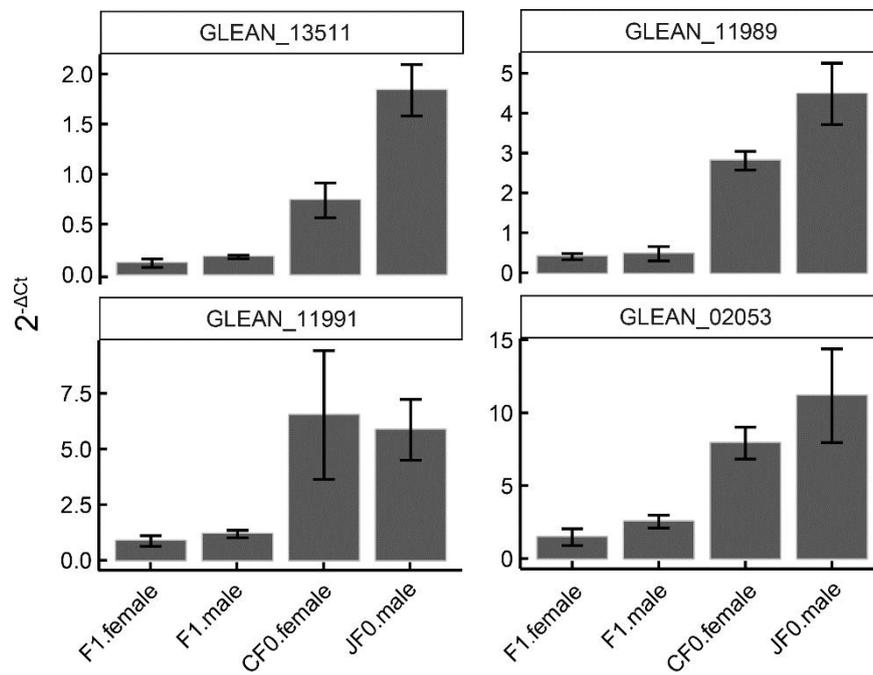
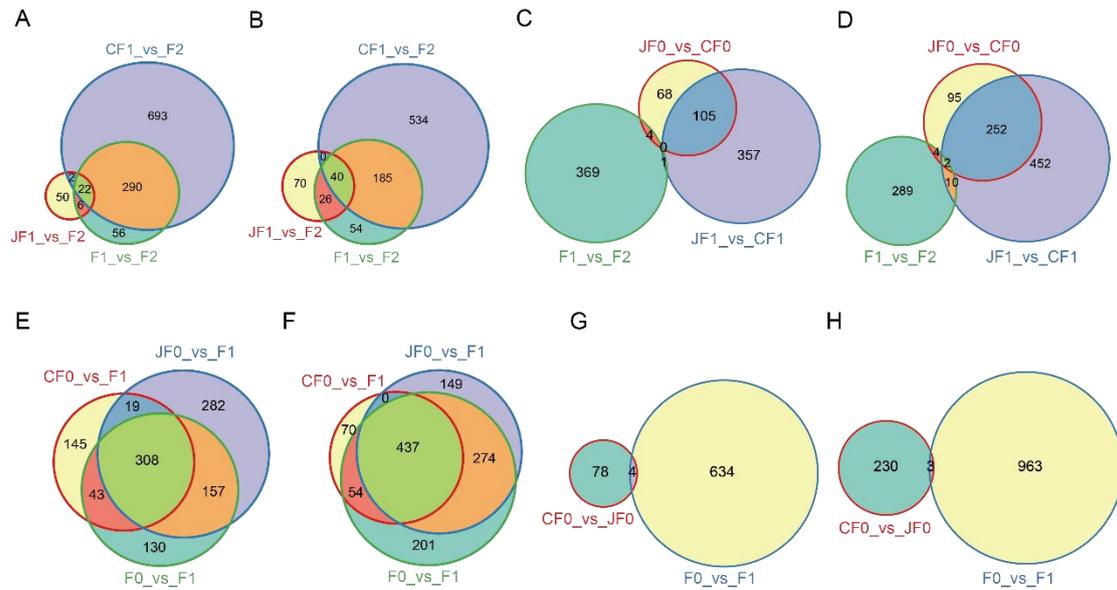


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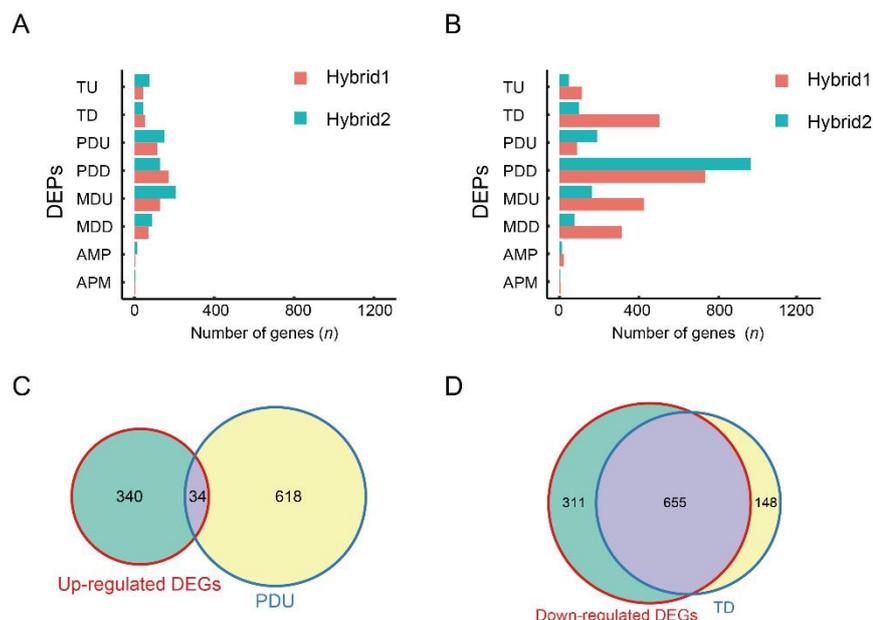


Supplementary Figure S1 Expression profiles of four down-regulated genes between F1 hybrids and their parents in binary hybrid system detected by qRT-PCR.



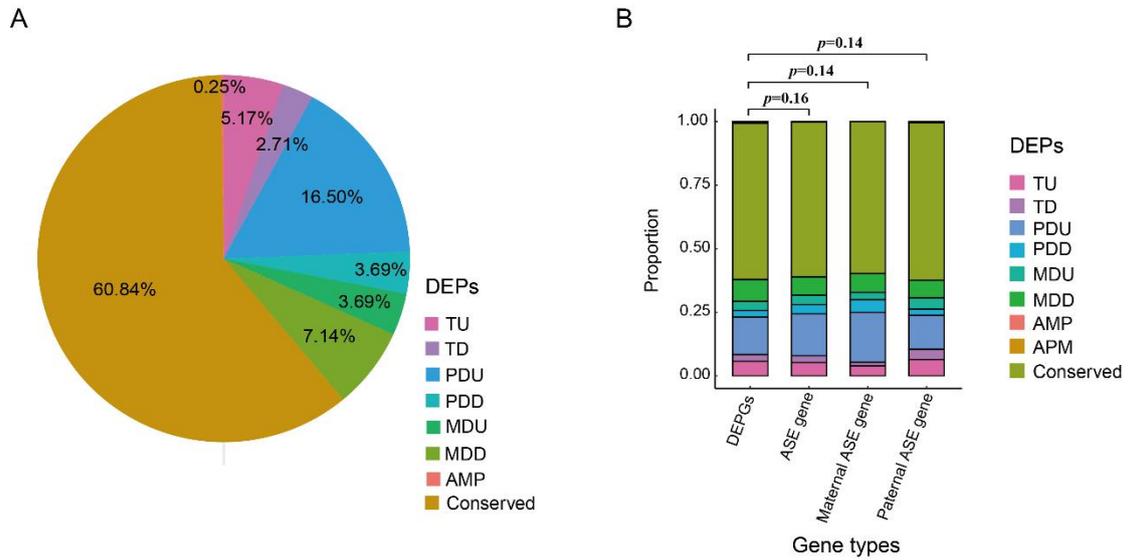
Supplementary Figure S2 Source of DEGs between parents and hybrids in two hybrid systems

A, B: Overlapping up-regulated (A) and down-regulated (B) DEGs between hybrid individuals and their parents (F1_vs_F2), maternal parent (JF1_vs_F2), or paternal parent (CF1_vs_F2) in quaternary hybrid system. C, D: Overlapping up-regulated (C) and down-regulated (D) DEGs between different varieties (JF0_vs_CF0, JF1_vs_CF1) and between hybrid individuals and their parents (F1_vs_F2) in quaternary hybrid system. E, F: Overlapping up-regulated (E) and down-regulated (F) DEGs between hybrid individuals and their parents (F0_vs_F1), maternal parent (CF0_vs_F1), or paternal parent (JF0_vs_F1) in binary hybrid system. G, H: Overlapping up-regulated (G) and down-regulated (H) DEGs between different varieties (JF0_vs_CF0) and between hybrid individuals and their parents (F0_vs_F1) in binary hybrid system.



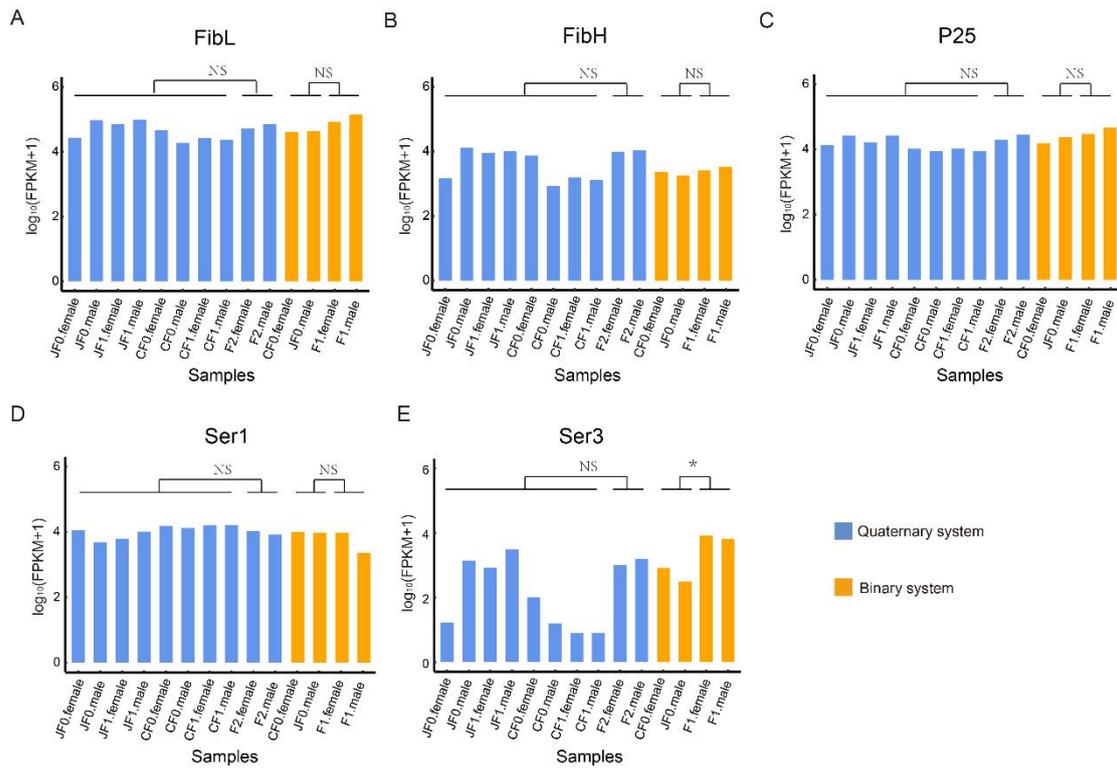
Supplementary Figure S3 DEPGs of inbred individuals and overlap between most abundant DEPGs and DEGs of hybrid offspring

A: Distribution of DEPGs in Japanese inbred individuals. B: Distribution of DEPGs in Chinese inbred individuals. C: Overlapping up-regulated DEGs and PDU genes in quaternary system hybrid offspring. D: Overlapping down-regulated DEGs and TD genes in binary system hybrid offspring.



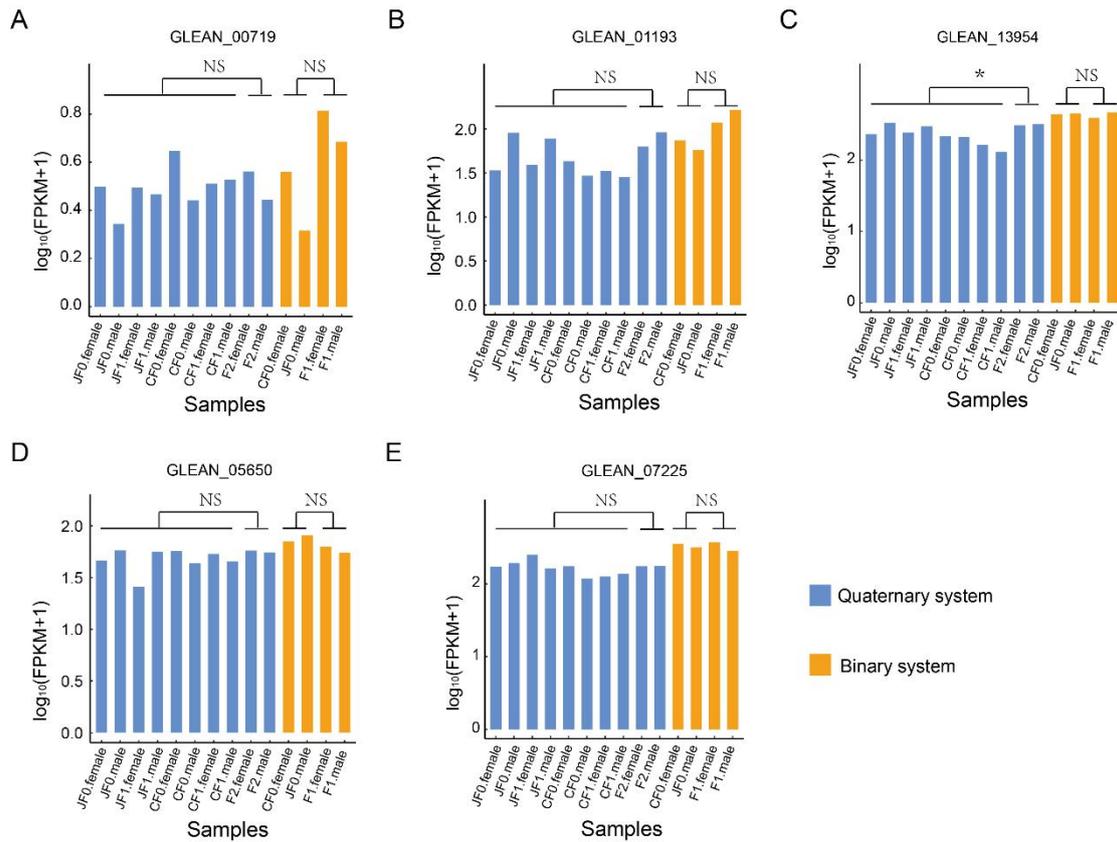
Supplementary Figure S4 ASE genes may be dispensable in hybrid vigor formation

A: Distribution of every DEPG in ASE gene set. B: Distribution of every DEPG in total DEPGs and three ASE gene sets. Significant differences are indicated as NS: not significant, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, chi-square test.



Supplementary Figure S5 Five key genes closely related to silk composition

A: *FibL*: fibroin light chain precursor. B: *FibH*: fibroin heavy chain precursor. C: *P25*: fibrohexamerin precursor. D: *Ser1*: sericin 1-like isoform X8. E: *Ser3*: sericin 3 isoform X1. Significant differences are indicated as NS: not significant, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, two-tailed t -test.



Supplementary Figure S6 Five key genes involved in glutamate and aspartate acid metabolic pathways

A: *GLEAN_00719*: glutamine synthetase 2 cytoplasmic-like isoform X1. B: *GLEAN_01193*: glutamate synthase. C: *GLEAN_13954*: glutamate dehydrogenase. D: *GLEAN_05650*: aspartate aminotransferase. E: *GLEAN_07225*: asparagine synthetase. Significant differences are indicated as NS: not significant, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, two-tailed t -test.

Supplementary Table S1 Statistics of whole-genome sequencing quality control and mapping results.

Hybrid system	Sample	Clean reads	Paired reads	Mapped paired reads	Mapped ratio
Quaternary	J1f (JF0)	48,230,399	46,488,384	39,468,508	84.90%
	J2m (JF0)	50,596,835	48,826,898	41,622,712	85.25%
	J1J2f (JF1)	45,644,207	44,029,204	37,172,206	84.43%
	J1J2m (JF1)	39,697,690	38,303,288	32,778,400	85.58%
	C1f (CF0)	46,590,694	44,882,564	37,081,342	82.62%
	C2m (CF0)	50,288,732	47,951,530	38,957,652	81.24%
	C1C2f (CF1)	42,296,628	40,729,030	34,831,612	85.52%
	C1C2m (CF1)	39,597,515	38,182,876	32,536,764	85.21%
	J1J2*C1C2f (F2)	53,344,752	51,424,514	43,986,836	85.54%
	J1J2*C1C2m (F2)	50,126,627	48,363,824	41,005,156	84.78%
Binary	C*Jf (F1)	50,968,274	49,828,096	44,280,160	88.87%
	C*Jm (F1)	48,397,367	47,336,276	41,695,946	88.08%

Supplementary Table S2 Statistics of transcriptome sequencing quality control and mapping results.

Hybrid system	Sample	Clean reads	Paired reads	Mapped paired reads	Mapped ratio
Quaternary	J1f (JF0)	45,049,910	39,672,226	26,678,436	67.25%
	J2m (JF0)	49,633,669	41,685,902	23,477,988	56.32%
	J1J2f (JF1)	52,892,637	46,108,750	29,259,524	63.46%
	J1J2m (JF1)	45,721,077	39,256,064	23,291,048	59.33%
	C1f (CF0)	93,479,243	62,852,526	53,276,988	84.77%
	C2m (CF0)	64,963,288	57,451,056	48,543,542	84.50%
	C1C2f (CF1)	78,090,335	59,488,036	49,456,110	83.14%
	C1C2m (CF1)	87,859,094	71,465,946	58,981,240	82.53%
	J1J2*C1C2f (F2)	42,861,687	37,150,644	22,343,474	60.14%
	J1J2*C1C2m (F2)	47,612,185	41,201,478	23,854,262	57.90%
Binary	Cf (CF0)	51,343,054	44,395,038	33,815,662	76.17%
	Jm (JF0)	49,147,700	43,498,564	33,122,658	76.15%
	C*Jf (F1)	51,911,562	44,204,456	31,468,410	71.19%
	C*Jm (F1)	46,731,446	38,908,412	27,752,124	71.33%

Supplementary Table S3 DEGs, DEPGs, and genes with DMRs.

Supplementary Table S4 GO enrichment for nine different gene sets.

Supplementary Table S5 KEGG enrichment for nine different gene sets.

Supplementary Tables S3–S5 are listed as separate excel files due to their large size.

Supplementary Table S6 Summary of DEPGs in hybrid offspring in two hybrid systems.

DEPs	Quaternary JF1	Quaternary CF1	Quaternary F2	Binary F1
TU	8(1.47%)	16(6.45%)	255(15%)	154(9.55%)
TD	39(7.18)	11(4.44%)	112(6.59%)	803(49.78%)
PDU	65(11.97%)	58(23.39%)	159(9.36%)	74(4.59%)
PDD	27(4.97%)	27(10.89%)	379(22.31%)	355(22.01%)
MDU	40(7.37%)	55(22.18%)	652(38.38%)	84(5.21%)
MDD	358(65.93%)	77(31.05%)	114(6.71%)	116(7.2%)
AMP	5(0.92%)	1(0.40%)	4(0.24%)	11(0.68%)
APM	1(0.18%)	3(1.21%)	24(1.41%)	16(0.99%)
Total	543	248	1,699	1,613

Supplementary Table S7 Distribution of each DEPG group in three ASE gene sets.

Type	TU	TD	MDU	MDD	PDU	PDD	AMP	APM	Conserved
ASE gene	21	11	15	29	67	15	1	0	247
Maternal ASE gene	8	3	6	15	40	10	0	0	122
Paternal ASE gene	13	8	9	14	27	5	1	0	125

Supplementary Table S8 Summary of silk gland whole-genome bisulfite sequencing of quaternary hybrid system.

Sample	Clean data (Gb)	Mapping ratio	C conversion ratio	CpG Covered percentage	Covered_CG depths
J1f (JF0)	13.52	39.80%	99.51%	65.74%	9.03
J2m (JF0)	19.26	41.90%	98.15%	66.50%	10.52
J1J2f (JF1)	16.86	33.10%	98.67%	65.83%	7.83
J1J2m (JF1)	19.45	37.50%	98.53%	70.76%	11.37
C1f (CF0)	16.35	43.40%	99.48%	65.76%	11.06
C2m (CF0)	16.30	45.90%	99.49%	66.78%	11.08
C1C2f (CF1)	17.93	45.00%	99.11%	67.62%	11.39
C1C2m (CF1)	17.39	45.60%	99.42%	67.46%	11.31
J1J2*C1C2f (F2)	18.55	43.40%	99.55%	73.80%	11.29
J1J2*C1C2m (F2)	17.94	42.30%	99.61%	73.92%	11.19