Supporting Information

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SI Text

A codling moth ortholog of *EF-1α* (LG5 in *Bombyx mori*), used as a reference gene for testing sex-linkage of selected codling moth genes by quantitative real-time PCR (qPCR), was cloned, sequenced (see Table S2 for its accession number) and used for BAC library screening (Table S3). Moreover, the codling moth BAC library was screened for an ortholog of the *Acetylcholinesterase 2* (*Ace-2*) gene (GenBank accession no. DQ267976), which is also autosomal in *B. mori* (Table S1). The *Ace-2* ortholog was used to evaluate the accuracy of qPCR for linkage testing. Subsequent FISH experiments with BAC clones containing either *Elongation factor 1 alpha* (*EF-1α*) or *Ace-2* and the Z-linked *Resistant to dieldrin* (*Rdl*) gene confirmed an autosomal location for both the *EF-1α* and *Ace-2* genes in the codling moth (Fig. S1 *A* and *B*).

Determination of sex-linkage by means of qPCR was first verified using the *kettin* and *Ace-2* genes that were already mapped to the codling moth Z chromosome and an autosome, respectively (Fig. 1 and Fig. S1*B*). The *kettin*-to-*EF-1α* gene dose ratio was about twice as high in males as in females, thus proving the Z-linkage of *kettin*. Conversely, results of quantitative analysis of the *Ace-2* gene did not differ significantly between males and females, which is consistent with the autosomal location of the codling moth *Ace-2* gene as confirmed by BAC-FISH. These results show that the qPCR is a useful and reliable tool for the gene dose based determination of sex-linked versus autosomal inheritance (Fig. 3, Fig. S2, and Table S4).

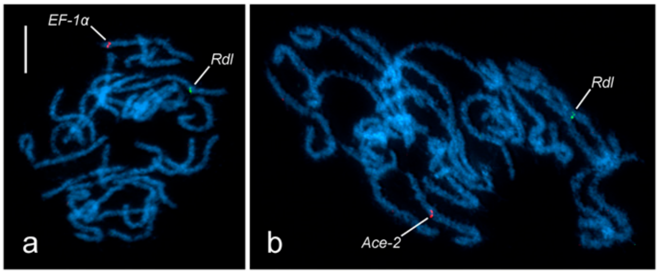

Fig. S1. BAC-FISH localization of two unmapped genes in comparison with a Z-linked gene on chromosome preparations of the codling moth, *Cydia pomonella*. Chromosomes were counterstained with DAPI (light blue). Hybridization signals of BAC probes (green and red) indicate the physical positions of loci marked by abbreviated names. (*A* and *B*) Pachytene spermatocyte complements. (*A*) Cohybridization of the BAC probe containing the Z-linked *Rdl* gene with BAC probe containing the *EF-1α* gene proved autosomal localization of *EF-1α*. (*B*) Sex-linkage of the *Ace-2* gene was excluded by cohybridization of BAC probe containing *Ace-2* with BAC probe in turn containing the Z-linked *Rdl* gene. (Scale bar: 10 μm.)

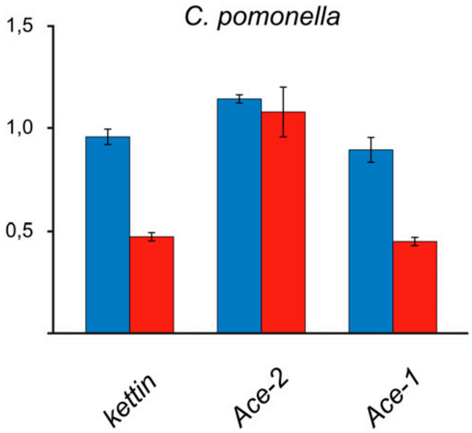

Fig. S2. qPCR determination of sex-linkage of the *Ace-1* gene in the codling moth, *Cydia pomonella*. Male (blue columns) and female (red columns) doses of *kettin, Ace-2*, and *Ace-1* genes normalized to the autosomal reference gene *EF-1α* are compared. A vertical bar at each column indicates the SD from three independent replicates. The *kettin* to *EF-1α* gene dose ratio was twice as high in males as in females due to the Z-linkage of *kettin* gene, whereas the relative dose of the autosomal *Ace-2* gene did not differ significantly between males and females. A twofold difference in the *Ace-1*-to-*EF-1a* gene dose ratios between males and females suggests Z-linkage of the *Ace-1* gene.

Table S1. ***B. mori*** **orthologs of genes isolated in this study**

Name	Symbol	Public ID*	Chromosome position*	Scaffold position*
Shaker	*Shkr*	BMgn003851	chr1: 20911282–20921258	Bm_scaf72: 1473937–1483913
lactate dehydrogenase	*Ldh*	BMgn012336	chr1: 17338625–17350610	Bm_scaf26: 2274069–2286054
Phosphogluconate dehydrogenase	*Pgd*	BMgn012298	chr1: 15112863–15127673	Bm_scaf26: 48307–63117
Period	*per*	BMgn000485	chr1: 12956618–13004501	Bm_scaf8: 7358664–7406547
Triosephosphate isomerase	*Tpi*	BMgn000559	chr1: 9023502–9027095	Bm_scaf8: 3425548–3429141
Resistant to dieldrin	*Rdl*	BMgn000568	chr1: 8060590–8089612	Bm_scaf8: 2462636–2491658
kettin	*ket*	BMgn000622	chr1: 6513219–6533895	Bm_scaf8: 915265–935941
ABC transporter family F protein ABCF2	*ABCF2*	BMgn002004	chr1: 4621452–4632826	Bm_scaf23: 4621452–4632826
apterous	*ap*	BMgn002127	chr1: 3487639–3516414	Bm_scaf23: 3487639–3516414
Ribosomal protein P0	*RpP0*	BMgn003309	chr15: 16146287–16150050	Bm_scaf42: 3154684–3158447
Acetylcholinesterase 1	*Ace-1*	BMgn003320	chr15: 15498774–15629164	Bm_scaf42: 2507171–2637561
Ribosomal protein L10	*RpL10*	BMgn003337	chr15: 14491197–14493497	Bm_scaf42: 1499594–1501894
mago nashi	*mago*	BMgn003398	chr15: 14026190–14028026	Bm_scaf42: 1034587–1036423
nanchung	*nan*	BMgn003369	chr15: 13344997–13360742	Bm_scaf42: 353394–369139
Isocitrate dehydrogenase 2	*Idh-2*	BMgn007586	chr15: 11046109–11053231	Bm_scaf66: 1438373–1445495
ABC transporter family C protein ABCC2	*ABCC2*	BMgn007793	chr15: 8949057–8952178	Bm_scaf3: 1002086–1005207
Ribosomal protein S5	*RpS5*	BMgn007710	chr15: 7586843–7587692	Bm_scaf3: 2366572–2367421
Notch	*N*	BMgn007929	chr15: 2349576–2409970	Bm_scaf3: 7544294–7604688
Elongation factor 1 alpha	*EF-1α*	BMgn003608	chr5: 17105811–17109595	Bm_scaf9: 615825–619609
Acetylcholinesterase 2	*Ace-2*	N/A	chr9	nscaf3045/nscaf3047

*KAIKObase version 3.2.2 (http://sgp.dna.affrc.go.jp/KAIKO; Accessed February 3, 2012).

Table S2. List of the partial sequences of tortricid genes obtained in this study

Species	Name*	GenBank acc. no.	Degenerate primer (forward)	Degenerate primer (reverse)
Cydia pomonella	*Shaker*	JQ771337	AAYGARTAYTTYTTYGAYAGRAA	ACRTGRTTRAARTTYTGNGAYTGCAT
	Ldh	JQ771341	GGNCARGTNGGNATGGC	CCDATNGCCCANGANGTRTA
	Pgd	JQ771338	GTNATGGGNCARAAYCTNAT	TGNCCNGTCCARTTNGTRTG
	Tpi	JQ771343	GGIAAYTGGAARATGAAYGG	CCICCIACYAARAAICCRTC
	Rdl	JQ771335	GAYTTYTAYTTYAGRCARTTYTGG	ATCCARTACATNAGRTTRAARCA
	kettin	JQ771344	AARGTIGAYACITTYGARTA	ATTGGGTATTATCGGAACG
	ABCF2	JQ771334	CARTGYGTNATGGARGTNGAYGA	GCRTCDATNGTYTCCATRTC
	apterous	JQ771339	GCNGTNGAYAGRCARTGGCA	CCAYTTNGCNCGNGCRTTYTGRAACC
	RpP0	JQ771358	ATGGGTAGGGAGGACAARGC	AGACCRAAGCCCATGTCGTC
	Ace-1	JQ771354	CGATACAAGGCATTCTGCCA	AAGTTTTGGTGCGCTAAGG
	RpL10	JQ771357	GACAAGCGTTTCWSYGGMAC	TTYCARATGAAGGTDYTGT
	mago	JQ771353	AAYTAYAARAAYGAYACNATGAT	TADATNGGYTTDATYTTRAARTG
	nanchung	JQ771346	CCNTTYGTNGTNATGATHTA	TANGTRTTNCCCATCATNGC
	Idh-2	JQ771360	GARATGGAYGGNGAYGARATG	RTGYTCRTACCADATYTTNGC
	ABCC2	JX258668	AARAGYCCNGTNTTYGGNATG	TTNRCNGTNGCYTCRTCCAT
	RpS5	JQ771355	GRTGGAGYTGYTAYGATGT	GAGTTWGATGARCCTTRGC
	Notch	JX307647	AAYAAYGCNGARTGYAAYTGGGA	ATYTGRAANACNCCCATNGCRTC
	EF-1α	JX258662	AARGARGCNCARGARATGGG	GCNACNGTYTGYCTCATRTC
Lobesia botrana	*Ace-1*	JQ771363	ACNGGNAARAARGTNGAYGCNTGG	GCRAARTTNGCCCARTAYCTCAT
	mago	JQ771369	AAYTAYAARAAYGAYACNATGAT	TADATNGGYTTDATYTTRAARTG
	Notch	JX258667	AAYAAYGCNGARTGYAAYTGGGA	ATYTGRAANACNCCCATNGCRTC
	EF-1α	JX258665	AARGARGCNCARGARATGGG	GCNACNGTYTGYCTCATRTC
Eupoecilia ambiguella	*Ace-1*	JQ771362	ACNGGNAARAARGTNGAYGCNTGG	GCRAARTTNGCCCARTAYCTCAT
	mago	JQ771368	AAYTAYAARAAYGAYACNATGAT	TADATNGGYTTDATYTTRAARTG
	Notch	JQ771361	AAYAAYGCNGARTGYAAYTGGGA	ATYTGRAANACNCCCATNGCRTC
	EF-1α	JX258666	AARGARGCNCARGARATGGG	GCNACNGTYTGYCTCATRTC

*For full gene names, see Table S1.

Table S3. List of the *C. pomonella* BAC clones mapped in this study

		Primers used for synthesis of hybridization probes		Primers used to confirm the presence of respective gene	
Gene*	BAC clone	Forward	Reverse	Forward	Reverse
Shaker	20G10	M13-26	M13-24	AGTCCAAGTTCTCGCATCGA	TACTCTGGCCACTGTGGTCG
Ldh	34N14	ATCGCCAGTAACCCCGTGG	CGCTGCTGTCTCCGTGTT	ATCGCCAGTAACCCCGTGG	CGCTGCTGTCTCCGTGTT
Pgd	03A23	M13-26	M13-24	TGCTAATGAAGCAAAAGGAACA	GCGCTGTGTGTCCATGTATT
period	23C16	ATAGACTTCGTCCACCCTTTG	CTGGATTTGCTGTCATTGTAGT	ACCTTCATACCCTTCCTGTTG	TAAAAGACGACCACTCCGTTT
Tpi	32P12	GGIAAYTGGAARATGAAYGG	ATIGCCCAIACIGGYTCRTA	CATTGGCGAGACCCTGGA	GTTCGTAGGCCAGCACCA
Rdl	23P13	M13-26	M13-24	AGGCAGTTCTGGACAGATCCACG	TGTATCGGATGTCCCGCATGGTG
kettin	33L16	GTCACAGGCAGACCTTACC	ATTGGGTATTATCGGAACG	GAAGCTGACGCGATTCGAT	TTAGGGGCTACCACTTGCT
ABCF2	25J19	M13-26	M13-24	CTCAAGACCAGCTAATGGACGTG	TCGTCCAGCAGTAGCAAGTGTGG
apterous	01K03	M13-26	M13-24	GCGGTGGACAGACAGTGGCA	GCCGGCAGTAGACCAGGTTG
RpP0	12O03	M13-26	M13-24	ATGGGTAGGGAGGACAAAGC	CCTTGATGAATTCCTTGATAG
RpL10	08A23	M13-26	M13-24	TTCTGGGAGACCAGCAGCAC	AACTTGATGGTGGCCTTGAC
mago	28B17	TGATCGGAGAGGAGCATATC	TAGATGGGCTTAATCTTGAAATG	TGATCGGAGAGGAGCATATC	TTTCAAATCCTGCACAAGGT
nanchung	40B18	CAGAATGGTGATGGGTGACTTGC	AGCTTCTATCTCGTGGTCGGTGC	CAGAATGGTGATGGGTGACTTGC	AGCTTCTATCTCGTGGTCGGTGC
Idh-2	12E19	M13-26	M13-24	CGCCTGATGAACAGAGAGTT	ATTTCCACCTTTCCAGGTTT
ABCC2	23H24	ACAATATCGGGCTTGTCCAC	TGTCCCACGGAGAAATTACC	ACAATATCGGGCTTGTCCAC	TGTCCCACGGAGAAATTACC
RpS5	32D15	GATGGAGCTGTTACGATGTC	TCGTCTGCGACGCACTCCGCG	GATGGAGCTGTTACGATGTC	TCGTCTGCGACGCACTCCGCG
Notch	19N22	M13-26	M13-24	CGGCCCGGACGGACAAGAGAT	ATGGACGCAGCAGCACCTTGA
EF-1α	09J15	M13-26	M13-24	TGATTACACTGTTTGGGGAGTC	TCCTTCATCTTGATTACTTCCG
Ace-2	11F21	AAGACAATGCGCGGGTATTTG	TCCTTCATCTTGATTACTTCCG	TGATTACACTGTTTGGGGAGTC	TCCTTCATCTTGATTACTTCCG

*For full gene names, see Table S1.

Table S4. Results of quantitative PCR

			Target-to-reference gene dose ratio, R							P	
Species	Target	Sex*	Sample I[†]	Sample II[†]	Sample III[†]	Mean ± SD	E_{target}[‡]	$E_{\text{reference}}$[‡]	Corrected mean ± SD[‡]	H_0 (1:1)[§]	H_0 (2:1)[§]
C. pomonella	*kettin*	M	2.05	1.80	1.96	1.94 ± 0.13	0.93	0.87	0.96 ± 0.04	0.001	0.798
		F	0.99	0.95	0.85	0.93 ± 0.07	0.93	0.87	0.47 ± 0.02	—	—
	Ace-2	M	2.15	2.24	2.21	2.20 ± 0.04	0.90	0.85	1.14 ± 0.02	0.523	—
		F	1.80	1.92	2.42	2.05 ± 0.33	0.90	0.85	1.08 ± 0.12	—	—
	Ace-1	M	2.23	1.92	2.24	2.13 ± 0.18	0.93	0.86	0.90 ± 0.06	0.004	0.841
		F	1.01	0.95	1.05	1.01 ± 0.05	0.93	0.86	0.45 ± 0.02	—	—
	mago	M	1.20	1.36	1.39	1.32 ± 0.10	—	—	—	0.464	—
		F	1.40	1.22	1.06	1.23 ± 0.17	—	—	—	—	—
	Notch	M	0.77	0.94	0.90	0.86 ± 0.11	—	—	—	0.005	0.757
		F	0.39	0.47	0.41	0.43 ± 0.06	—	—	—		
L. botrana	*Ace-1*	M	0.90	0.90	0.91	0.91 ± 0.01	—	—	—	0.001	0.145
		F	0.48	0.47	0.53	0.49 ± 0.03	—	—	—		
	mago	M	0.97	0.82	1.00	0.93 ± 0.10	—	—	—	0.788	—
		F	0.91	0.89	0.94	0.91 ± 0.02	—	—	—		
	Notch	M	1.27	0.97	1.00	1.08 ± 0.16	—	—	—	0.009	0.236
		F	0.49	0.34	0.50	0.44 ± 0.09	—	—	—		
E. ambiguella	*Ace-1*	M	1.33	1.40	1.31	1.34 ± 0.05	—	—	—	<0.001	0.514
		F	0.71	0.63	0.61	0.65 ± 0.05	—	—	—		
	mago	M	1.08	1.12	0.96	1.05 ± 0.08	—	—	—	0.001	0.309
		F	0.55	0.38	0.44	0.46 ± 0.08	—	—	—		
	Notch	M	2.11	2.35	2.27	2.24 ± 0.12	0.84	0.89	1.24 ± 0.06	<0.001	0.242
		F	1.05	0.94	0.88	0.96 ± 0.08	0.84	0.89	0.57 ± 0.04		

*M, male; F, female.

[†] Mean value ($n = 3$) in three independent samples (I–III).

[‡] If R was much higher than 1 in males, then it was corrected by the actual PCR efficiencies (E) calculated from the slope of the standard curve.

[§] Null hypothesis (H_0) of no difference (1:1) or a twofold difference (2:1) in the means between males and females was tested by unpaired two-tailed t test for unequal variances ($P > 0.05$ means no significant difference from the 1:1 and 2:1 ratios, respectively).

Table S5. **List of primers used for qPCR**

Species	Gene	Forward	Reverse
Cydia pomonella	*kettin*	ACCAGAAGGTACGTGGGCGA	CACGTTACCCGTGGCTTGGG
	Ace-1	CTGCCACATTCATGCGTTCA	ACCCAAAGCATAACAGCTGC
	mago	TGATCGGAGAGGAGCATATC	TTTCAAATCCTGCACAAGGT
	Notch	CAACGCCTTCCCCATCTTCAA	TTGTAACGGCGCAGAGGAAGC
	EF-1α	TACACTGTTTGGGGAGTCAGCT	TTCCCAATATCTTGAGCGCGT
	Ace- 2	CTGGTTCAAGGGATGGCAGA	ACCAATACCGCCGATTTTGT
Lobesia botrana	*Ace-1*	CCTGTTGAAAGTTGGGGAGACG	GGCCTGGGTCTAGGTGTGAC
	mago	CCCTTCTGGGTCGCGAGATTG	TGCACCCCTGCGTAATGGATG
	Notch	TCCAAGCATTCGCTATCGCC	GGGAACCATGTTATACCGG
	EF-1α	AGGTGCGAATACAACAATGG	GCAAGGCTGAAGGCAAGTG
Eupoecilia ambiguella	*Ace-1*	ACACTGCCTCATTCATGCGT	ACCCAGAGCATGACAGCTG
	mago	CGGAGACCTTCTGGATCACGGG	AGTTCACCAGAATTGCCCGTCT
	Notch	TCCAAGCATTCGCTATCGCC	GGGAACCATGTTATACCGG
	EF-1α	CGTTCCAATACCGCCGATTTTG	TTGGTTCAAGGGATGGAACGT