

Supplementary Information for

Intergenerational transfer of DNA methylation marks in the honey bee

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Figures S1 to S14
Table S1
Legend for Dataset S1

Other supplementary materials for this manuscript include the following:

Dataset S1

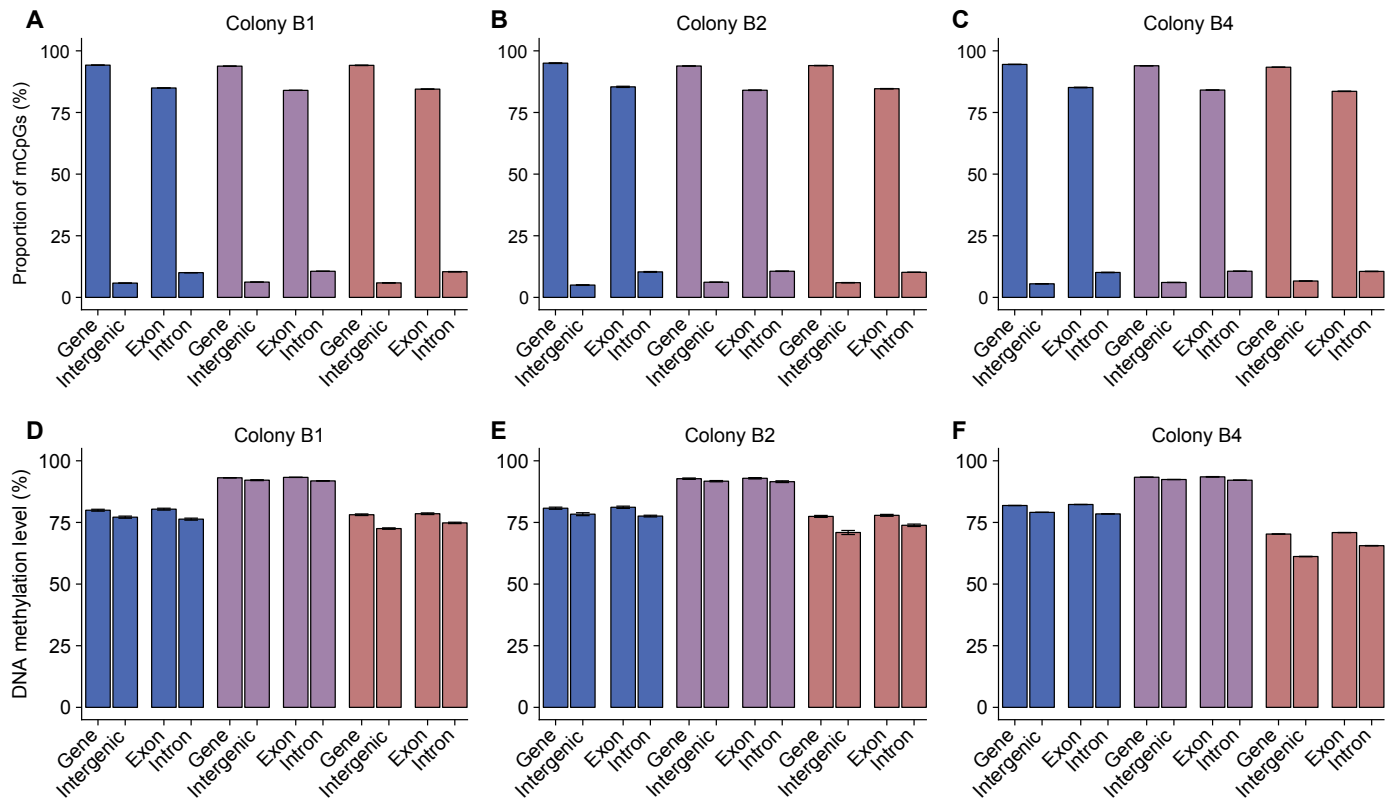


Fig. S1. DNA methylation patterns in various genomic contexts in drones, semen and workers. (A–C) Proportion of mCpGs (%) in various genomic contexts across all patriline for drone (blue), semen (purple) and worker (red) samples in colony B1 (A), B2 (B) and B4 (C). (D–F) DNA methylation level (% of C out of (C+T) at each CpG site) of mCpGs across all patriline for drone (blue), semen (purple) and worker (red) samples in colony B1 (D), B2 (E) and B4 (F). Bar plots represent mean \pm SE.

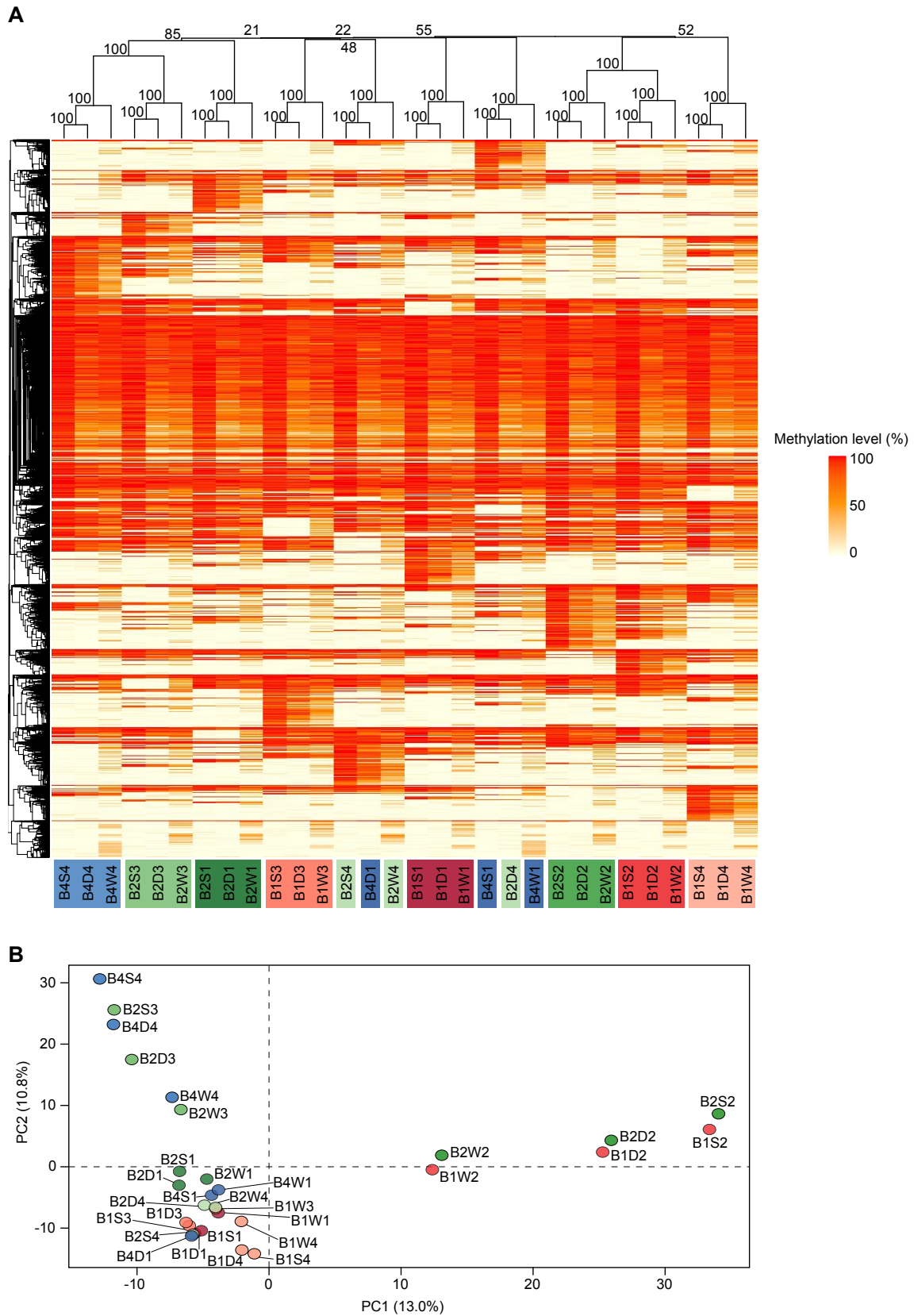


Fig. S2. Clustering of samples according to their methylation status across all colonies. (A) Hierarchical clustering (average agglomerative method on correlation distances) of methylation status of all shared mCpGs across all patriline and all colonies for drone (D), semen (S) and worker (W) samples. Bootstrap support values (%) are depicted above each node. Heatmap shows the level of DNA methylation for each site (ranging from 0%, yellow to 100%, red). **(B)** Principal component analysis (PCA) of DNA methylation level of all shared mCpGs across all patriline and all colonies for drone (D), semen (S) and worker (W) samples. Percentage of variance explained is depicted on each axis. Different colors represent different patriline.

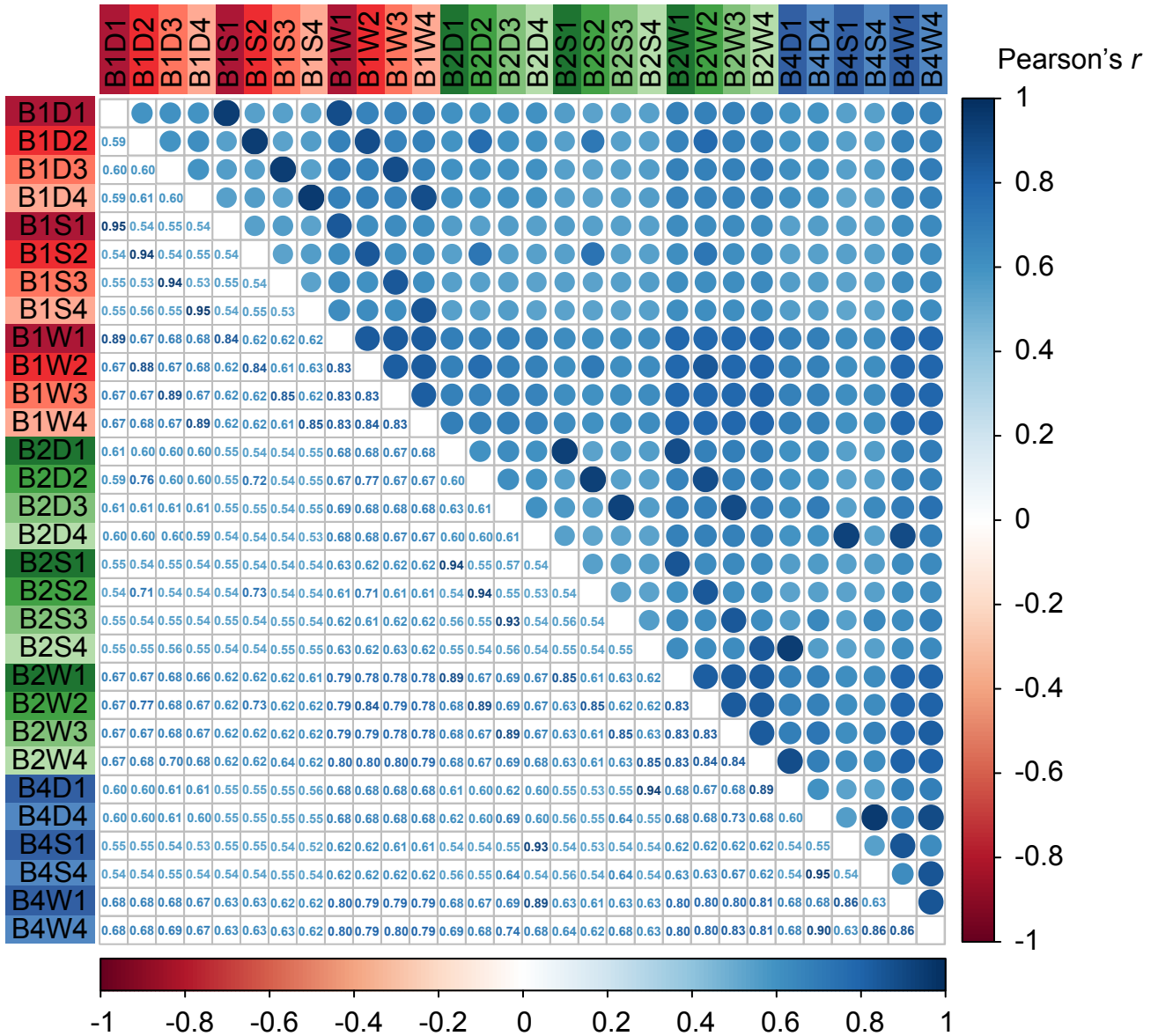


Fig. S3. Correlogram of methylation patterns between all samples across all colonies. Correlation (Pearson's r) of DNA methylation levels between all pairwise sample comparisons (all $p < 0.00001$). The color and size of the circles in the upper panel are proportional to the value of the Pearson product-moment correlation coefficient depicted in the lower panel (ranging from -1 , dark red to 1 , dark blue). Patriline groups are represented by different colors.

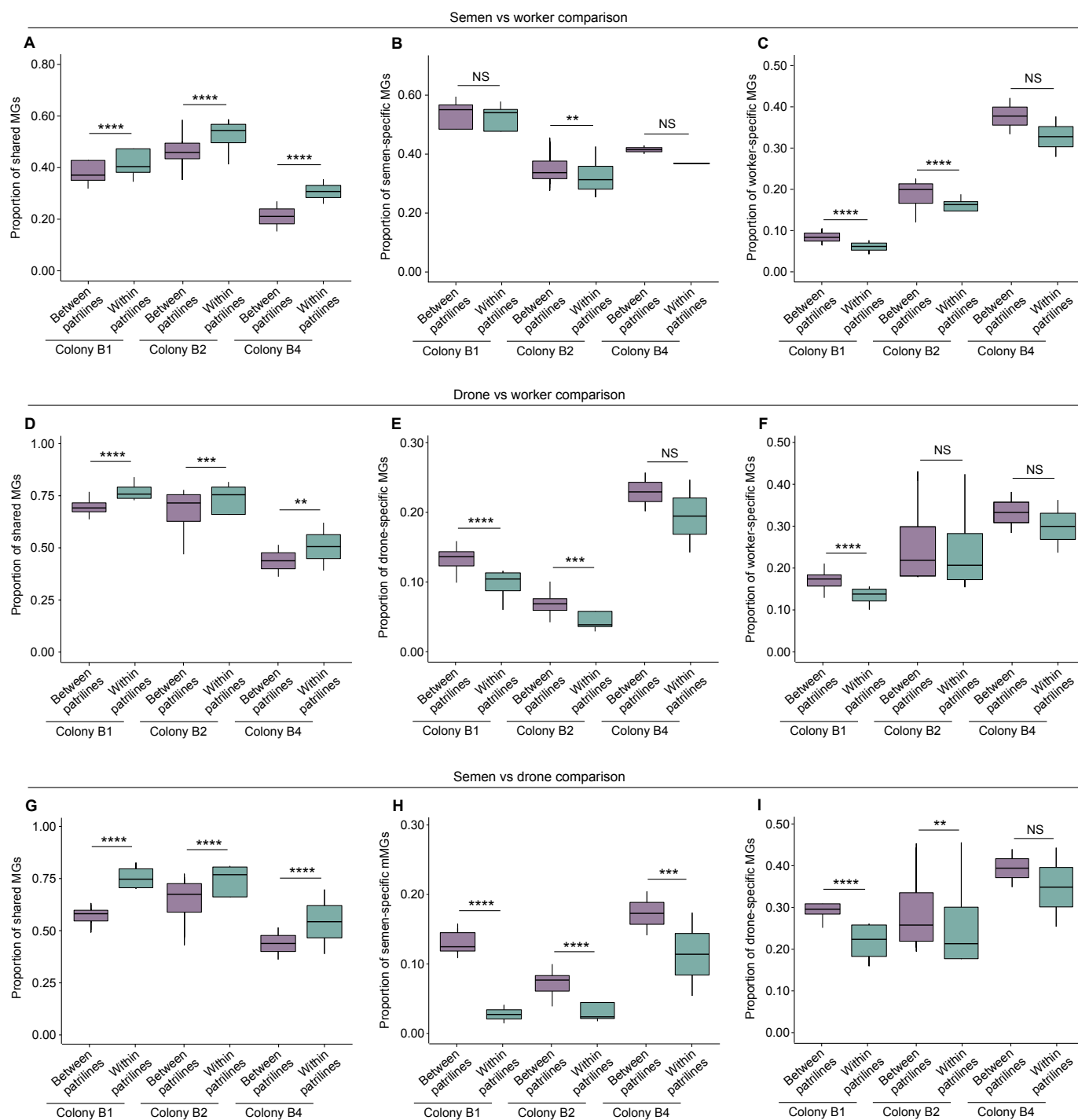


Fig. S4. Overlap of methylated genes (MGs) within and between patrines in colonies B1, B2 and B4. (A–C) Semen vs worker comparison showing the proportion of (A) shared MGs, (B) semen-specific MGs and (C) worker-specific MGs. (D–F) Drone vs worker comparison showing the proportion of (D) shared MGs, (E) drone-specific MGs and (F) worker-specific MGs. (G–I) Semen vs drone comparison showing the proportion of (G) shared MGs, (H) semen-specific MGs and (I) drone-specific MGs. Box plots represent median, interquartile range (IQR) and $1.5 \times$ IQR. GLMMs: NS, not significant; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

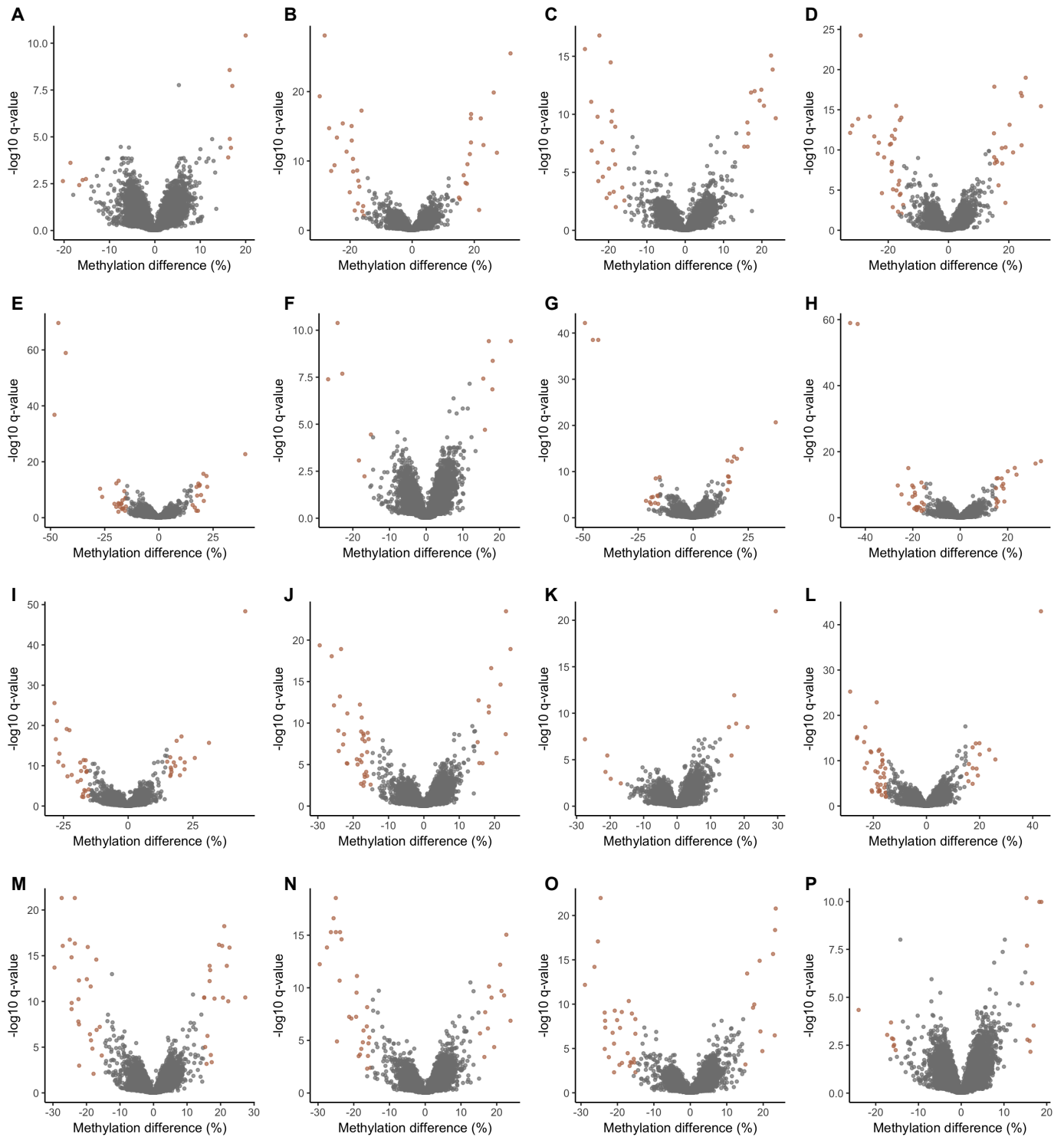


Fig. S5. Differential methylation between each semen and worker sample pairwise comparison in colony B1. (A–D) Semen sample from patriline 1 versus worker sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Semen sample from patriline 2 versus worker sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Semen sample from patriline 3 versus worker sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Semen sample from patriline 4 versus worker sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

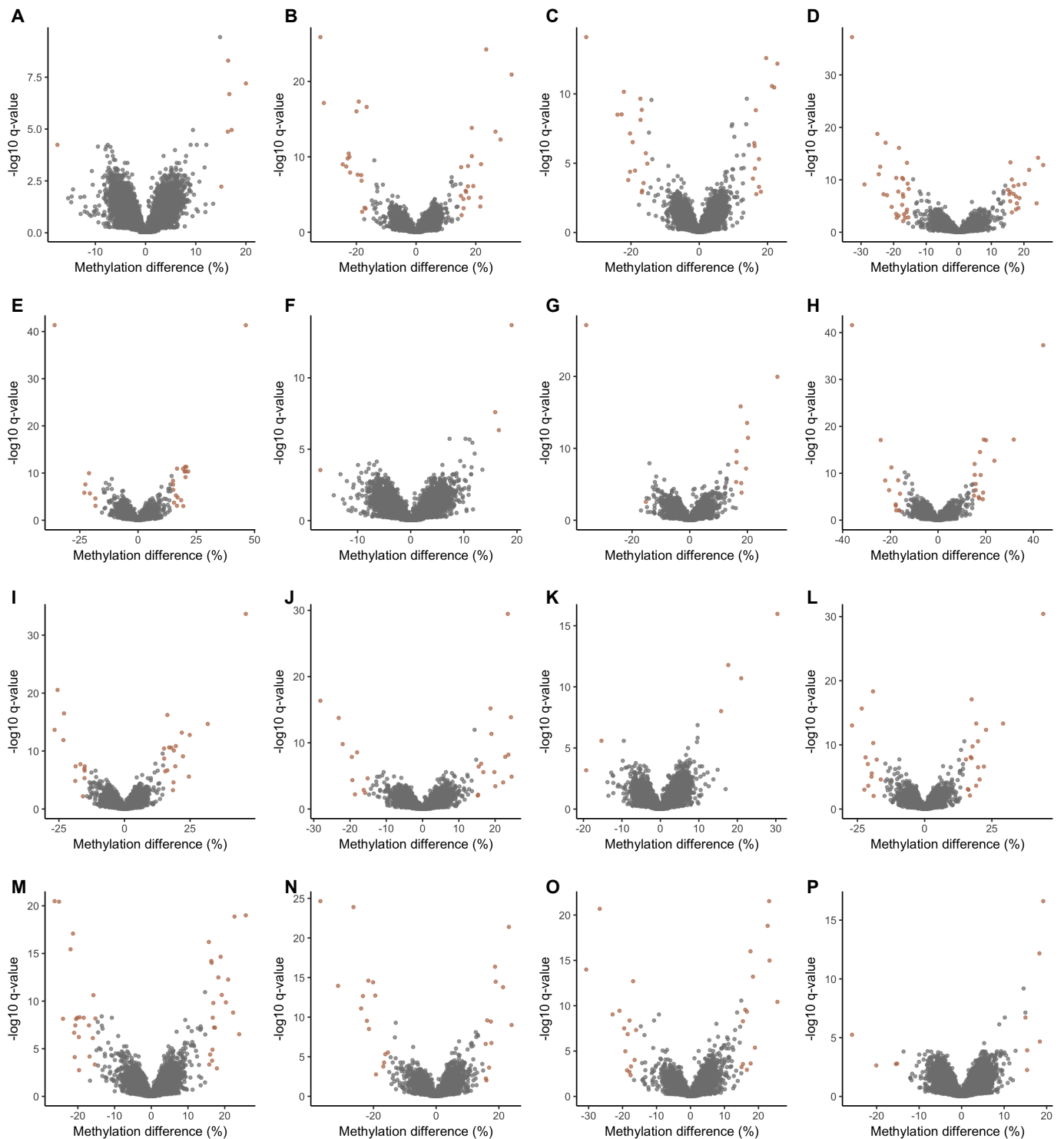


Fig. S6. Differential methylation between each drone and worker sample pairwise comparison in colony B1. (A–D) Drone sample from patriline 1 versus worker sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Drone sample from patriline 2 versus worker sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Drone sample from patriline 3 versus worker sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Drone sample from patriline 4 versus worker sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

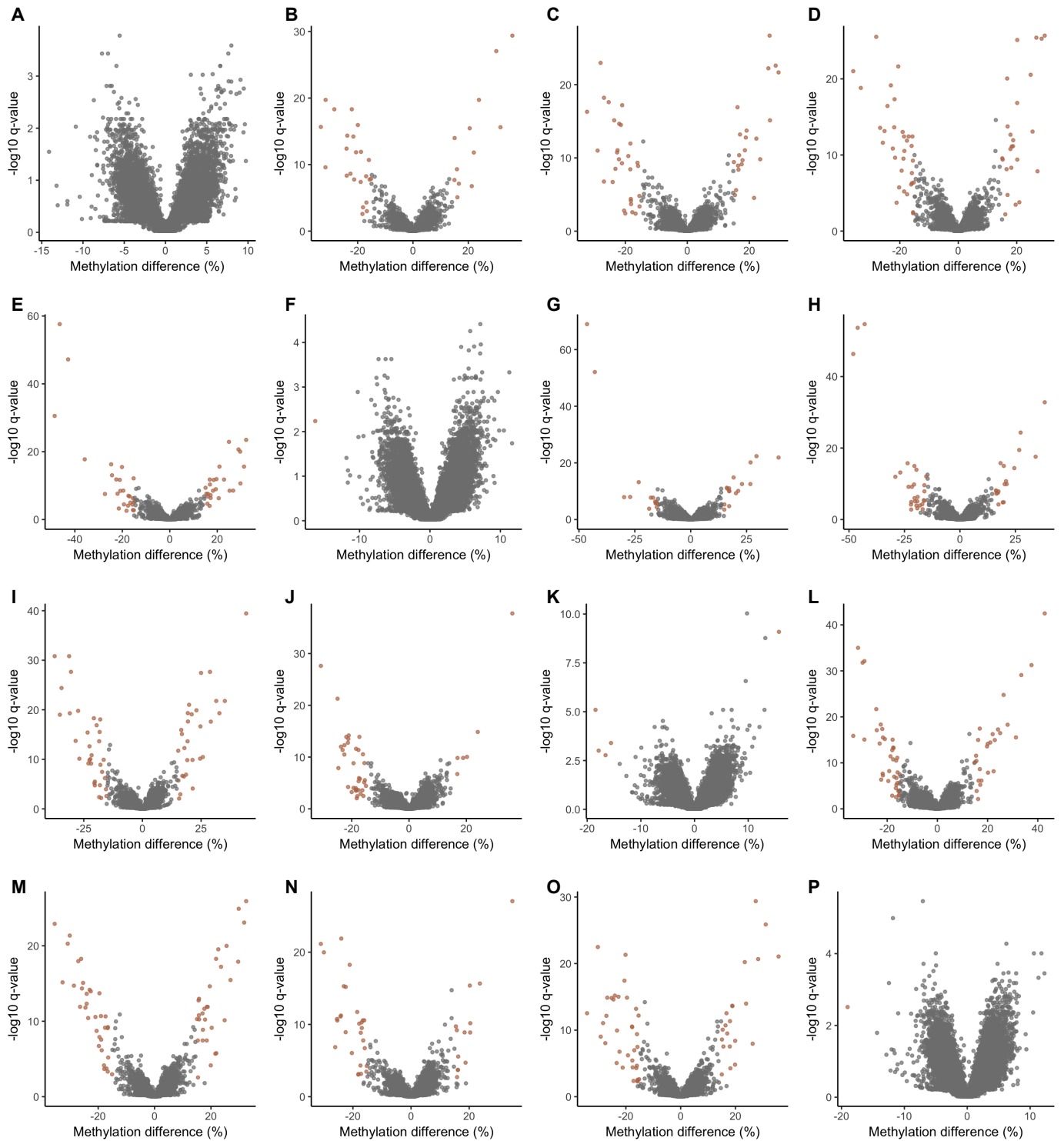


Fig. S7. Differential methylation between each semen and drone sample pairwise comparison in colony B1. (A–D) Semen sample from patriline 1 versus drone sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Semen sample from patriline 2 versus drone sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Semen sample from patriline 3 versus drone sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Semen sample from patriline 4 versus drone sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs (q -value ≤ 0.01 , methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

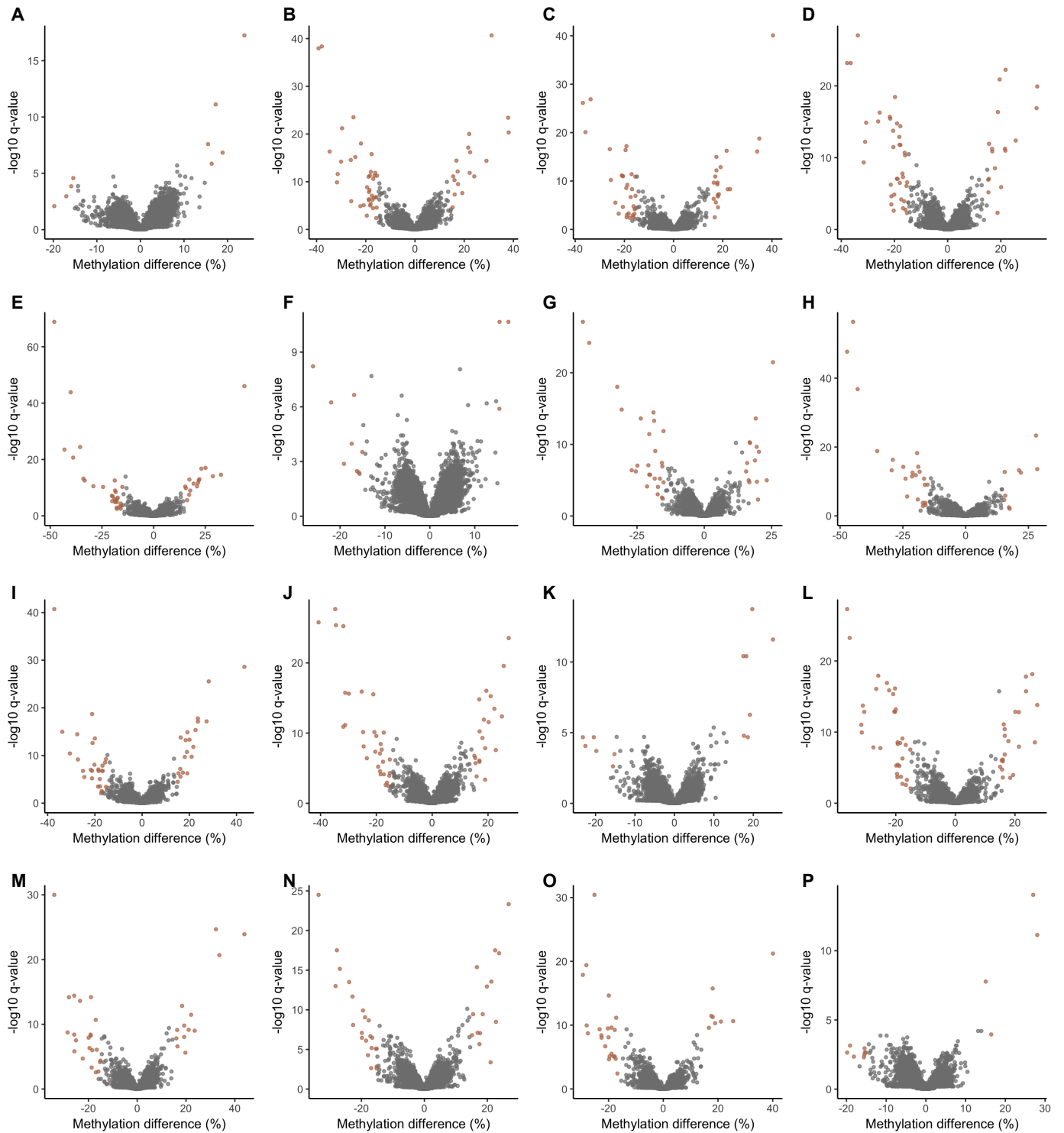


Fig. S8. Differential methylation between each semen and worker sample pairwise comparison in colony B2. (A–D) Semen sample from patriline 1 versus worker sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Semen sample from patriline 2 versus worker sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Semen sample from patriline 3 versus worker sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Semen sample from patriline 4 versus worker sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

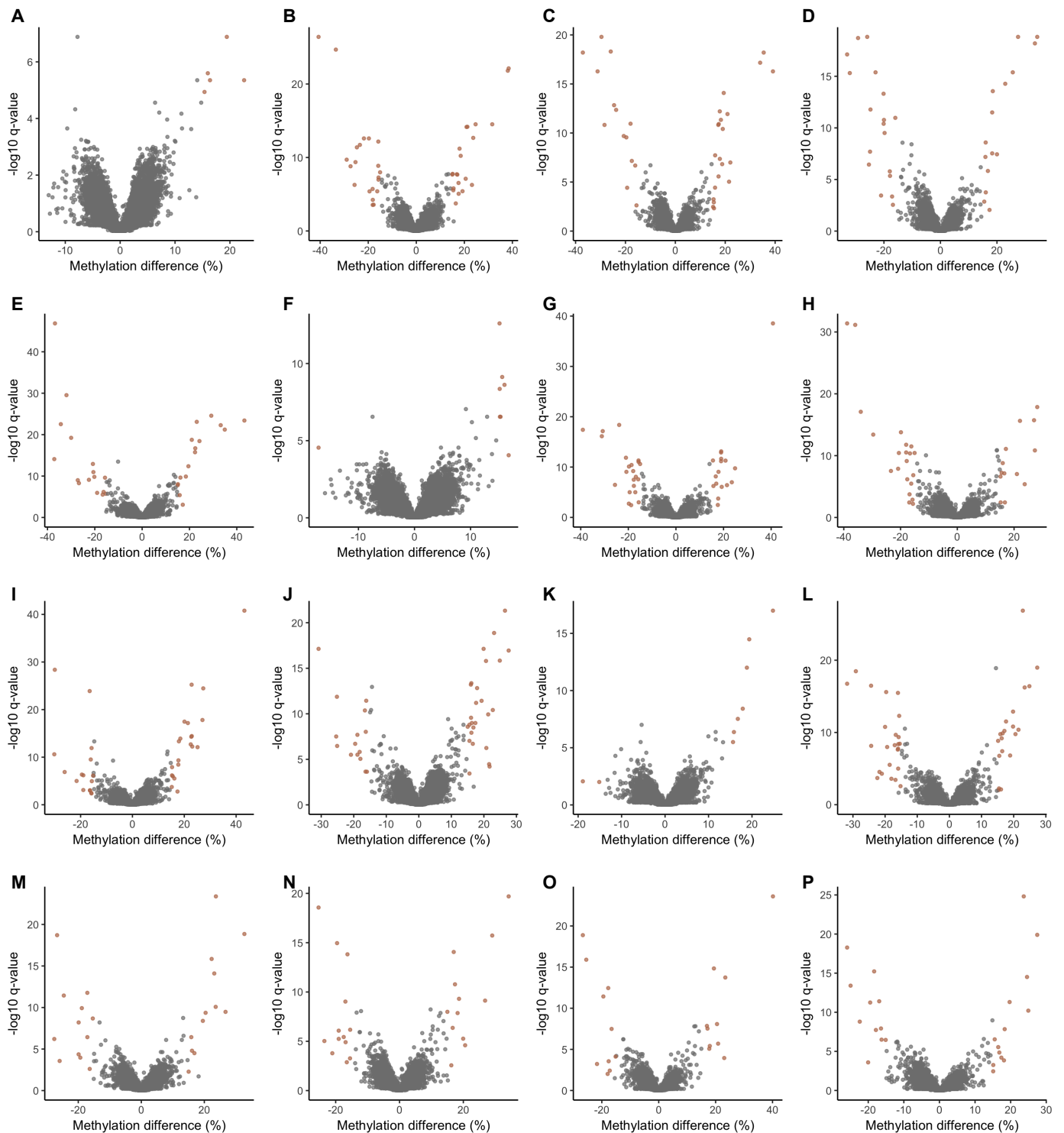


Fig. S9. Differential methylation between each drone and worker sample pairwise comparison in colony B2. (A–D) Drone sample from patriline 1 versus worker sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Drone sample from patriline 2 versus worker sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Drone sample from patriline 3 versus worker sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Drone sample from patriline 4 versus worker sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs (q -value ≤ 0.01 , methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

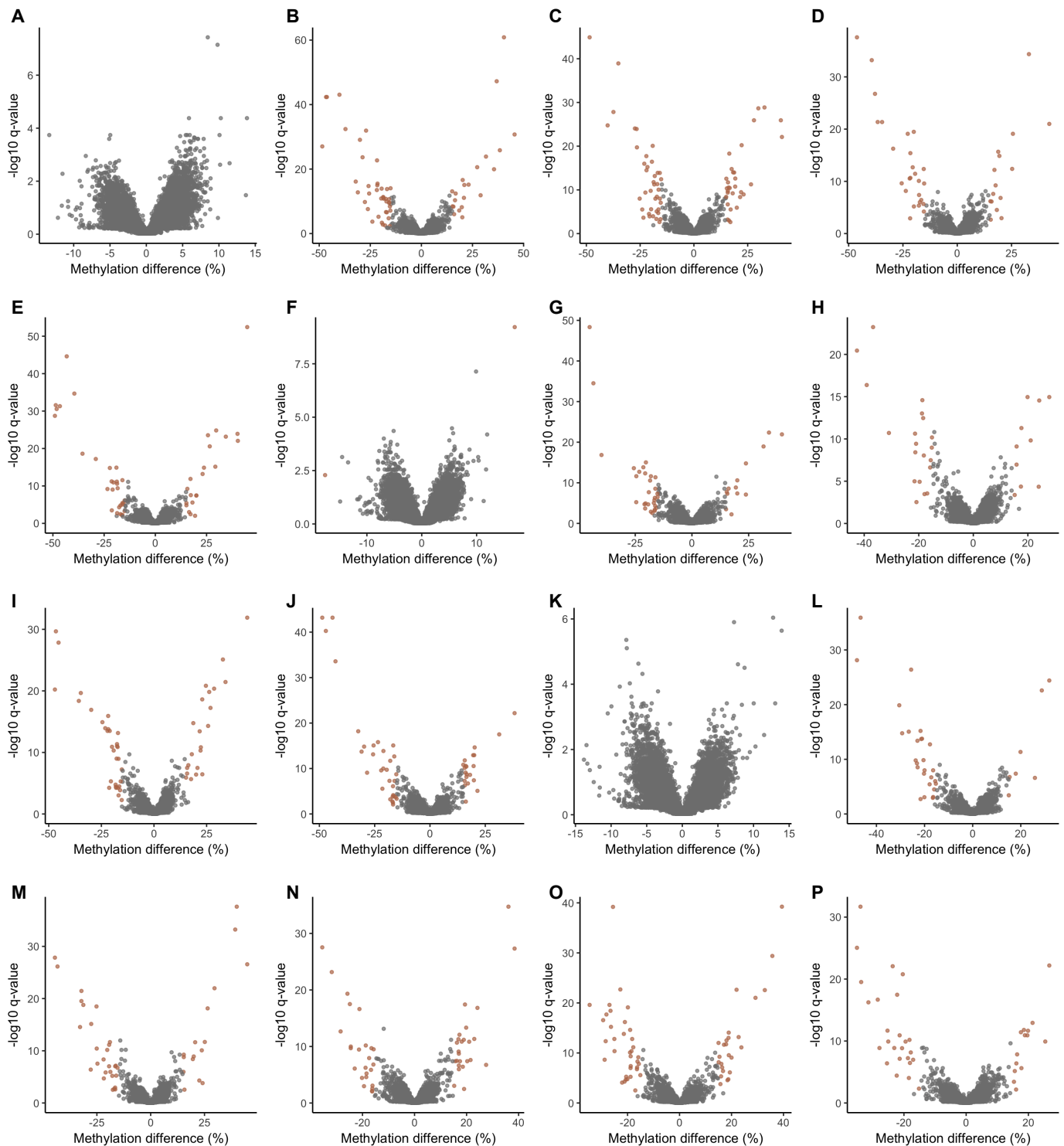


Fig. S10. Differential methylation between each semen and drone sample pairwise comparison in colony B2. (A–D) Semen sample from patriline 1 versus drone sample from patriline 1 (A), 2 (B), 3 (C) and 4 (D). (E–H) Semen sample from patriline 2 versus drone sample from patriline 1 (E), 2 (F), 3 (G) and 4 (H). (I–L) Semen sample from patriline 3 versus drone sample from patriline 1 (I), 2 (J), 3 (K) and 4 (L). (M–P) Semen sample from patriline 4 versus drone sample from patriline 1 (M), 2 (N), 3 (O) and 4 (P). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

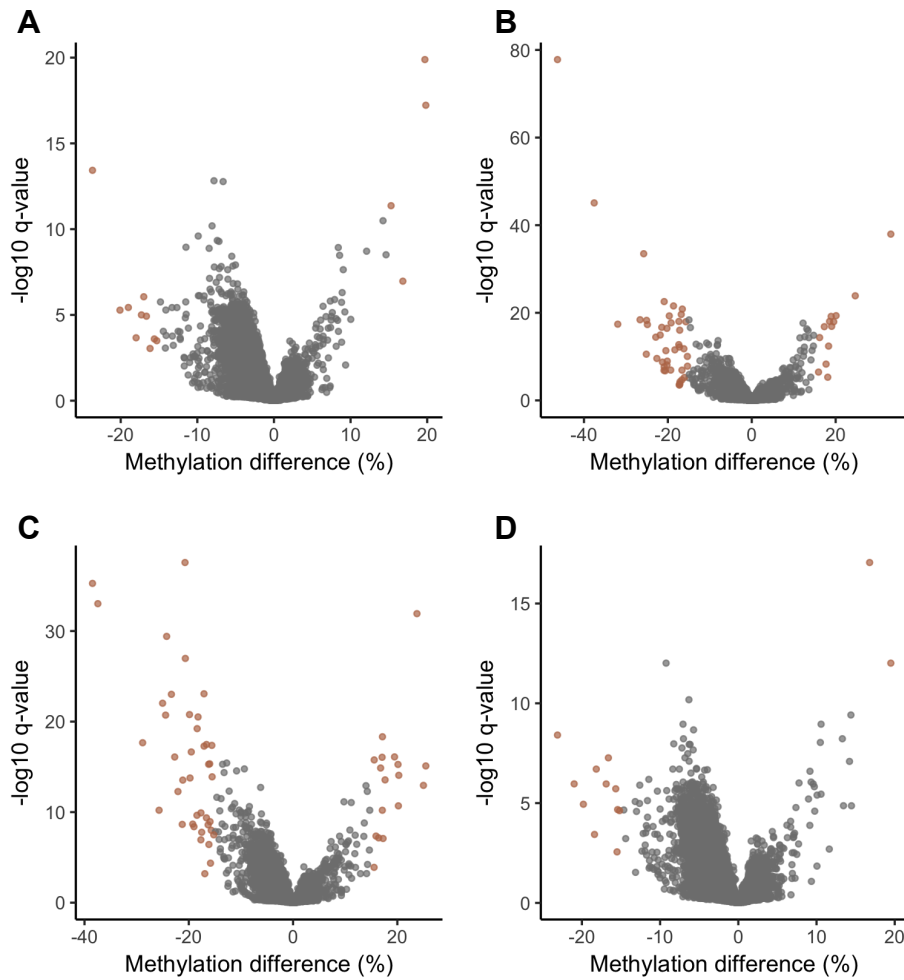


Fig. S11. Differential methylation between each semen and worker sample pairwise comparison in colony B4. (A,B) Semen sample from patriline 1 versus worker sample from patriline 1 (A) and 4 (B). (C,D) Semen sample from patriline 4 versus worker sample from patriline 1 (C) and 4 (D). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

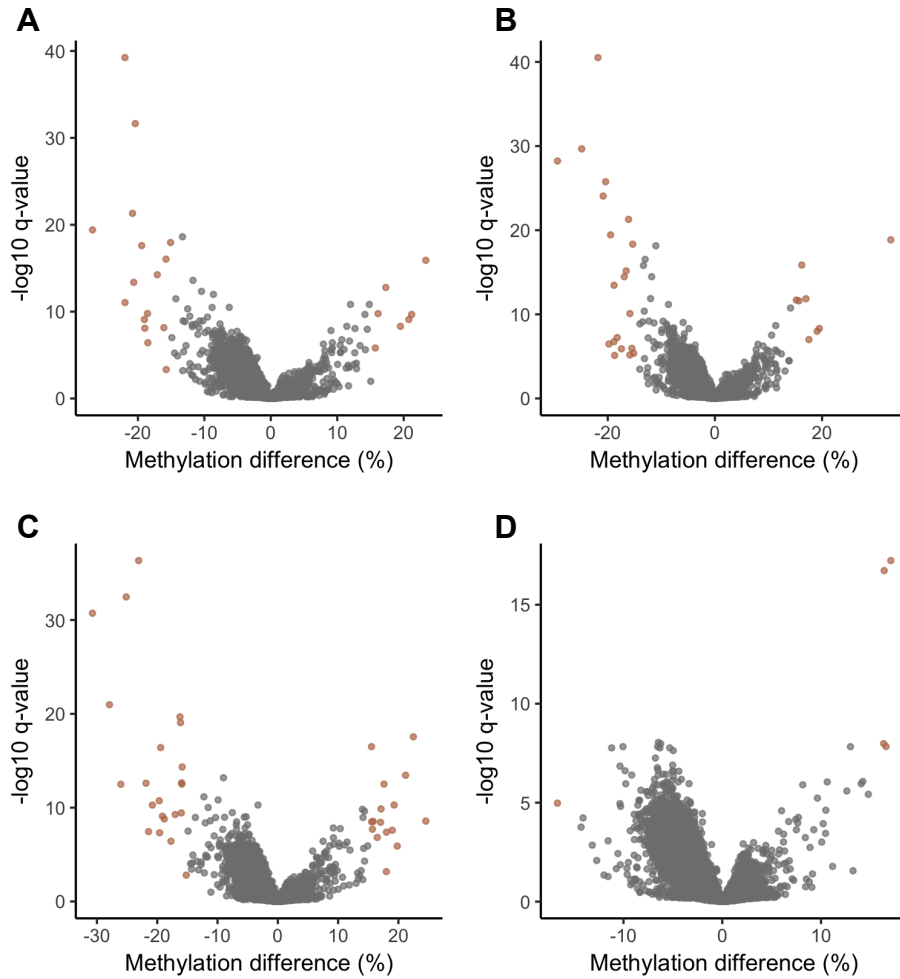


Fig. S12. Differential methylation between each drone and worker sample pairwise comparison in colony B4. (A,B) Drone sample from patriline 1 versus worker sample from patriline 1 (A) and 4 (B). (C,D) Drone sample from patriline 4 versus worker sample from patriline 1 (C) and 4 (D). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

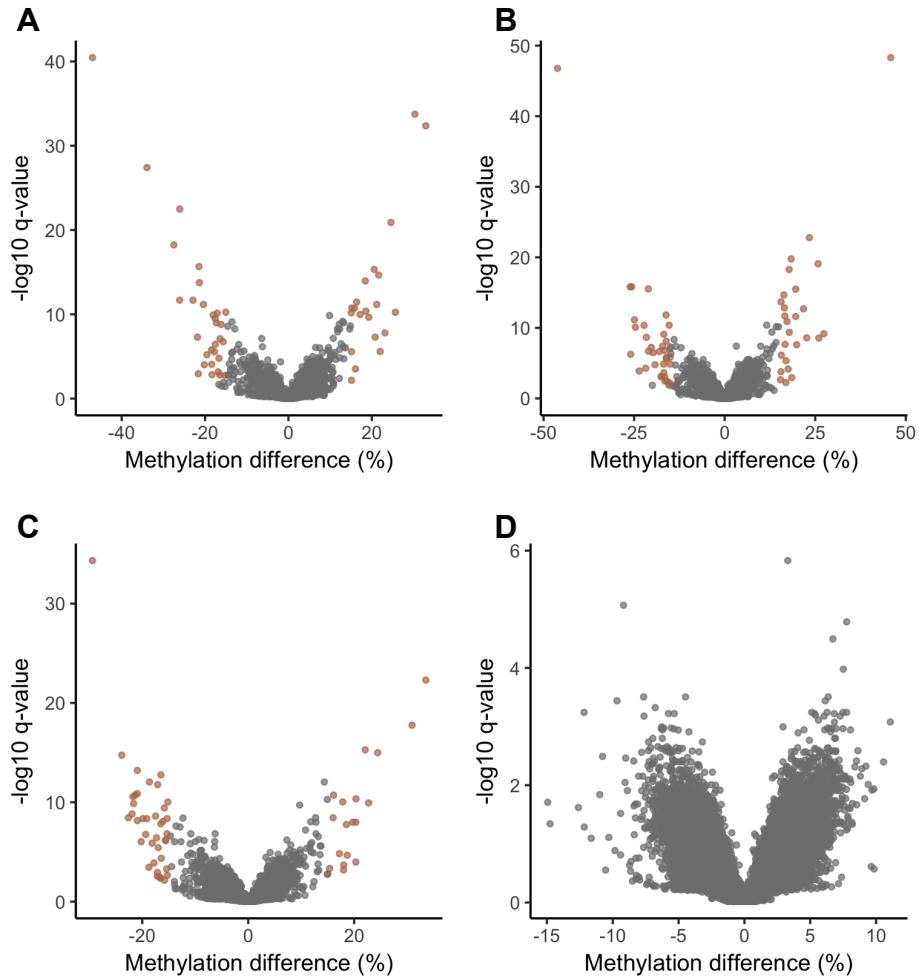


Fig. S13. Differential methylation between each semen and drone sample pairwise comparison in colony B4. (A,B) Semen sample from patriline 1 versus drone sample from patriline 1 (A) and 4 (B). (C,D) Semen sample from patriline 4 versus drone sample from patriline 1 (C) and 4 (D). Volcano plots represent DMRs ($q\text{-value} \leq 0.01$, methylation difference $\geq 15\%$; orange dots) and non-significant regions (grey dots).

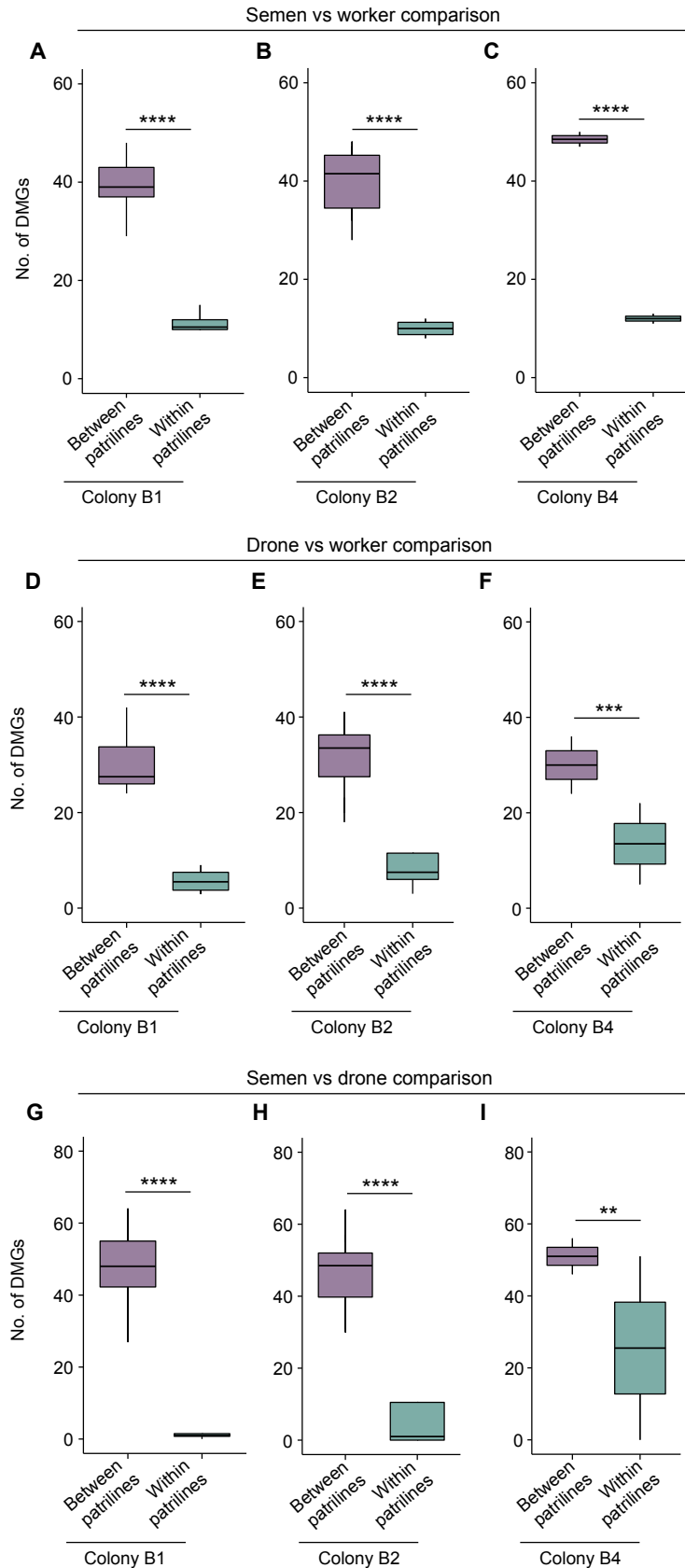


Fig. S14. Differentially methylated genes (DMGs) within and between patriline. (A–C) Number of DMGs between semen and workers from different patriline and from the same patriline in colony B1 (A), B2 (B) and B4 (C). (D–F) Number of DMGs between drones and workers from different patriline and from the same patriline in colony B1 (D), B2 (E) and B4 (F). (G–I) Number of DMGs between semen and drones from different patriline and from the same patriline in colony B1 (G), B2 (H) and B4 (I). Box plots represent median, interquartile range (IQR) and $1.5 \times$ IQR. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Table S1. Sequencing statistics.

Sample	Colony	Caste	Tissue	Conversion efficiency (%)	Mapping efficiency (%)	Genome coverage	No. sufficiently covered CpGs	No. mCpGs	Methylation density (%)	Methylation level (%)
B1S1	B1	Drone	Semen	99.45	74.22	29.34	6,179,883	58,610	0.95	93.04 ± 0.04
B1S2	B1	Drone	Semen	99.41	77.01	27.60	6,252,003	59,062	0.94	93.10 ± 0.04
B1S3	B1	Drone	Semen	99.46	77.68	32.90	7,483,205	63,556	0.85	92.91 ± 0.04
B1S4	B1	Drone	Semen	99.44	76.96	30.01	6,957,427	59,721	0.86	93.16 ± 0.04
B1D1	B1	Drone	Thorax	99.51	78.32	23.96	5,588,461	51,860	0.93	80.89 ± 0.07
B1D2	B1	Drone	Thorax	99.48	74.38	23.99	5,496,463	52,458	0.95	79.61 ± 0.07
B1D3	B1	Drone	Thorax	99.47	71.73	26.07	6,098,613	55,729	0.91	79.03 ± 0.07
B1D4	B1	Drone	Thorax	99.47	75.90	28.21	6,735,804	56,580	0.84	79.63 ± 0.07
B1W1	B1	Worker	Thorax	99.45	71.36	24.34	6,589,359	47,943	0.73	77.16 ± 0.09
B1W2	B1	Worker	Thorax	99.43	66.86	23.20	5,780,150	47,329	0.82	77.61 ± 0.08
B1W3	B1	Worker	Thorax	99.40	70.51	22.55	5,457,224	46,051	0.84	78.68 ± 0.08
B1W4	B1	Worker	Thorax	99.38	70.63	25.55	6,547,916	53,618	0.82	77.73 ± 0.08
B2S1	B2	Drone	Semen	99.43	78.83	30.78	7,065,952	61,958	0.88	92.09 ± 0.04
B2S2	B2	Drone	Semen	99.45	79.35	26.25	6,109,971	57,810	0.95	93.36 ± 0.04
B2S3	B2	Drone	Semen	99.45	77.57	27.11	5,937,051	57,227	0.96	92.44 ± 0.04
B2S4	B2	Drone	Semen	99.45	80.04	26.81	5,964,465	56,514	0.95	92.85 ± 0.04
B2D1	B2	Drone	Thorax	99.51	66.33	22.27	5,591,105	39,941	0.71	81.17 ± 0.08
B2D2	B2	Drone	Thorax	99.44	61.86	22.89	6,360,013	40,902	0.64	80.89 ± 0.07
B2D3	B2	Drone	Thorax	99.56	70.25	24.31	6,726,369	45,729	0.68	79.33 ± 0.08
B2D4	B2	Drone	Thorax	99.52	59.31	19.95	4,981,330	32,505	0.65	81.19 ± 0.08
B2W1	B2	Worker	Thorax	99.38	72.34	24.11	5,594,500	54,894	0.98	78.22 ± 0.08
B2W2	B2	Worker	Thorax	99.41	73.29	26.76	6,564,152	59,548	0.91	76.69 ± 0.08
B2W3	B2	Worker	Thorax	99.45	74.62	24.45	5,966,357	56,855	0.95	77.07 ± 0.08
B2W4	B2	Worker	Thorax	99.54	74.06	24.62	6,238,512	56,700	0.91	76.22 ± 0.08
B4S1	B4	Drone	Semen	99.46	82.74	27.40	6,845,163	62,039	0.91	92.99 ± 0.04
B4S4	B4	Drone	Semen	99.39	82.18	27.02	6,497,011	60,242	0.93	93.56 ± 0.03
B4D1	B4	Drone	Thorax	99.51	71.93	22.72	5,543,042	48,966	0.88	80.79 ± 0.07
B4D4	B4	Drone	Thorax	99.51	68.56	23.29	5,810,610	52,502	0.90	82.64 ± 0.06
B4W1	B4	Worker	Thorax	99.52	73.99	55.81	9,035,583	73,236	0.81	69.50 ± 0.09
B4W4	B4	Worker	Thorax	99.53	73.28	55.69	8,989,812	73,937	0.82	69.86 ± 0.09

Dataset S1 (separate file). Genotypes at five microsatellite loci of all drones, queens (inferred) and workers in each patriline in colonies B1, B2 and B4.