**Supplementary Figure S1 | Correspondence between embryonic stages deduced from transcriptome similarity.** Lines connect embryos with Reciprocal Best Transcriptome Similarity (RBTS). Multiple lines which are drawn from single embryonic stages (solid blue dot) indicate that there are no statistically significant differences between the lines. The line width reflects the reproducibility of the RBTS by different distance calculation methods (*e.g.* the thickest line indicates that the RBTS result was reproducible by all 4 distance calculation methods: '1 – Pearson correlation coefficient', '1 – Spearman correlation coefficient', 'Euclidean distance', and 'Manhattan distance'). Although the reason remains to be clarified, a clear correspondence can be seen between *X. laevis* and *D. rerio* embryos. Only data which were reproduced by all of the normalization methods (MAS5, gcRMA, PMdChip) are illustrated. See the Methods section for details about RBTS.
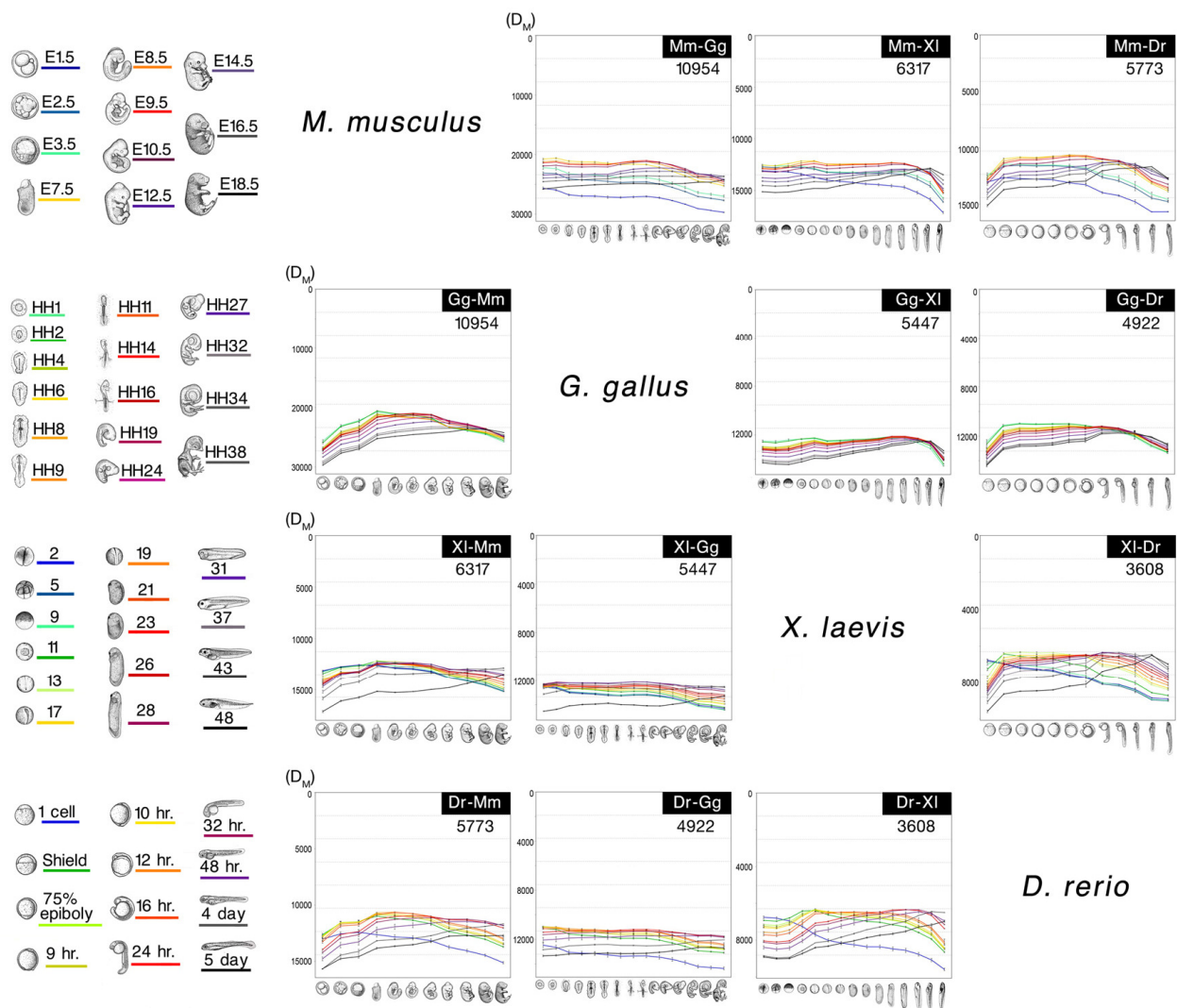
Supplementary Figure S2 | Transcriptome similarities of *M. musculus*, *G. gallus*, *X. laevis*, and *D. rerio* embryos. Total Manhattan distance (D_M) values calculated for pairs of embryos from different species at different developmental stages. gcRMA normalization within the same species was performed during preprocessing of the gene chip data. A lower D_M value on the y-axis indicates higher transcriptome similarity. The sampled stages are shown on the left (different coloured lines indicate different developmental stages). Note that the D_M scores calculated for early-versus-early stages (left part of the broken blue lines in each graph) are not the lowest. Error bars indicate standard deviations. D_M scores are not directly comparable between graphs because the D_M scale is affected by the number of genes in each comparison (numbers in the upper right corner of each line chart; see the Methods section for more details). Mm, *Mus musculus*; Gg, *Gallus gallus*; Xl, *Xenopus laevis*; Dr, *Danio rerio*.

2
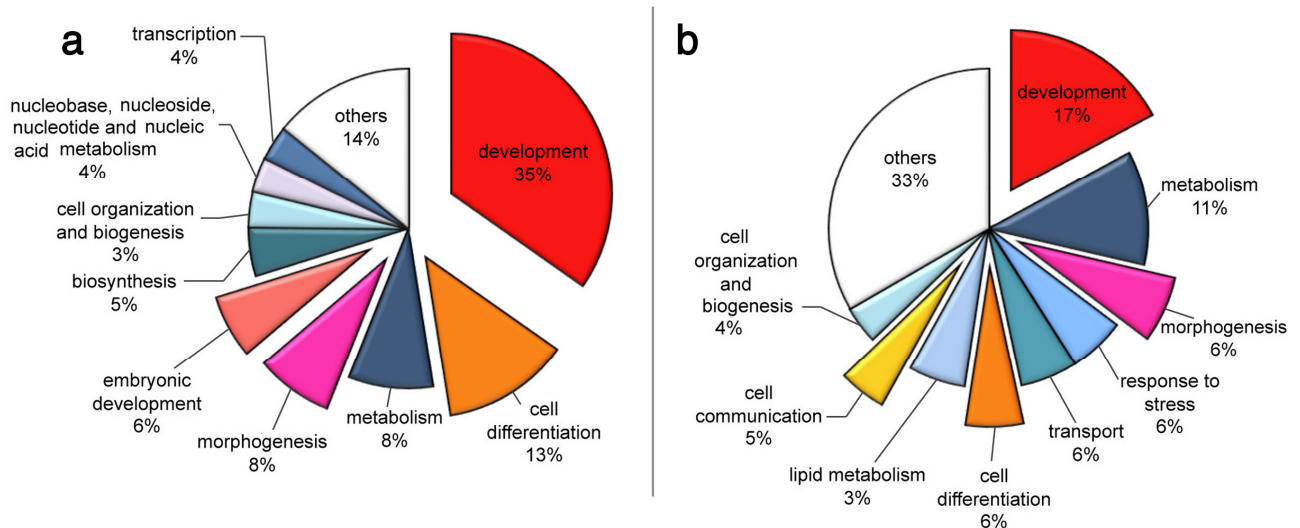
Supplementary Figure S3│ Gene Ontology (GO) Slim analysis of similarly or differentially expressed co-orthologous groups in the putative phylotypic period. After calculating the expression similarity of 1573 1-1-1-1 Reciprocal Best BLAST Hit (RBBH) orthologues, the genes which were expressed in all of the conserved stages (E9.5 for *M. musculus*, HH16 for *G. gallus*, stage 28 for *X. laevis*, 24 hours post-fertilization (hpf) for *D. rerio*) were extracted, except for constitutively expressed genes (defined by a 'Present' result in the MAS5 analysis of any of the early to late stages). **(a)** GO values of the top 10% of similarly expressed orthologous genes (*n* = 11,349 GO terms). Among the 110 genes which were extracted, the GO values of the top 10% expression similarities were analysed by using the GO-Slim program (http://www.genome.iastate.edu/tools/catego/). GO values which were related to embryogenesis were floated. **(b)** GO values of the lowest 10% of similarly expressed orthologous genes (*n* = 11,252 GO terms). Among the 110 genes which were extracted, the GO values of the bottom 10% expression similarities were analysed by using the GO-Slim program. Note that significantly higher (Fisher's exact test; *P* = 0.0022) ratios of developmental GO terms were found in the top 10% of genes (a). For the GO-Slim analysis, generic GO slims (ftp://ftp.geneontology.org/pub/go/GO_slims/goslim_generic.obo) without 3 root classes were used. Similar results were obtained when the other data sets (E9.5 for *M. musculus*, HH16 for *G. gallus*, stage 31 for *X. laevis*, and 24 hpf for *D. rerio*) were used.

3
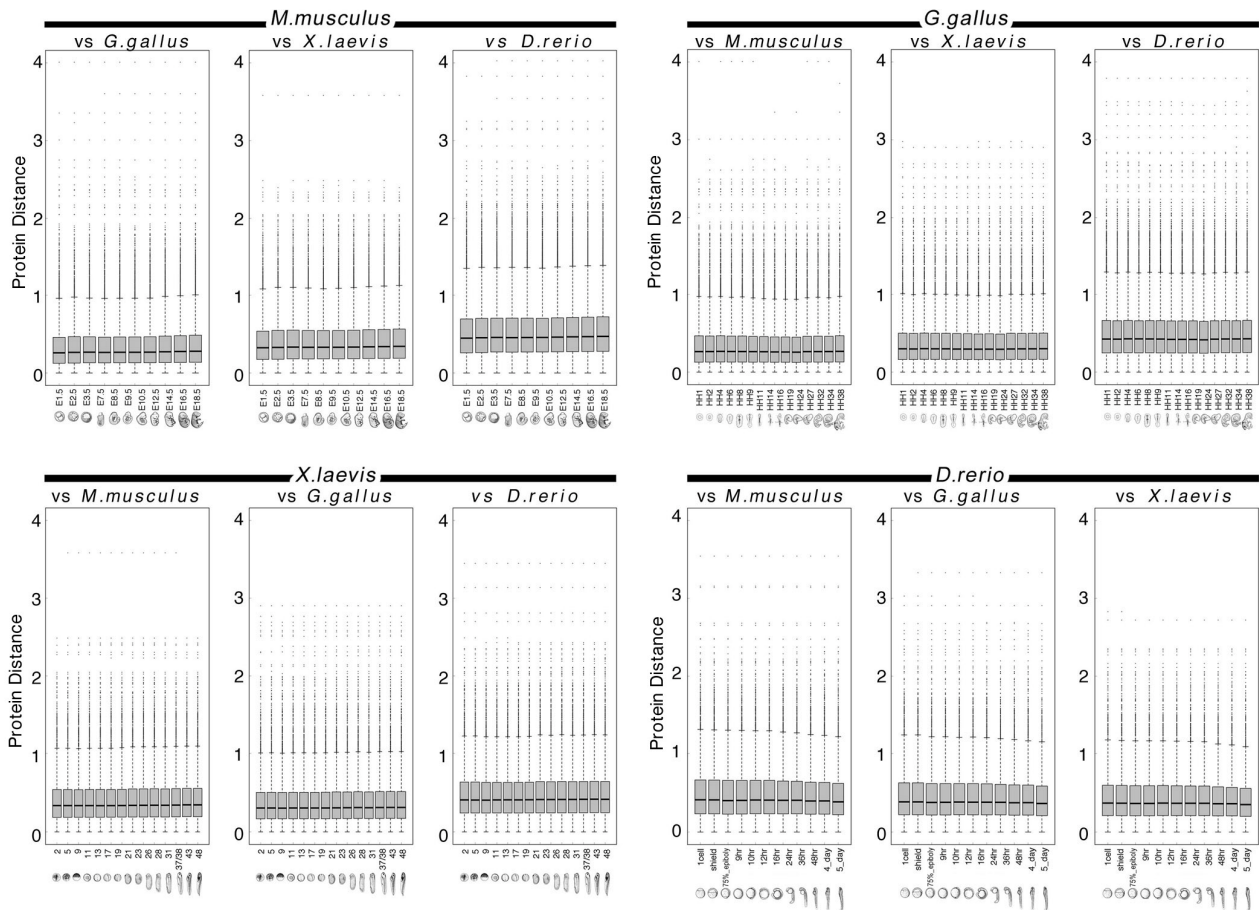
Supplementary Figure S4 | No significant biases in the Protein Distances of expressed genes were observed when comparing vertebrate embryogenesis. Box (quantile) plot of the distribution of the Protein Distances of expressed genes (defined by a 'Present' result in the MAS5 analysis using default parameters) at each developmental stage, excluding genes which were expressed constitutively during embryogenesis. Note that the distributions largely overlap with each other. Kruskal-Wallis tests of the mean, mode, or median values indicated that none of the developmental stages had critically biased protein distances (none of them were below $P < 0.01$, where the corrected alpha level $= 8.3 \times 10^{-4}$). The Protein Distance was calculated between, but not within, the 4 vertebrate species, using the Protdist program (http://evolution.genetics.washington.edu/phylip/doc/protdist.html).
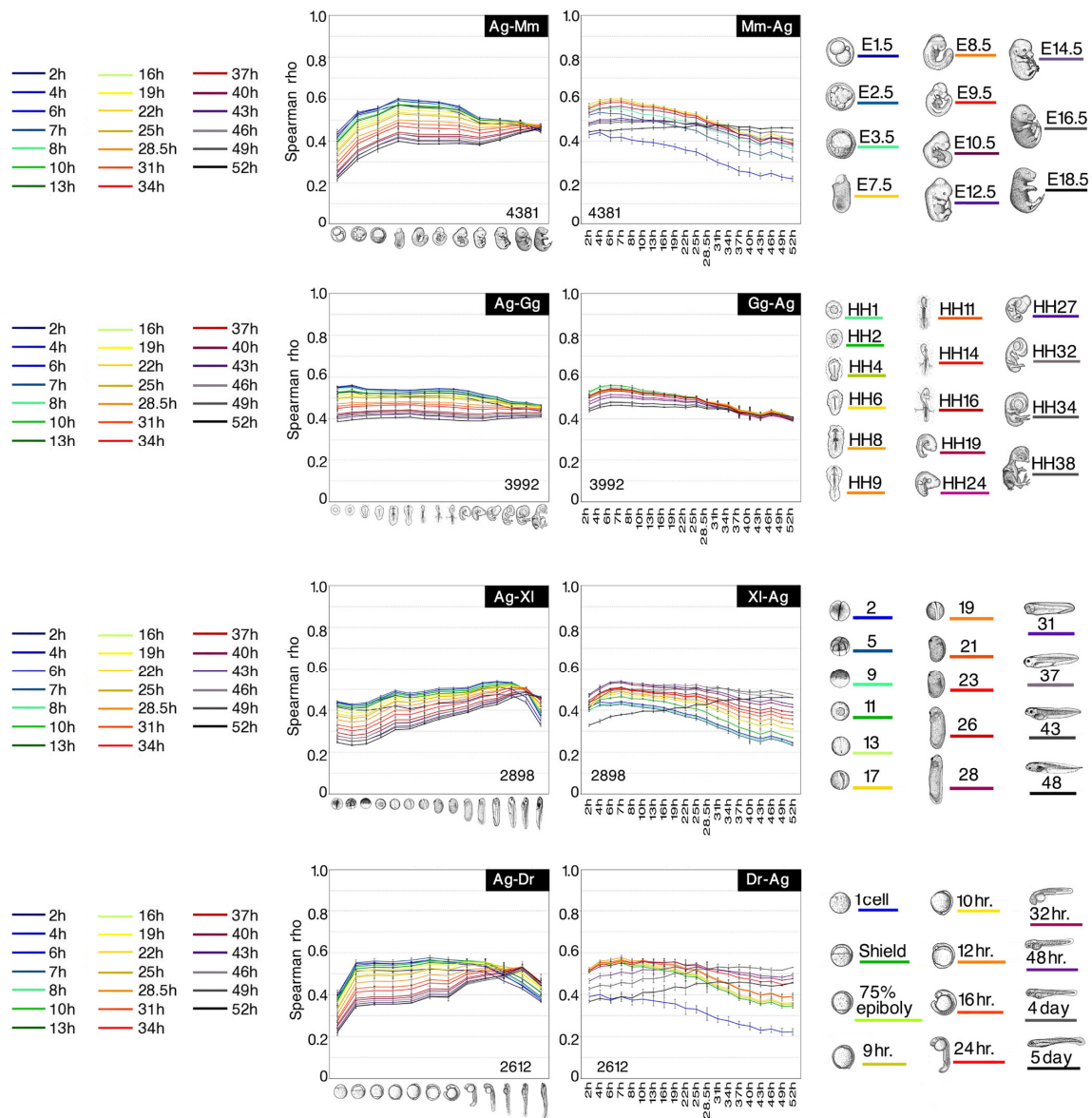
Supplementary Figure S5│ Transcriptome similarities between *Anopheles gambiae* and vertebrate embryos. Spearman correlation coefficients (ρ) of transcriptome data from pairs of embryos from different species at different developmental stages. Higher ρ values indicate higher transcriptome similarity. Although the highest ρ scores were seen around 6–8 h (segmentation stages) *A. gambiae* embryos (Mm-Ag, Gg-Ag, Xl-Ag, Dr-Ag), rigorous Total Sum Distance analysis did not show consistent results among different methods of distance calculation. Note that the peaks for vertebrate embryos are similar to those in Fig. 1. The sampled stages are shown on the left and right. Different coloured lines indicate different developmental stages. The number at the bottom of each line chart indicates the number of orthologous genes which were compared. These results did not change with different methods of normalization or calculating distance. Error bars indicate standard deviations. Ag, *Anopheles gambiae*; Mm, *Mus musculus*; Gg, *Gallus gallus*; Xl, *Xenopus laevis*; Dr, *Danio rerio*.

5

Supplementary Table S1 │ Combinations of embryos with the highest sum transcriptome similarity.

Transcriptome similarity calculated by Pearson Correlation Coefficient
Mus_E9.5 Chick_HH16 Xlaevis_31 Drerio_24hpf
Mus_E9.5 Chick_HH14 Xlaevis_28 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_28 Drerio_24hpf
Transcriptome similarity calculated by Spearman Correlation Coefficient
Mus_E9.5 Chick_HH14 Xlaevis_31 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_28 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_31 Drerio_24hpf
Mus_E9.5 Chick_HH14 Xlaevis_28 Drerio_24hpf
Transcriptome similarity calculated by total Euclidean distance
Mus_E9.5 Chick_HH16 Xlaevis_28 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_31 Drerio_24hpf
Transcriptome similarity calculated by total Manhattan distance
Mus_E9.5 Chick_HH14 Xlaevis_28 Drerio_16hpf
Mus_E7.5 Chick_HH2 Xlaevis_28 Drerio_16hpf
Mus_E9.5 Chick_HH14 Xlaevis_31 Drerio_24hpf
Mus_E9.5 Chick_HH11 Xlaevis_28 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_28 Drerio_24hpf
Mus_E9.5 Chick_HH16 Xlaevis_31 Drerio_24hpf
Mus_E9.5 Chick_HH14 Xlaevis_28 Drerio_24hpf

Combinations of embryos with 1 developmental stage per species (11 Mm stages $\times$ 15 Gg stages $\times$ 15 Xl stages $\times$ 12 Dr stages = 29,700 combinations) were analysed to identify the group with the highest sum transcriptome similarity (Mm $\Leftrightarrow$ Gg + Mm $\Leftrightarrow$ Xl + Mm $\Leftrightarrow$ Dr + Gg $\Leftrightarrow$ Xl + Gg $\Leftrightarrow$ Dr + Xl $\Leftrightarrow$ Dr). Two-sample Wilcoxon tests (Bonferroni corrected alpha level = 2.27×10^{-11}) were performed to test statistical significance. The combinations of embryos listed here are data sets which were reproduced by any of the normalization methods tested (MAS5, gcRMA, PMdChip) and do not have any statistically significant differences in sum transcriptome similarity. Note that all of the data sets contained the following combinations: (1) Mus_E9.5 + Chick_HH16; Xlaevis_28 + Drerio_24 hpf; and (2) Mus_E9.5 + Chick_HH16; Xlaevis_31 + Drerio_24 hpf. Mm/Mus, *Mus musculus*; Gg/Chick, *Gallus gallus*; Xl/Xlaevis, *Xenopus laevis*; Dr/Drerio, *Danio rerio*.

Supplementary Table S2 | Morphological characteristics of embryos with the smallest total sum distance of transcriptomes[46,47,48,49].

	Event	M. musculus E9.5	G. gallus HH16	*X. laevis* stage 28/31	*D. rerio* 24 hpf
Axial structures	Bilaterality along antero-posterior axis	+	+	+ / +	+
	Rhombomeres	+	+	+ / +	+
	Neural crest cells	+	+	+ / +	+
	Notochord	+	+	+ / +	+
	Somites	+	+	+ / +	+
	Neural tube or neural folds partially fused	+	+	+ / +	+
Pharyngeal	Pharyngeal arches	+	+	+ / +	+
	Thyroid anlage	+	+	− / −	+
	Hypophysis anlage	?	−	+ / +	+
Olfactory	Olfactory placodes	+	+	+ / +	+
Otic	Otic placode	+	+	+ / −	+
Optic	Lens placode	+	+	? / +	−
Cardiovascular system	Heart with chambers	+	?	− / −	?
	Aortic arches	+	−	− / +	−
Intestinal	Hindgut	−	+	+ / +	−
	Hepatic primordium	+	+	+ / +	?
	Liver diverticulum	+	+	+ / +	−
	Gallbladder primordium	+	−	− / −	−
	Pancreas primordium	−	−	− / −	+
	Spleen anlage	−	−	− / −	−
Kidney	Mesonephric duct anlagen	+	+	+ / +	+
Limb	Forelimb (or pectoral fin) bud	+	−	− / −	−
Epidermal	Epidermis	+	+	+ / +	+
	Hair or feathers	−	−	− / −	−

Key: + observed; – not observed; ? rudimentary.

References

46   Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. Stages of embryonic development of the zebrafish. *Dev Dyn* **203**, 253-310, (1995).

47   Faber, P. D. N. J. Normal Table of Xenopus Laevis (Daudin): A Systematical and Chronological Survey of the Development from the Fertilized Egg Till the End of Metamorphosis. *Garland Pub* (1994).

48   Hamburger, V. & Hamilton, H. L. A series of normal stages in the development of the chick embryo. 1951. *Dev Dyn* **195**, 231-272, (1992).

49   Matthew H. Prof. Kaufman, M. H. K. *The atlas of mouse development.* 2nd edn, (Academic Press, 1992).