Supplementary Information

"The fate of W chromosomes in hybrids between wild silkmoths, *Samia cynthia* ssp.: no role in sex determination and reproduction"

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Supplementary Table S1 Hatchability of eggs in the control (C), parental (P) and F_1 crosses, and backcrosses (B_1) between *Samia cynthia walkeri* (SCW) and *Samia cynthia pryeri* (SCP)

Cross No.	Cross type	Crosses		No. of pairs	No. of eggs laid mean ± SD[#]	Egg hatchability (%) mean ± SD[#]
		female	male			
1	C	SCW	SCW	4	243.3 ± 44.2a,b,c	89.6 ± 7.1a,b
2	C	SCP	SCP	3	178.7 ± 39.6c	80.1 ± 4.0b
3	P	SCP	SCW	5	179.0 ± 72.9c	96.1 ± 2.0a
4	P	SCW	SCP	3	228.7 ± 11.9b,c	94.1 ± 4.6a,b
5	B_1	SCP♀ × SCW♂	SCW	3	362.3 ± 65.7a,b	8.8 ± 6.1e
6	B_1	SCW	SCP♀ × SCW♂	5	345.8 ± 22.5a,b	46.0 ± 6.7c
7	B_1	SCW♀ × SCP♂	SCW	3	232.7 ± 46.0a,b,c	50.1 ± 4.9c
8	B_1	SCW	SCW♀ × SCP♂	3	185.3 ± 88.7c	40.4 ± 4.9c,d
9	F_1	SCP♀ × SCW♂	SCP♀ × SCW♂	6	351.3 ± 38.8a	12.5 ± 4.9e
10	F_1	SCW♀ × SCP♂	SCW♀ × SCP♂	6	218.3 ± 47.7c	30.5 ± 7.5d

[#]Compared using one-way ANOVA followed by Tukey's multiple comparisons test; values in columns marked with the same letters are not significantly different ($P > 0.05$).

Supplementary Table S2 Sex chromosome constitutions in respective F_2 hybrid individuals produced by reciprocal crosses between *Samia cynthia* ssp.

Crosses: SCP♀ × SCW♂			Crosses: SCW♀ × SCP♂		
F_2 larva	Sex chromosomes	Type[a]	F_2 larva	Sex chromosomes	Type[b]
Male 1	neo-Z/Z/Chr13	expected g)	Male 1	Z/Z/Chr13/neo-W	unexpected l)
Male 2	neo-Z/Z/Chr13/W	unexpected k)	Male 2	Z/neo-Z/neo-W	unexpected k)
Male 3	neo-Z/Z/Chr13	expected g)	Male 3	Z/Z/Chr13/Chr13	expected h)
Male 4	neo-Z/neo-Z/W	unexpected l)	Male 4	Z/neo-Z/Chr13	expected g)
Male 5	neo-Z/neo-Z/W	unexpected l)	Male 5	Z/Z/Chr13/Chr13	expected h)
Male 6	neo-Z/neo-Z/W	unexpected l)	Male 6	Z/neo-Z/Chr13	expected g)
Male 7	neo-Z/neo-Z	expected h)	Male 7	Z/Z/Chr13/neo-W	unexpected l)
Male 8	neo-Z/Z/Chr13	expected g)	Male 8	Z/Z/Chr13/neo-W	unexpected l)
Female 1	neo-Z/Chr13/W	expected e)	Female 1	neo-Z/neo-W	expected e)
Female 2	neo-Z/Chr13	unexpected i)	Female 2	neo-Z/neo-W	expected e)
Female 3	Z/Chr13/Chr13	unexpected j)	Female 3	Z/Chr13/Chr13	unexpected j)
Female 4	Z/Chr13/Chr13/W	expected f)	Female 4	Z/Chr13/Chr13	unexpected j)
Female 5	neo-Z/Chr13/W	expected e)	Female 5	Z/Chr13/neo-W	expected f)
Female 6	Z/Chr13/Chr13	unexpected j)	Female 6	neo-Z/Chr13	unexpected i)
Female 7	neo-Z/Chr13	unexpected i)	Female 7	Z/Chr13/neo-W	expected f)
Female 8	Z/Chr13/Chr13/W	expected f)	Female 8	neo-Z/neo-W	expected e)

[a]Symbols e) to l) indicate the types of sex chromosome constitutions in Figure 3e-l.

[b]Symbols e) to l) indicate the types of sex chromosome constitutions in Figure 4e-l.

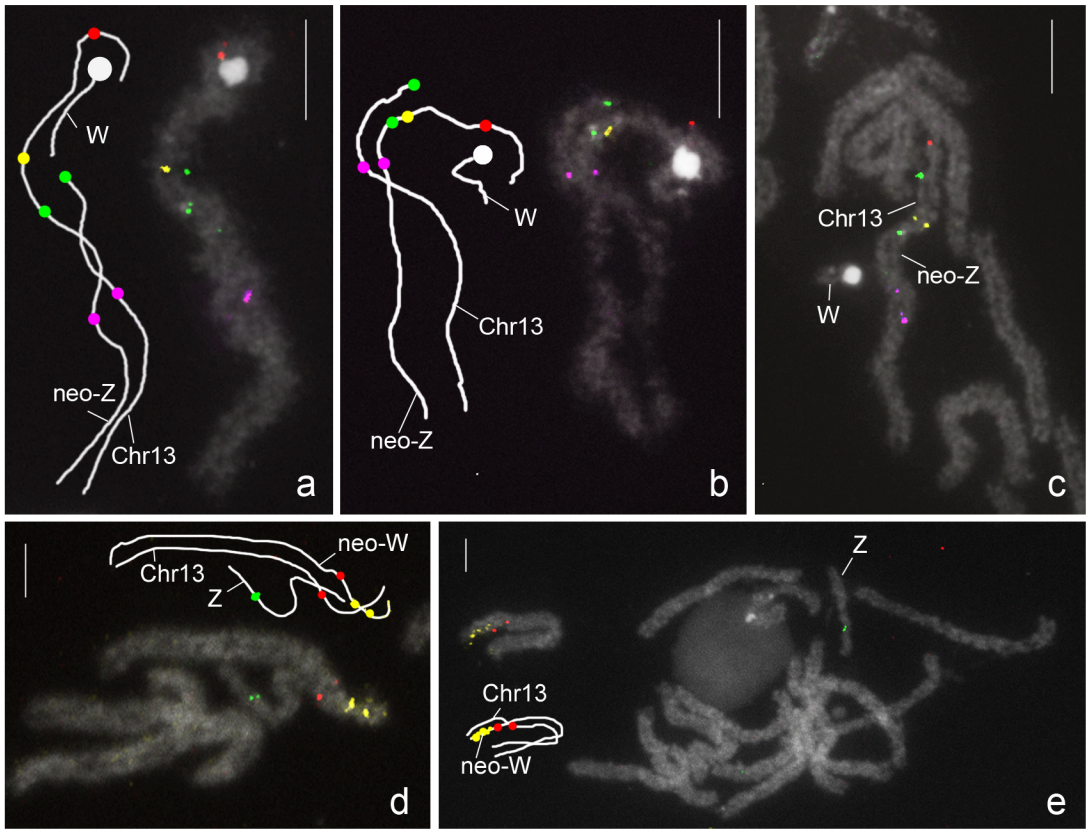
Supplementary Table S3 GenBank accession numbers of repetitive sequences derived from the *Samia cynthia pryeri* W chromosome and *GRP2* sequences of two *S. cynthia* subspecies (*walkeri* and *pryeri*) used in this study

Species	Symbol	Chromosome	Accession No.
S. c. pryeri	minor W-repeat (SCPW67)	W chromosome	LC033565
S. c. pryeri	major W-repeat (SCPW50)	W chromosome	LC033564
S. c. pryeri	*GRP2*	Chromosome 13	LC033563
S. c. walkeri	*GRP2*	neo-Z chromosome	LC033562
S. c. walkeri	*GRP2*	neo-W chromosome	LC033561

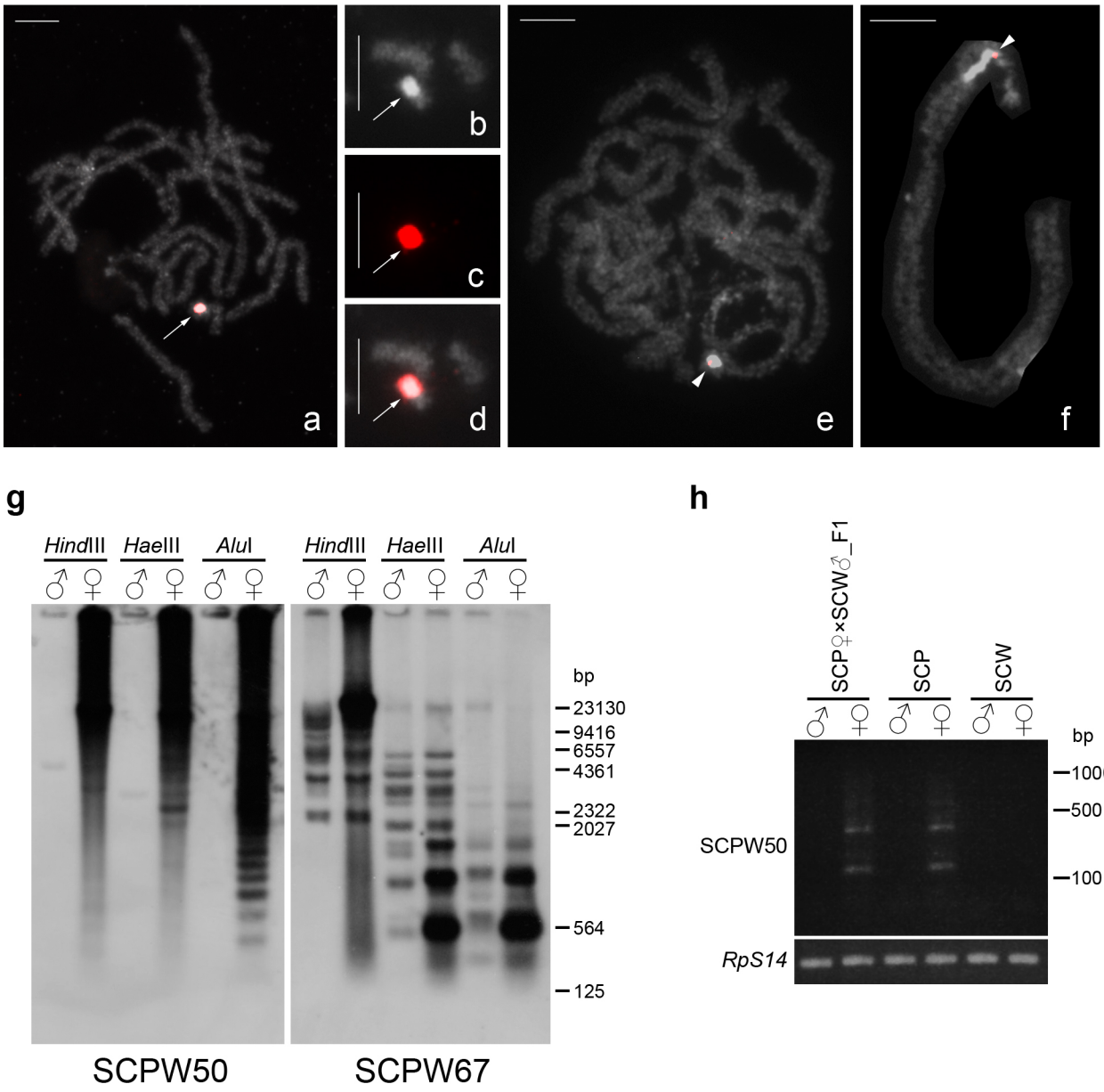
Supplementary Table S4 Hatchability of eggs and W (neo-W) chromosome genotype of parents in individual crosses between F_2 hybrids (data used in scatterplots presented in Figure 5)

Crosses between F_2 hybrids (SCP♀×SCW♂)				Crosses between F_2 hybrids (SCW♀×SCP♂)			
Pair No.	Egg hatchability	W chromosome[#]		Pair No.	Egg hatchability	neo-W chromosome[#]	
		female	male			female	male
1	0.4461	+	-	1	0.3691	-	+
2	0.4088	-	-	2	0.0192	+	-
3	0.0776	-	+	3	0.0000	+	+
4	0.2628	+	+	4	0.0047	+	-
5	0.2690	-	-	5	0.3624	-	-
6	0.2336	-	+	6	0.1554	+	-
7	0.0000	+	-	7	0.0101	+	-
8	0.4548	+	+	8	0.0046	+	-
9	0.0000	-	+	9	0.0000	+	-
10	0.2148	-	+	10	0.0000	-	+
11	0.0610	+	-	11	0.0971	+	+
12	0.0000	+	+	12	0.1370	-	+
13	0.3599	+	-				
14	0.1557	-	-				
15	0.0588	-	+				
16	0.0000	+	-				
17	0.7500	+	-				
18	0.0000	+	+				
19	0.3750	+	+				
20	0.0000	-	+				
21	0.0000	+	-				

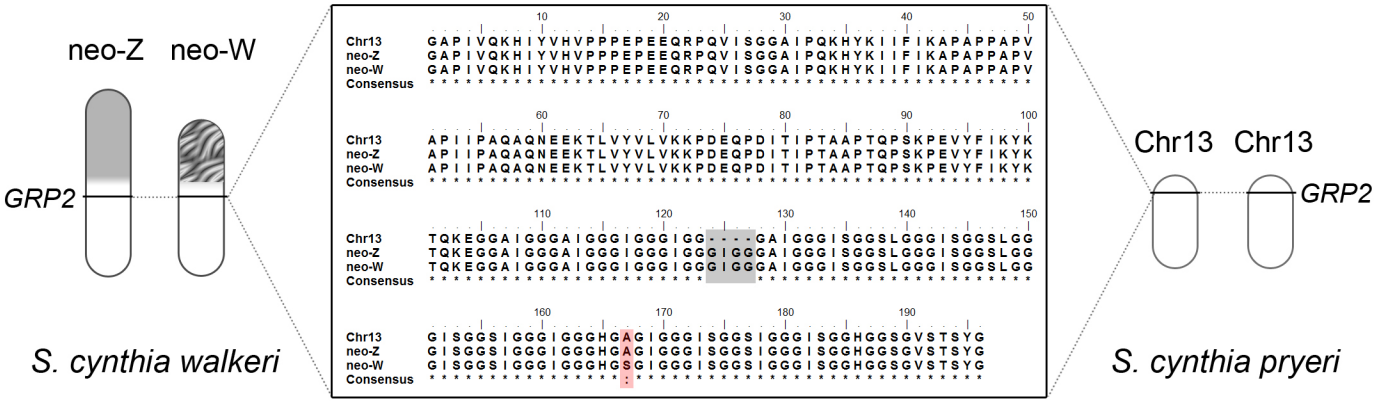
[#]The presence (+) or absence (-) of W (or neo-W) chromosome in respective F_2 hybrids.



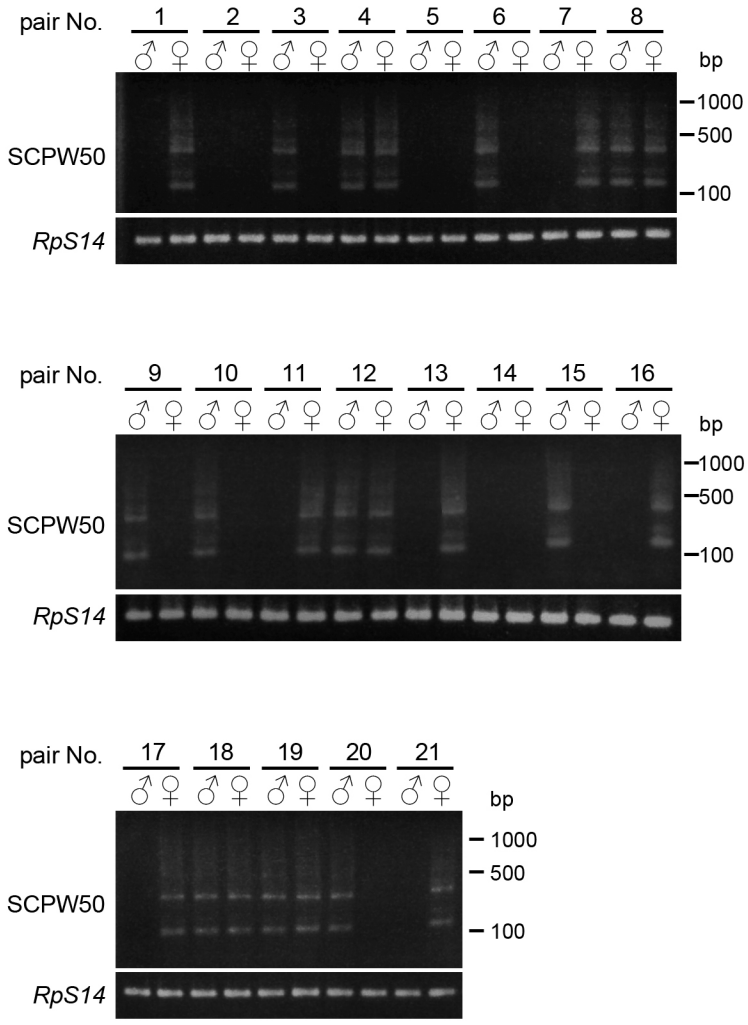
Supplementary Figure S1 FISH with sex chromosome derived probes in pachytene chromosomes of F_1 females from crosses between *Samia cynthia pryeri* females and *S. c. walkeri* males (**a**-**c**), and between *S. c. walkeri* females and *S. c. pryeri* males (**d**-**e**). Chromosomes were stained with DAPI (grey). (**a**-**c**) Orange-labelled probe of the 19B8 fosmid clone (yellow signals) and Red-labelled probe of the 45A6 fosmid clone (red signals) mapped to the ancestral part of the neo-Z chromosome, and Green-labelled probe of the 56J8 fosmid clone (green signals) and Cy5-labelled probe of the 56J22 fosmid clone (magenta signals) to chromosome 13 or the corresponding autosomal part of the neo-Z chromosome (for details about fosmid probes, see Yoshido *et al*., 2013). The *S. c. pryeri* W chromosome is easily distinguished by strong DAPI staining. (**d**-**e**) Cy3-labelled probe of the 32B23 fosmid clone (red signals) mapped to chromosome 13 or the corresponding autosomal part of the neo-W chromosome, and Green-labelled probe of the 45A6 fosmid clone (green signals) to the Z chromosomes. Cy3-labelled W-painting probe (yellow signals) highlighted the ancestral part of the *S. c. walkeri* neo-W chromosomes. Bar = 5.0 µm.



Supplementary Figure S2 (**a**-**f**) FISH with Cy3-labelled probes of the SCPW50 (**a**-**d**) and SCPW67 (**e**-**f**) clones derived from the *Samia cynthia pryeri* W chromosome DNA sequences. Bar = 5.0 µm. (**a**) The SCPW50 probe highlighted the W chromosome (arrow) in pachytene complement of *S. c. pryeri*. (**b**-**d**) A detail of the WZ bivalent: **b** DAPI image; **c** SCPW50 probe; **d** merged image. Arrows indicate the highly heterochromatic part of the W chromosome identified with the probe. (**e**) The SCPW67 probe hybridized to a small part of *S. c. pryeri* W chromosome (arrowhead) in pachytene complement of F_1 female of the cross between *S. c. pryeri* female and *S. c. walkeri* male. (**f**) A detail of a sex chromosome trivalent (W + neo-Z + chromosome 13) showing a discrete hybridization signal of the probe (arrowhead). In F_1 females, similar trivalents without the W ball-like structure were rarely observed (see Yoshido *et al*., 2013). (**g**) Southern hybridization with the SCPW50 (left) and SCPW67 (right) probes. Genomic DNAs of *S. c. pryeri* were digested using three restriction enzymes, *Hind*III, *Hae*III, and *Alu*I, respectively. Size specification, done with λ-*Hind*III digest, is shown by numbers (bp) on the right-hand side. (**h**) Gel showing results of PCR using a primer set designed according to the SCPW50 sequence. Respective genomic DNAs extracted from both sexes of F_1 hybrids of crosses between *S. c. pryeri* female and *S. c. walkeri* male (SCP♀×SCW♂_F1), *S. c. pryeri* (SCP) and *S. c. walkeri* (SCW) were used as templates. A partial sequence of the Ribosomal protein 14 (*RpS14*) gene was used as a positive control. 1000, 500, and 100 indicate molecular size markers (bp).



Supplementary Figure S3 Comparison of glycin rich protein 2 (GRP2) amino acid sequences derived from a part of the orthologous sequences of the *GRP2* gene obtained from the neo-Z and neo-W chromosomes in *S. cynthia walkeri* and chromosome 13 in *S. cynthia pryeri*. The subspecies sequences differ by an insertion/deletion at positions 124-127 (grey boxed). A non-synonymous substitution was identified at the 167 position of GRP2 (red boxed) derived from the neo-W chromosome GRP2 sequence.



Supplementary Figure S4 Gels showing PCR detection of the W chromosome marker in genomic DNAs of 21 pairs of F_2 hybrids from crosses between *Samia cynthia pryeri* females and *Samia cynthia walkeri* males using a primer set specific for the SCPW50 sequence. A partial sequence of the *Ribosomal protein 14* (*RpS14*) was used as a positive control. 1000, 500, and 100 indicate molecular size markers (bp).