 ng/μL)
Plate No.	1_1	1_2	2_1	2_2	3_1	3_2	4_1	4_2	Control
Injection egg No.	48	48	48	48	48	48	48	48	96
Development egg No.	36	39	0	13	23	25	22	16	51
Chocolate color embryo No.	36	39	0	13	23	25	22	16	0
Black color embryo No.	0	0	0	0	0	0	0	0	51
Hatched No.	0	0	0	0	0	0	0	0	44

Table S7. Primers used to quantity *BmTh* gene expression in p50T and *sch* embryos before hatch with real-time PCR

Primer name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
BmRBL3-RF1	TCGTCATCGTGGTAAGGTCAA	TTTGTATCCTTTGCCCTTGGT
BmRBL3-RF2	CGGTGTTGTTGGATACATTGAG	GCTCATCCTGCCATTTCTTACT
BmTh-RF1	CGCCACGCTTCTGATCTCGATAAC	TTGACACCCAGCGTCTGAATTTG
BmTh-RF2	TTTCCACACACCTGAACCTGACTG	TGCACAGTCCGAACTCAACCGTAA