The evolution of high-order genome architecture revealed from 1,000 species

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SUMMARY

Spatial genome organization plays a crucial regulatory role, but its evolution remains unknown. Leveraging Hi-C data from 1,025 species, we trace the evolutionary trajectories of 3D genome, through two higher-order architectures, 'global folding' (spatial organization of karyotype) and 'checkerboard' (chromatin compartments). Early life forms, including prokaryotes and unicellular eukaryotes, mostly display random configurations. Through the evolution of plants, global folding became the prominent architecture with eudicots and remained dominant. Animals, however, progressively developed stronger checkerboard throughout vertebrate and invertebrate evolution, and even during early embryogenesis, suggesting a conserved gene co-regulation mechanism. In contrast, plants prefer linear gene clusters over checkerboard. Both strategies of gene arrangement reinforce the biological principle 'structure determines function': divergent evolutionary paths converge on architectural solutions to meet the greater regulatory demands.

KEYWORDS

Genome structure; evolution

INTRODUCTION

Among the regulatory mechanisms, three-dimensional (3D) genome structures have become a key area of interest. The advent of genome-wide chromatin conformation capture (Hi-C) techniques has revolutionized our ability to study how 3D genome folding influences the fine-tuning of gene regulation[1,2]. Hi-C measures physical proximity between pairs of sites across the genome, resulting in a 'contact map' of interactions, linking gene loci with regulatory elements. These structures are now understood to play a pivotal role in gene expression[3,4]. However, despite these advances, the role of 3D genome folding in evolution remains poorly understood, largely due to the scarcity of datasets, which are typically restricted to closely-related species[5–10], confining previous studies to narrow evolutionary lineages.

To address this limitation, we leverage Hi-C data from thousands of species (mainly from the Darwin Tree of Life project[11]) to more fully explore the evolutionary significance of 3D genome. By doing so, we aim to extend the basic principle established by the discovery of the double helix – that structure determines function – to higher levels of genome organization. By algorithmically restoring long-range chromatin interactions, we identified two higher-order genome architectures at the whole-genome scale, which we

refer to as 'global folding' and 'checkerboard', which vary across evolutionary time. By exploring 3D genome organization across the entire tree of life, this study provides new insights into how living systems evolve intricate structures.

RESULTS

Two whole genome-scale genome architectures: global folding and checkerboard

To investigate the large-scale genome architectures across the tree of life, we analyzed Hi-C data from 1,025 species, spanning bacteria, fungi, plants, and animals (Fig. 1; Data. 1), representing around 3,800 million years of evolutionary history. The vast phylogenetic diversity of these species and their enormous variation in genome size (from 2.1 Mb in *Thermococcus kodakarensis* to 14.6 Gb in *Triticum aestivum*; Fig. S1) make it impossible to identify conserved synteny blocks, which are the foundation for cross-species comparison of traditional localized genome structures such as TADs and loops[5,8]. To address this limitation, we developed a two-step pre-processing approach aimed at providing a broader perspective rather than a focus on local sequences. First, we automatically scaled chromosomal maps to a comparable size. Second, we devised NormDis, a **Norm**alization algorithm to remove **Dis**tance-dependent biases, which re-weights and makes visible long-range chromatin interactions, which in Hi-C data are invariably obscured by the far more prevalent short-range contacts (see Methods and Supplementary Methods; examples shown in Fig. S2). After applying NormDis and examining the normalized Hi-C maps, it became apparent that across evolutionary history two major whole-genome-scale architectures consistently occurred. We refer to these as 'checkerboard' and 'global folding'; we schematically illustrate these terms in Fig. S3 and define them below.

Global folding describes how an entire set of chromosomes are spatially organized within the nucleus, with the folding of each chromosome typically (but, as discussed below, not always) anchored by three points: two at the ends (telomeres) and one in between, which strongly co-localizes with centromeres (Fig. S4). For example, in yeast (*Saccharomyces cerevisiae*), X-shaped patterns on the normalized map indicate a polarized conformation, in which center and end anchors cluster separately (Fig. S3A). However, we also observed other global folding patterns beyond 'X' shapes. To systematically classify these patterns, we employed a multi-step computational framework on both intra- and inter-chromosomal maps (Fig. 1; see Methods). First, we applied centralization and symmetrization to enhance topological clarity. Next, we performed feature extraction on the chromosomal maps based on sparse principal component analysis[12], to eliminate noises and uncommon features. Finally, we categorized five intra- and five inter-global folding types through t-SNE[13] and DBSCAN[14] (Fig. 2A and S3). The global folding score (GFS) was assessed by the similarities between input normalized maps and identified folding types (documented in Data. 2 and 3).

Checkerboard patterns are the regular crisscrossed textures on normalized maps, which reflect their spatial compartmentalization (Fig. S3B). In human[2] and mice[15], these patterns correspond to chromatin compartments, which separate euchromatic from heterochromatic regions. However, we found that checkerboard 'strength' varies widely across species, with some showing pronounced contrast between the different compartments comprising the checkerboard; others, by contrast, showed weaker or negligible contrast. To quantify checkerboard strength, we developed an entropy metric based on chromatin interaction polarity (Fig. 1; see Methods). Since genomic regions from the same compartment tightly interact[2], their spatial interactions should more closely resemble each other, whereas regions from different compartments tend to repel, resulting in a lower degree of similarity. The degree of this interaction polarization, measured by information entropy, serves as a robust indicator of checkerboard 'strength'; in effect, its degree of modularity (documented in Data. 4).

These two higher-order genome architectures, global folding and checkerboard, are remarkably conserved across mature tissues from same species (Fig. S5). This is quantitatively supported by smaller differences among tissues of given species (average standard deviation (std) of four tested species,

checkerboard score (CBS): 0.06, intra-global folding score (GFS): 0.57, inter-GFS: 0.27; detailed scores are documented in Data. 5) than inter-species comparisons (std of CBS: 0.17, intra-GFS: 0.80, inter-GFS: 0.90), making them ideal for cross-species comparisons.

Intra- and inter-chromosomal global folding

After analyzing 19,904 intra- and 485,102 inter-chromosomal maps from 1,025 species, we identified five major intra- (Center, Large-Center, Center-End-Axis, Center-Whole, and End-Whole) and five inter-chromosomal (Center-Center, End-End, Center-End-Axis, Center-Whole, and End-Whole) global folding patterns (Fig. 2A, examples in Fig. S3A). Each pattern is widely distributed, supported by a minimum of 2,134 chromosomal maps. Some of them, as labeled by the illustrations in Fig. 2A, align with previously reported structures[16], while others represent novel architectures. For example, the newly discovered center-whole pattern is characterized by vertical or horizontal interaction signals anchored at the center, while end-whole is marked by signals at the ends. Although the biophysical architectures underlying these patterns remain unclear, they reveal the existence of structures beyond those previously identified. Other architectures may also emerge as more species data become available.

The prevalent global folding type varies among taxonomic kingdoms (Fig. 2B; examples on Fig. S6). Animals (both invertebrates and vertebrates) generally exhibit weaker global folding, with no specific type significantly enriched (minimum Wilcoxon signed-rank test *p*-value: 0.39, except for large-center pattern in vertebrates *p*-value: 0.023, *p*-values are documented in Data. 6). This indicates a relatively disordered organization at the whole-genome scale. In contrast, plants, prokaryotes (intra-chromosomal maps), and fungi (inter-chromosomal maps), strongly favor a center-end-axis architecture (maximum *p*-value: 0.031; Fig. 2B; Data. 6), which reflects a conformation in which the centromeres and telomeres cluster separately, while chromosome arms are arranged in parallel.

Despite the prevalent global folding, exceptions exist within each kingdom. For example, *Bufo bufo*, the common toad, exhibits a center-end-axis pattern, an architecture otherwise rare in vertebrates (Fig. S6). Nevertheless, closely related species tend to share similar global folding structures, as indicated by the significantly smaller differences across smaller taxonomic divisions (for example orders) than larger ones (kingdoms; mean intra-chromosomal GFS difference: 0.872 for orders and 1.195 for kingdoms, Mann-Whitney U test *p*-value: 0.005; mean inter-chromosomal GFS difference: 0.992 and 1.196, respectively, *p*-value: 0.032; Fig. S7; detailed differences are documented in Data. 7). This suggests that lineage-specific events may have shaped global folding patterns which were then stably inherited. An example is the emergence of condensin II, which resulted in the loss of global folding architectures in multiple organisms[16]. Notably, global folding appears independent of chromosomal morphology, as we found no significant correlations between GFS and chromosome length (Pearson correlation coefficient (PCC) < 0.13; Fig. S8).

We next investigate the interplay between intra- and inter-chromosomal global folding within same species. Usually, the same intra- and inter-chromosomal global folding type co-occur (Fig. 2C; average Spearman correlation coefficient, SCC: 0.40). Nevertheless, cross-type pairs are also observed, particularly those that share the same anchor points (average SCC: 0.22; examples on Fig. S9A). For example, the intra-chromosomal “center” pattern (a central chromatin domain), is often accompanied by the inter-chromosomal “center-whole”, where the center anchor interacts with an entire chromosome (SCC: 0.47; examples in Fig. S9B). These observations suggest that the anchor points play a central role in shaping global folding.

Within a single species, different chromosomes could also adopt distinct global folding patterns, a phenomenon we term global folding polymorphism. For instance, in *Clonorchis sinensis*, the first two long chromosomes display center-end-axis architectures, whereas the remaining five short ones lack large-scale folding structures (Fig. 2D). Polymorphic folding is more common in species with irregularly smaller

chromosomes, referred to as micro-chromosomes in prior studies[17] (SCC between polymorphic score and chromosome length difference ratio: 0.29, *p*-value $< 10^{-16}$; Fig. S11A). Reptiles (Lepidosauria) and birds (Aves), in which micro-chromosomes are commonly abundant[17], exhibit the highest polymorphism scores (reptiles: 1.228; birds: 1.203; Fig. S11D and Data. 8), indicating that micro-chromosomes might contribute to the diverse folding patterns within species.

Checkerboard score reflects the degree of genome compartmentalization

The checkerboard score (CBS) quantifies the intensity of checkered textures on normalized maps, serving as a proxy for chromatin compartmentalization. Among 1,025 species, CBS values range from weak (~2.3) to strong (~3) (Fig. 2E; documented in Data. 4). As with global folding patterns, checkerboard patterns do not correlate with chromosome length (PCC with length: -0.087; Fig. S12). This suggests that checkerboard organization is a biologically regulated architecture rather than a byproduct of chromosome morphology.

Species with low CBS typically exhibit alternative chromatin organizations as either strong global folding or randomized chromatin interactions. For example, *Aedes aegypti* (CBS: 2.39; Fig. 2F) displays a strong center-end-axis global folding pattern, which may restrict long-range chromatin interactions and thereby hinder the formation of checkerboard. Maps of *Caenorhabditis elegans* (CBS: 2.53) lack organized compartmentalization and instead show random spot-like interactions, probably resulting from the unrestricted thermal collisions of chromatin. Additionally, some species exhibit intermediate, transitioning chromatin architectures. For instance, the African clawed frog, *Xenopus laevis* (CBS: 2.67), and the Oak Beauty moth, *Biston stratarius* (CBS: 2.75) show mixed Hi-C patterns, where certain loci display checkerboard patterns, while others exhibit global folding or random interactions (Fig. 2G). This suggests that genomes may undergo a gradual evolutionary shift, transitioning from weaker to stronger checkerboard patterns.

Checkerboard patterns emerge from selective interactions between genomic regions, leading to a well-organized chromatin structure (Fig. 2H). For example, in human (CBS: 2.97), the genome is divided into two compartments, where loci within the same compartment interact tightly, while those in different compartments repel[2]. Interestingly, some species with slightly lower checkerboard scores, such as the chaffinch, *Fringilla coelebs* (CBS: 2.90), display an alternative “one compartment” architecture (Fig. 2H), in which a single compartment dominates, while the other regions remain less structured. The “one compartment” architecture appears more prevalent in reptiles (Lepidosauria) and birds (Aves) than in mammals (Mammalia) (Mann Whitney U test *p*-value of reptiles: 0.034; birds: 0.018; Fig. 2I). This suggests that in these species, genomes might be capable of assembling only a single chromatin hub, reflecting an intermediate evolutionary stage preceding the well-established A/B compartments.

Evolution of genome architectures along the tree of life

To investigate the evolutionary dynamics of genome architectures, we analyzed 21 taxonomic groups across prokaryotes, fungi, plants and animals (Fig. 3). Each group includes Hi-C data from at least four species, ensuring statistical robustness and minimizing the influence of outliers (numbers shown in Fig. 1A).

Global folding strength does not follow a consistent evolutionary trend (Fig. 3A; detailed scores are documented in Data. 9). In plants, both intra- and inter-chromosomal global folding scores (GFSs) peak at Ranunculales, a basal eudicot lineage (median intra-GFS 3.60 vs. 0.95 for all species; inter: 4.02 vs. 1.04). After this peak, GFSs gradually decline. In vertebrates, GFSs reach a lower peak at amphibians (Amphibia; intra GFS: 0.99; inter: 2.02), decreasing further both in birds (Aves; 0.29; 0.38) and mammals (Mammalia; 0.54; 0.38). Among invertebrates, only roundworms (Nematoda; 2.38; 1.93) exhibit strong GFSs, while others lack pronounced global folding patterns. Overall, global folding strength fluctuates without a clear evolutionary trend, suggesting that it is not shaped by a universal evolutionary force.

We next examined whether GFS correlates with organismal complexity, using a direct measure of complexity, cell type number (CTN)[18,19] as well as an indirect one, body length[20] (a proxy for long-term effective population size, itself a strong correlate of complexity; values are documented in Data. 10). We observed a negative correlation between GFS and CTN (SCC of intra-GFS: -0.90, *p*-value: 0.02; inter: -0.78, *p*-value: 0.02), and also a weaker but still negative correlation with body length (intra: -0.36, *p*-value: 0.002; inter: -0.31, *p*-value: 0.051; Fig. 3C), suggesting that species with higher complexity tend not to be associated with strong global folding patterns.

In contrast to GFS, checkerboard score exhibits a clear evolutionary trend. In both invertebrate and vertebrate animals, as well as in eudicots, CBS steadily increases over evolutionary time (Fig. 3B; documented in Data. 11). In addition, CBS is strongly and significantly associated with organism complexity as evidenced by the strong correlations with CTN (SCC: 0.95; *p*-value: 0.0002) and body length (SCC: 0.55; *p*-value: 0.0002; Fig. 3D). As stronger checkerboard patterns reflect greater levels of chromatin compartmentalization, these findings are consistent with the hypothesis that gene expression becomes more precisely coordinated in species with greater complexity.

Distinct evolutionary trajectories of genome architectures

To explore the evolutionary trajectory of genome architectures, we traced both global folding and checkerboard patterns of extant species and inferred their ancestral states. By mapping species onto a two-dimensional matrix based on global folding and checkerboard score, we identified a spectrum of genome organizations, ranging from disordered maps (e.g., *Sphagnum tenellum*), strong global folding (e.g., *Papaver bracteatum*), strong checkerboard (e.g., *Homo sapiens*), and mixed architectures (e.g., *Verbascum thapsus*) (Fig. 4A). Generally, GFS negatively correlates with CBS (intra-chromosomal SCC: -0.26, *p*-value $< 10^{-16}$; inter SCC: -0.34, *p*-value $< 10^{-16}$, shown in Fig. S13). This supports the hypothesis that global folding physically obstructs checkerboard formation, likely due to conflicted spatial constraints.

To infer the genome architecture of the last eukaryotic common ancestor (LECA), we analyzed 14 unicellular eukaryotes (including five algae and eight fungi) and also 17 earliest multicellular animal groups, Porifera. Most of these early life forms exhibit random chromatin interaction maps (30 of 31), lacking strong global folding or checkerboard patterns (median GFS: 0.79, median CBS: 2.50 for unicellular eukaryotes, Fig. 4B; median GFS: 1.07, median CBS: 2.64 for Porifera, Fig. S14 and S15). Taken together, these observations suggest that LECA possessed a disordered genome structure with weak global folding and weak checkerboard patterns (Fig. 4C).

In bacteria and archaea, genome architectures vary. Bacteria either exhibit strong global folding (4 of 5 analyzed bacteria; average GFS: 3.26; Fig. 4B), or a partial checkerboard (*Escherichia coli*; CBS: 2.83; Fig. S16). These divergent architectures are diminished or disrupted by the knockout of structural proteins (see Supplementary File), indicating that the last bacterial common ancestor (LBCA), which lacked such proteins, had an unstructured genome architecture, similar to that in knockout bacteria (Fig. 4C). Archaea lack notable global folding patterns (average GFS: 1.00), but exhibit a spectrum of checkerboard patterns from weak to strong (Fig. 4B). As with bacteria, we conjecture that the last archaeal common ancestor (LACA) also lacked strong genome architectures. Notably, the checkerboard patterns in archaea are coarser than in mammals, with larger interaction domains (median size: 4.96% of chromosome length vs. 3.17% of mice, Mann Whitney U test *p*-value: 0.0009, and 3.14% of human, *p*-value: 0.0007; Fig. S17). Similarly, we inferred the genome architecture of the last universal common ancestor (LUCA), from which the fundamental prokaryotic domains (archaea and bacteria) diverged[21], to lack both global folding or checkerboard architectures (Fig. 4C).

From the random architectures of the common ancestors, animals and plants have evolved distinct spatial organizations. In both vertebrates and invertebrates, checkerboard patterns, indicative of functional compartmentalization, appear to increase over evolutionary time (Fig. 4D; Fig. S14 and S15). The trajectory begins with the disordered structures in fishes, transitions through “one-hub” architectures in reptiles and birds, and culminates at the A/B compartments in mammals. This may reflect an increased capacity to spatially cluster (and thereby coordinate the activity of) genes otherwise scattered throughout the genome.

Interestingly, we observed a similarly progressive emergence of checkerboard patterns during human early embryo development. Chromatin undergoes a disruption of global folding and a stepwise reconstruction of checkerboard architectures from the 2-cell stage (intra-GFS: 3.01, inter-GFS: 3.45, CBS: 2.31) to 6-week embryo (intra-GFS: 0.24, inter-GFS: 1.04, CBS: 2.93) (Fig. 4E; Fig. S18). The construction of checkerboard mirrors the evolutionary trend in animals, which suggests that the checkerboard pattern is a fundamental architectural feature required for gene regulation, enabling the emergence of increasing complexity.

Unlike animals, plants exhibit a markedly different evolutionary trajectory. During plant evolution, global folding surges dramatically, around the divergence of Liliopsida and Ranunculales (Fig. 4F). While checkerboard patterns increase modestly in eudicots (Fig. 4F, S14 and S15), they remain significantly weaker than in animals (median CBS of Rosids: 2.56, Mann Whitney U test *p*-value 2.5×10^{-11}; Asterids: 2.60, *p*-value 2.4×10^{-9} compared to mammals: 2.88; Fig. S19). The dominance of global folding may physically affect checkerboard formation, constraining the long-range spatial compartmentalization of plant genomes, as seen in animals.

Despite their weaker checkerboard patterns, plants still require coordinated gene expression, particularly for their complex secondary metabolic pathways[22]. Instead of spatial clustering functionally associated genes, it is plausible that plants adopt an alternative strategy. For example, in opium poppy, *Papaver somniferum*, genes involved in the noscapine and morphine biosynthesis pathways are linearly adjacent on the genome, forming a localized gene cluster[23]. Consistent with this being a more widespread phenomenon, we found that genes within the same pathways are more proximal in plants than in vertebrates, as evidenced by a significantly higher probability of being on the same chromosome (median probability: 6.3% in plants compared to 3.9% in vertebrates besides mammals and 4.1% in mammals; Mann Whitney U test *p*-value $< 10^{-16}$) and closer genomic distances (median distance between genes on same chromosome: 1.1×10^{7} bp in plants compared to 2.4×10^{7} bp in vertebrates and 3.6×10^{7} bp in mammals; *p*-value $< 10^{-16}$; Fig. 4G). This supports the hypothesis that in contrast to the spatial compartmentalization of genomes observed in animals, plant genomes have a greater degree of linear gene clustering.

DISCUSSION

Evolution of higher-order genome architectures

Our analysis of Hi-C data across 1,025 species suggests general principles governing 3D genome evolution, that originating from the random genome architectures of common ancestors, two organized, low-entropy genome architectures evolve, which we refer to as global folding and checkerboard. In prokaryotes, the majority of bacteria (4 of 5) exhibit global folding, whereas a majority of archaea (3 of 4) display coarse checkerboard patterns. This dichotomy extends to eukaryotes: plants evolved pronounced global folding, while invertebrate and vertebrate animals progressively strengthened checkerboard organization. The sustained organization of these architectures, energetically unfavorable yet prevalent, implies strong selective forces and specialized regulatory maintenance.

Protein complexity facilitates architectural sophistication

The evolution of genome architectures is paralleled with increasing complexity of structure-maintenance machinery. In bacteria, the center-end-axis global folding pattern is regulated by a single SMC protein, suggesting its relatively simple regulatory mechanisms. In contrast, global folding in *Arabidopsis thaliana* is controlled by a more complex, two-step regulation involving multiple proteins[24]. Similarly, species exhibiting the strongest checkerboard patterns demonstrate advanced mechanisms, such as the specialized architectural proteins in *Drosophila* species[25,26] and the specific N-terminal of CTCF in mammals[26,27]. These observations refute the notion of genome architectures as passive byproducts, instead positioning them as actively engineered systems shaped by specialized protein-mediated mechanisms.

Global folding and cellular plasticity

Although global folding lacks a correlation with species complexity, its association with cellular plasticity offers a compelling functional hypothesis. Plants, which exhibit the strongest global folding, retain widespread cellular totipotency[28], and human 2-cell stage embryos (highly plastic stem cells) similarly display evident global folding. This parallels with prokaryotic systems, where disrupting global folding patterns (e.g., via SMC knock out in *B. subtilis*[29] or *C. crescentus*[30]) impairs chromosome segregation and cell cycle progression, as also seen in yeast mutants[31]. Taken together, we propose that global folding may serve as a scaffold for maintaining cellular pluripotency, with potential implications for regenerative medicine and developmental biology.

Checkerboard and cellular specialization

The modular organization of functionally related genes is crucial for coordinated regulation, serving as a basis for cellular specialization. Two common mechanisms for achieving modularity are observed: linear genomic clustering or spatial clustering via checkerboard architectures. Linear clustering is particularly prevalent in species with random or global folding patterns, which lack fine-tuned spatial compartmentalization, as exemplified by operons in prokaryotes[32] and gene clusters in plants[23]. This arrangement may enable more rapid and dynamic response to fluctuating external stimuli, such as unpredictable weather, with less time and energetic expense; they would not need to be spatially coordinated first. This could be particularly advantageous for sessile organisms like plants[33]. However, linear clustering does impose evolutionary constraints, favoring genetic innovation through polyploidization, despite its rarity and associated risks of genome instability[34].

In contrast, checkerboard architecture offers a spatially mediated mechanism, which we found to positively correlate with species complexity. By spatially organizing scattered genes into close proximity, checkerboard provides greater flexibility in gene regulation, which, in turn, facilitates the increasingly diverse cell types. Nevertheless, the transition towards checkerboard accompanies the loss of pluripotency. Interestingly, besides animals, we also observed weak but notable checkerboard-like organization in some eudicots, albeit constrained by their dominant global folding architecture (Fig. 4F, illustrated in Fig. 5). This indicates that besides linear clustering, some eudicots have also evolved a capacity to spatially cluster co-expressed genes. For example, studies of *Arabidopsis thaliana* have shown that co-regulated genes can be brought together through 3D topologies, even over long chromosomal distance[35]. These findings indicate that, relative to global folding, checkerboard structures are a more flexible genome architecture, the use of which over evolution have facilitated the increases of organism complexity.

Trade-off between plasticity and specialization

The evolution of species complexity is a testament to the ingenuity of life. The distinct trajectories of 3D genome organization underscore the potential trade-offs between global folding (associated with cellular plasticity) and checkerboard organization (linked to specialization), through which each evolutionary lineage or embryonic developmental trajectory has forged its own path (Fig. 5).

Overall, our findings expand the axiom "structure determines function" to high-order genome architectures, which shape high-level biological processes, such as cellular specialization or plasticity. This bridges evolutionary biology and developmental biology, offering insights for regenerative medicine (e.g., enhancing cellular plasticity) and synthetic biology (e.g., engineering gene regulation networks). Future studies dissecting the interplay between these architectures in diverse lineages will further unravel how life's complexity is encoded in the 3D foldings of the genome.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Kai Ye (kaiye@xjtu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- This paper analyzes existing, publicly available data, accessible at Darwin Tree of Life project or NCBI. Detailed sources for each species are listed in Supplementary File 1.
- All original code has been deposited at https://github.com/xjtu-omics/HiArch and is publicly available at [DOI] as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: K.Y. and Y.C. Methodology: Y.C. Investigation: Y.C. and Y.L. Formal Analysis: Y.C. Funding Acquisition: K.Y. Writing – Original Draft: Y.C. Writing – Review & Editing: K.Y., S.B., X.Y. Supervision: K.Y.

DECLARATION OF INTERESTS

Authors declare that they have no competing interests.

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FIGURE TITLES AND LEGENDS

Fig. 1. Analytical framework for deciphering higher-order genome architectures

The top panel displays the taxonomic distribution of 1,025 species, spanning prokaryotes (archaea and bacteria), fungi, plants and animals (invertebrates and vertebrates). Computational pipeline for high-order genome architectures comprises three modules: 1) NormDis, which re-weights Hi-C intearactions to remove distance-dependent biases to reveal preferred interactions (marked in red) and repelled interactions (marked in blue). 2) global folding quantification: normalized maps are pre-processed through centralization and symmetrization, and then major global folding types were discovered via unsupervised clustering; 3) checkerboard strength, which is quantified by the information entropy of interaction similarities. Higher entropy reflects stronger segregation of active and inactive compartments. Details are described in Methods.

Fig. 2. Diversity of global folding (A-D) and checkerboard patterns (E-I) across species.

(A) Major global folding types. Five Intra- and five inter-chromosomal global folding types were identified through unsupervised clustering, shown by t-SNE projection. Previously characterized structures are annotated with schematics, while unlabeled ones represent novel foldings. The distribution of folding types is represented by their chromosomal map counts across all analyzed samples, reflecting their biological prevalence. (B) Enrichment of global folding across major taxonomic groups. Global folding scores for intra- and inter-chromosomal patterns across major taxonomic groups: prokaryotes (intra: n=9; inter: n=2), fungi (n=22 for both), plants (n=107), invertebrates (n=766), and vertebrates (n=126). Red asterisks (*) denote significant enrichment (one-tailed Wilcoxon signed-rank test, $p < 0.05$). (C) Interplay between intra- and inter-chromosomal folding types. Spearman correlation coefficients between folding types were calculated to quantify the association between distinct folding types. Coefficients were computed using global folding scores derived from 1,025 species. (D) Global folding polymorphism. Representative normalized map of Clonorchis sinensis demonstrates the coexistence of multiple global folding types within a single genome. (E) Distribution of checkerboard scores across all 1,025 species. Colored points pinpoint the checkerboard score for representative species. (F) Example species lacking checkerboard pattern. These species often exhibit global folding or randomized interaction patterns, reflecting relatively homogeneous interaction networks. (G) Intermediate species with mixed checkerboard and global folding/random regions. (H) Checkerboard organization. The second row exhibits hierarchically clustered contact maps, with black lines marking the segregation of compartments. The accompanying illustrations depict the spatial compartments, with different colors representing different compartments. (I) Interior contact scores of compartments, indicating their compaction levels. Positive values indicate condensed chromatin states, while negative values reflect dispersed domains.

Fig. 3. Evolutionary trends of high-order architectures and their correlations to species complexity.

(A) Intra- and inter-chromosomal global folding scores (GFS) across 21 phylogenetically ordered taxonomic groups (group sizes shown in Fig. 1A). (B) Checkerboard scores (CBS) for the same taxonomic groups. (C) Global folding scores show negative correlation with species complexity proxies: cell type number (CTN; n=8) and body length (n=26). (D) Checkerboard scores significantly correlate with species complexity proxies: cell type number (CTN; n=8, p=0.0002) and body length (n=26, p=0.0002), underscoring their role in facilitating biological complexity.

Fig. 4. Evolutionary and developmental trajectories of 3D genome architecture.

(A) Negative correlation between checkerboard score and intra-chromosomal global folding score across 1,025 species. The correlation with inter-global folding score is shown in Fig. S13. Example species illustrate the spectrum of architectures: Sphagnum tenellum (random), Papaver bracteatum (strong global folding), Homo sapiens (strong checkerboard), and Verbascum thapsus (mixed architectures). (B) Unicellular organisms (including bacteria, archaea, algae, fungi, and Discoba) mostly exhibit random architecture without both architectures. Left: normalized maps of representative bacteria and its mutant

(S. venezuelae) show that SMC knockout disrupts global folding. Right: example archaea with coarsen checkerboard patterns. (C) Evolutionary trajectory of 3D genome architectures. Schematic of genome architecture evolution from the disordered structures of common ancestors to diverse architectures of extant species: bacteria mostly adopt global folding; archaea have developed rudimentary checkerboard; each lineage of eukaryotes has forged its own paths, where animals exhibit checkerboard patterns along with spatial gene clustering, and plants displays global folding patterns with linear gene clustering. (D) Evolution of vertebrate genome architecture. Progressive strengthening of checkerboard architectures is observed in vertebrates. Representative groups are shown with exemplified maps: Actinopteri (weak checkerboard), Lepidosauria (intermediate checkerboard with one compartment) and Mammalia (strong checkerboard with two compartments). Other groups are shown in Fig. S14 and S15. (E) Genome architectural transition during early human embryogenesis: strong global folding of 2-cell stage to checkboard patterns of 6-week embryo. (F) Evolution of plant genome architecture. Divergent patterns appear in major plant clades: Chlorophyta (random map), Ranunculales & Liliopsida (dominant global folding) and Rosids & Asterids (persistent global folding with weak checkerboard). (G) Lineage-specific genomic arrangements of pathway genes. Pathway genes of plants (n=784 pathways) adopt linear clustering as indicated by the higher probability of locating on the same chromosome and shorter intra-chromosomal distance. Genes of vertebrates (n=2087 for vertebrates except Mammalia, n=2463 for Mammalia) exhibit a more scattered distribution. Statistical significance was assessed using Mann-Whitney U tests.

Fig. 5. Trade-offs between two high-order genome architectures during evolution or development.

The horizontal axis represents species complexity at maturity—the maximum attainable complexity of an organism. The vertical axis reflects developmental complexity, which is the degree of functional partitioning during ontogeny. While evolutionary and developmental complexity are inherently related and are not suitable for being represented as independent axes, we employ this simplified framework to illustrate the trajectories of 3D genome architecture during evolution and development. Animal evolution (solid red curve) exhibits progressive reliance on checkerboard architecture, driving the increase of species complexity but reducing developmental plasticity. Plant evolution (solid green curve) shows sustained global folding, maintaining cellular totipotency. Human embryogenesis (dashed red curve) recapitulates evolutionary transitions of genome architectures: early embryos prioritize global folding (2-cell stage), followed by checkerboard emergence (8-cell stage to 6-week embryo). The distinct evolutionary and developmental trajectories reflect a universal trade-off between genome architectures: checkerboard enables functional specialization at the cost of plasticity, while global folding preserves adaptability while limiting regulatory sophistication.

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STAR METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Species Hi-C data	Darwin Tree of Life project	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

METHOD DETAILS

Pre-processing of Hi-C matrix

A Hi-C dataset comprising 1,517 species was collected. The tree of life was downloaded from NCBI Taxonomy. Species that could not be found in the taxonomy dataset were excluded, resulting in a total of 1,475 species. The sources and detailed information, including species names and NCBI species indices, are listed in Supplementary Table 1.

Hi-C maps are capable of reflecting the quality of genome assembly (examples shown in Figure S20). We manually checked the assembly quality and removed 450 species and 710 chromosomes with low-quality assembles (documented in Supplementary Table 1). At last, 1,025 species with high-quality assembles are kept.

To normalize the distinct genomic lengths among species, Hi-C maps are automatically scaled based on two criteria: 1) to make the number of bins per chromosome approximately the same, and 2) to ensure sufficient contacts at both intra- and inter-chromosomal scales. Notably, chromosomes with extremely sparse contacts, which typically result from the absence of certain chromosomes (e.g., sex chromosomes), are removed.

NormDis: Normalization to remove proximity effects

The NormDis algorithm is applied to eliminate distance-dependent biases on Hi-C maps, which is introduced by the polymeric nature of DNA molecules. The main idea is to make Hi-C contacts distance-independent by normalizing contacts by expected contact number,

$$NC_{ij} = \frac{C_{ij}}{\overline{C_d}},$$

where C_{ij} is the raw Hi-C contact of row i and column j, NC_{ij} is the normalized contact, $\overline{C_d}$ is the expected contact of distance $d = |i - j|$.

Previous studies showed that Hi-C contacts associate with genomic distances in a log-log correlation[36]. Based on this, we tried to calculate $\overline{C_d}$ by linear fitting of logarithms of genomic distance and Hi-C contacts (Figure S21), calculated by:

$$\log(\overline{C_d}) = LF(log(C_d),\ log(distance)),$$

where LF represents the linear fitting optimized by the ordinary least squares. However, this linear correlation is observed only in a narrow interval of genomic distances (e.g., 100 kb to 10 Mb in *Acropora millepora*, as shown in Figure S21). Beyond this interval, deviations emerge, as shown at the distal regions of the *A. millepora* contact map. These deviations disrupt linear fitting accuracy, generating regional overestimations and underestimations that collectively impair normalization precision.

Diagonal mean values provide more accurate expected values for normalization,

$$\overline{C_d} = Mean(C_d).$$

However, near the matrix corners, the shorter diagonal length increases sensitivity to outliers (Figure S21), often resulting in normalization errors.

Consequently, we developed a scaled diagonal method that aggregates long-distance diagonals to ensure computational robustness while preserving accuracy, by

$$\overline{C_d} = Mean(C_{d'}), where d' = \{d_i | Floor\left(\frac{log_2^d + k}{k}\right) = Floor\left(\frac{log_2^{d_i} + k}{k}\right)\}.$$

where k controls the number of diagonals to be aggregated. Notably, based on the linear nature of chromosome, expected contacts should be theoretically decreasing monotonically with genomic distances. However, some species, for example *Acropora millepora*, exhibit anomalously elevated long-range contacts, likely reflecting its unique global folding patterns (Figure S21). To preserve these signals, we enforce monotonic decay in the expected contact values.

Long-distance contacts are exposed after NormDis. To test whether these contacts reflect real physical structures within nuclei, we conducted a DNA FISH experiment, labeling centromeres and telomeres of opium poppy (*Papaver somniferum*; Figure S2B). Normalized maps of poppy display a special pattern, including the anti-diagonal lines on intra-maps and X-shape on inter-maps. These patterns indicate that centromeres and telomeres of long chromosome arms independently cluster at the two side of nuclei, while the short-arm telomeres freely float in between. This structure is evident by the reconstructed structures and the DNA FISH images, which indicates the consistence of normalized maps and real physical structures.

Detection and measurement of global folding

Preprocessing for global folding

To unveil the global folding patterns from the normalized maps, a preprocessing pipeline is applied (Figure S22A; detailed in Supplementary Methods). Notably, maps sharing the same global folding type may exhibit distinct visual patterns, due to variations in the genomic positioning of their center anchors. It is exemplified in *Papaver bracteatum* (Figure S22A), where two separate maps from different chromosomes nevertheless preserve the same anti-diagonal center-end-axis pattern. To address this, we developed CenterFinder, a tool to identify center anchors (Figure S22B). It offers two modes tailored to different types of global folding:

1. The 'Inter row sum' mode identifies center anchors by detecting local maxima in inter-chromosomal contact row sums, making it optimal for anchors exhibiting prominent inter-chromosomal interactions.
2. The “Intra X” mode identifies center anchors by locating the anti-diagonal midpoints, specifically designed for intra-chromosomal center-end-axis patterns.

Typically, anchor with the strongest signal is designated as the default center anchor, but it can be manually adjusted. The mode used and manually re-chosen anchors are documented in Supplementary Table 12 and 13.

Global folding pattern mining

Global folding pattern mining extracts biologically representative patterns from high-noise maps via robust statistical analysis (Figure S22C). We initially filtered for strong global folding maps by selecting the top 5% of inter- and top 20% of intra-chromosomal maps exhibiting minimal dissimilarity between average-pooled and symmetrized maps, effectively excluding maps lacking strong folding patterns.

After the above pre-processes and selection, maps still contain considerable noises, which will hinder subsequent pattern mining. To address this issue, we apply sparse representation techniques to reduce the dimension of each map, as well as to reduce noise. Specifically, we assume that each processed map can be approximately reconstructed as a linear combination of a small set of features. Formally, for the i-th sample, $x_i \in R^{256}$, we assume that

$$x_i = Uv_i + n_i,$$

where $U \in R^{256 \times k}$ denotes the dictionary matrix, shared across all maps, and randomly initiated; k is the element number of the dictionary; $v_i \in R^k$ is a k dimensional representation of x_i; and n_i denotes the corresponding noise. It should be noted that x_i represents distinct sparse patterns, thus it is reasonable to assume that v_i can be low-dimensional (*i.e.*, k is a small number) and sparse. We can simultaneously

perform sparse representation on all maps, by solving the following sparse principal component analysis model:

$$\min_{U,V} \|X - UV\|_F^2 + \lambda \|V\|_1,$$

where $X = [x_1, x_2, \cdots x_N]$ represents N input maps; $V = [v_1, v_2, \cdots v_N]$ represents the sparse representation for all maps; and λ controls the trade-off between reconstruction accuracy and sparsity of V. Both the dictionary U and the sparse representation V will be jointly optimized using a bi-level iterative algorithm. After optimized sparse representation V is achieved, we can view v_i as noise-reduced k-dimension representations of x_i.

With noise-reduced k-dimension representations, we then apply t-SNE[13] to visualize and DBSCAN[14] to cluster. The average pattern of each cluster is designated as the representative pattern for each global folding types. In total, we identified five types for intra-chromosome and five for inter-chromosome (Figure 2A).

Although the dictionary matrix in sparse dimension reduction is randomly initiated, we re-run the pattern mining and still got the same types (Figure S23), indicating the robustness of our procedures.

We then compute the similarities of maps to identified types, serving as the global folding scores (GFS), by

$$GFS = \sum_i X_i \cdot P_i,$$

where X_i is the i-th value from map, P_i is the i-th value from type. Higher global folding score denotes higher alignment to folding type. GFSs for 1,025 species are documented in Supplementary Table 2 and 3.

Measurement of checkerboard strength

The checkerboard pattern, displayed as the crisscrossed textures on normalized maps, signifies the physical separation into genomic compartments. Checkerboard 'strength' reflects the degree of separation, characterized by two components: 1) the tight connections within the interior compartment, and 2) repelled contacts between different compartments. Accordingly, we measure the checkerboard strength by firstly computing the similarities between genomic bins to reveal the connections (high similarities) or repulsions (low similarities), and then measuring the strength of connections and repulsions (Figure S24).

The "cosine" distance metric is utilized for assessing the similarities,

$$D_{ij} = cosine\left(C_{ix}, C_{xj}\right) = \frac{\sum_{x=1}^{n} C_{ix} C_{xj}}{\sqrt{\sum_{x=1}^{n} C_{ix}} \sqrt{\sum_{x=1}^{n} C_{xj}}}$$

where C_{ij} is the raw contact of bin i and bin j, $cosine$ is the cosine distance, D_{ij} is the distance between bin i and bin j. Lower distances indicate more similar connections, whereas higher distances reflect weak or repelled contacts between bins. Maps exhibiting pronounced checkerboard patterns show a bimodal distribution of similarity values (high and low; Figure S24), resulting in a high information entropy,

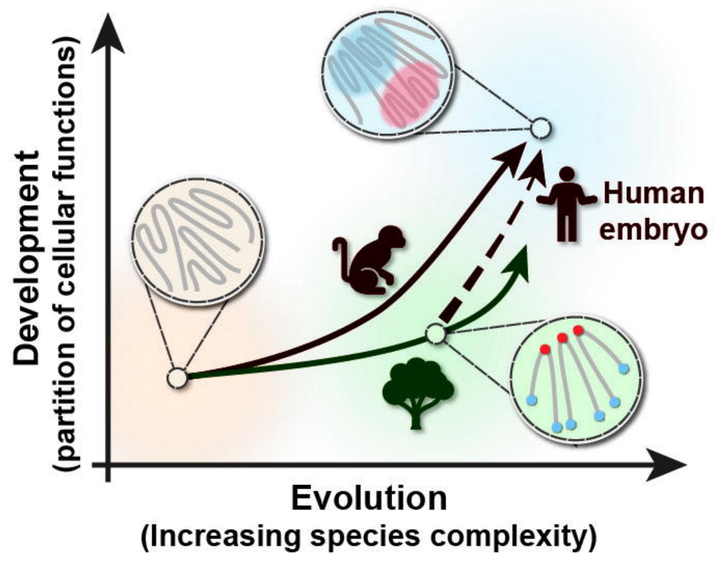
$$CBS_d = Entropy\left(D_{|i-j|=d}\right) = -\sum_x P(D_x) \log_2 P(D_x)$$

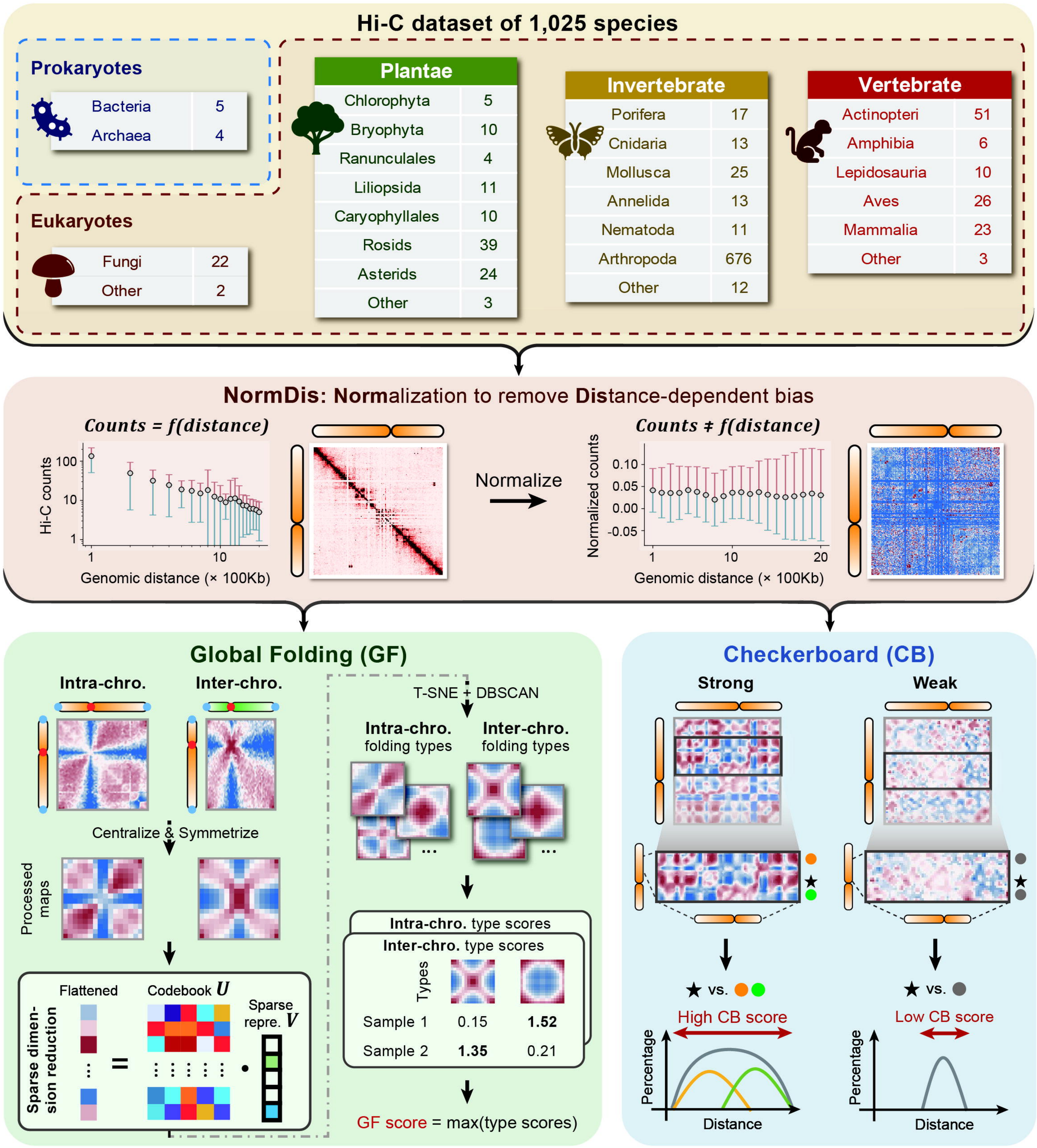
where $D_{|i-j|=d}$ is the cosine distances with genomic distance d. In contrast, random maps exhibit intermediate similarities, reflecting neither preferential attraction nor repulsion between genomic bins, and result in lower entropies.

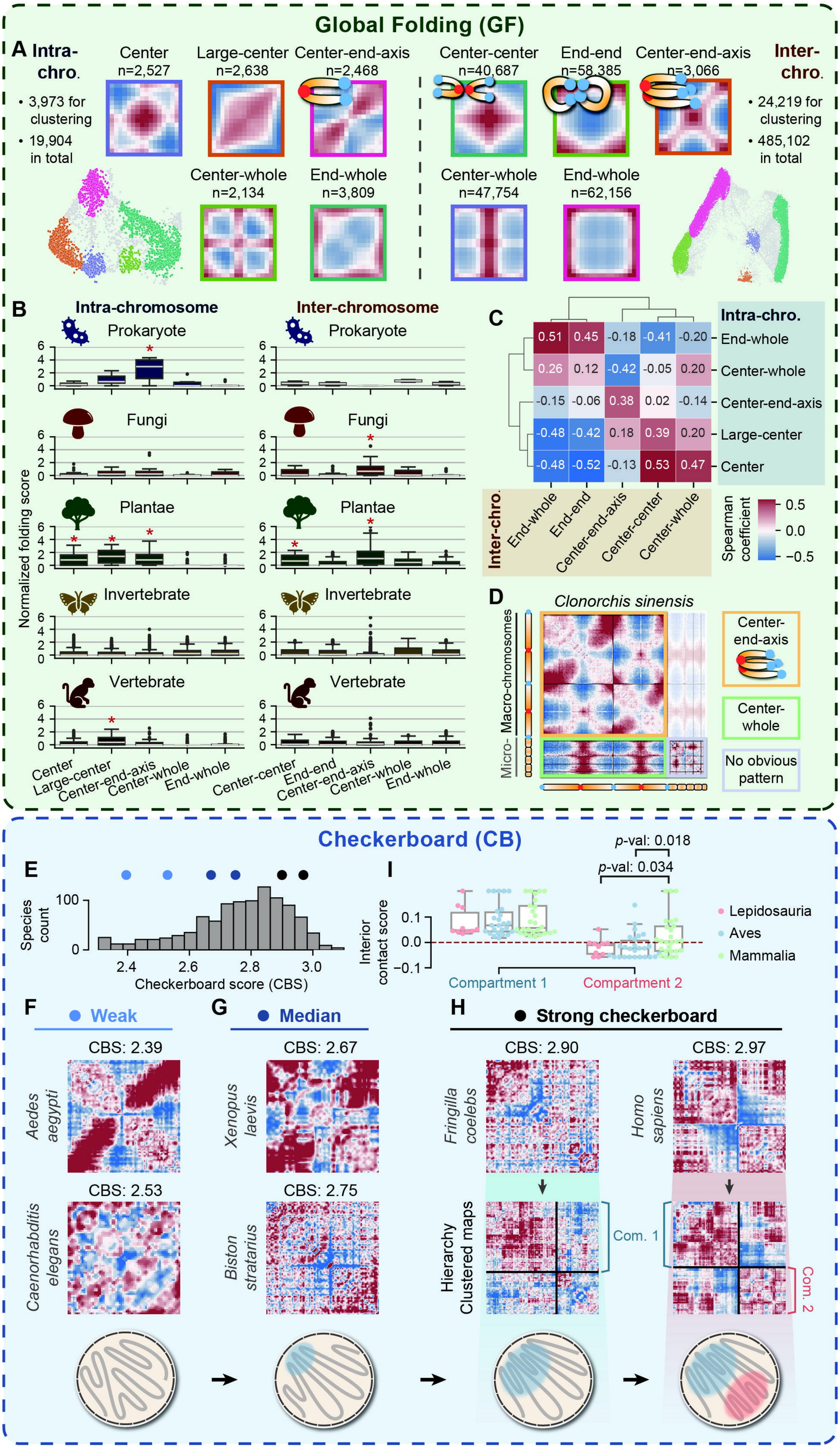
Even without a checkerboard (Figure S24), map with strong global folding can produce high entropy in whole-map calculations due to opposing short-distance and long-distance similarities. We addressed this by calculating distance-stratified entropies and averaging them into the checkerboard score,

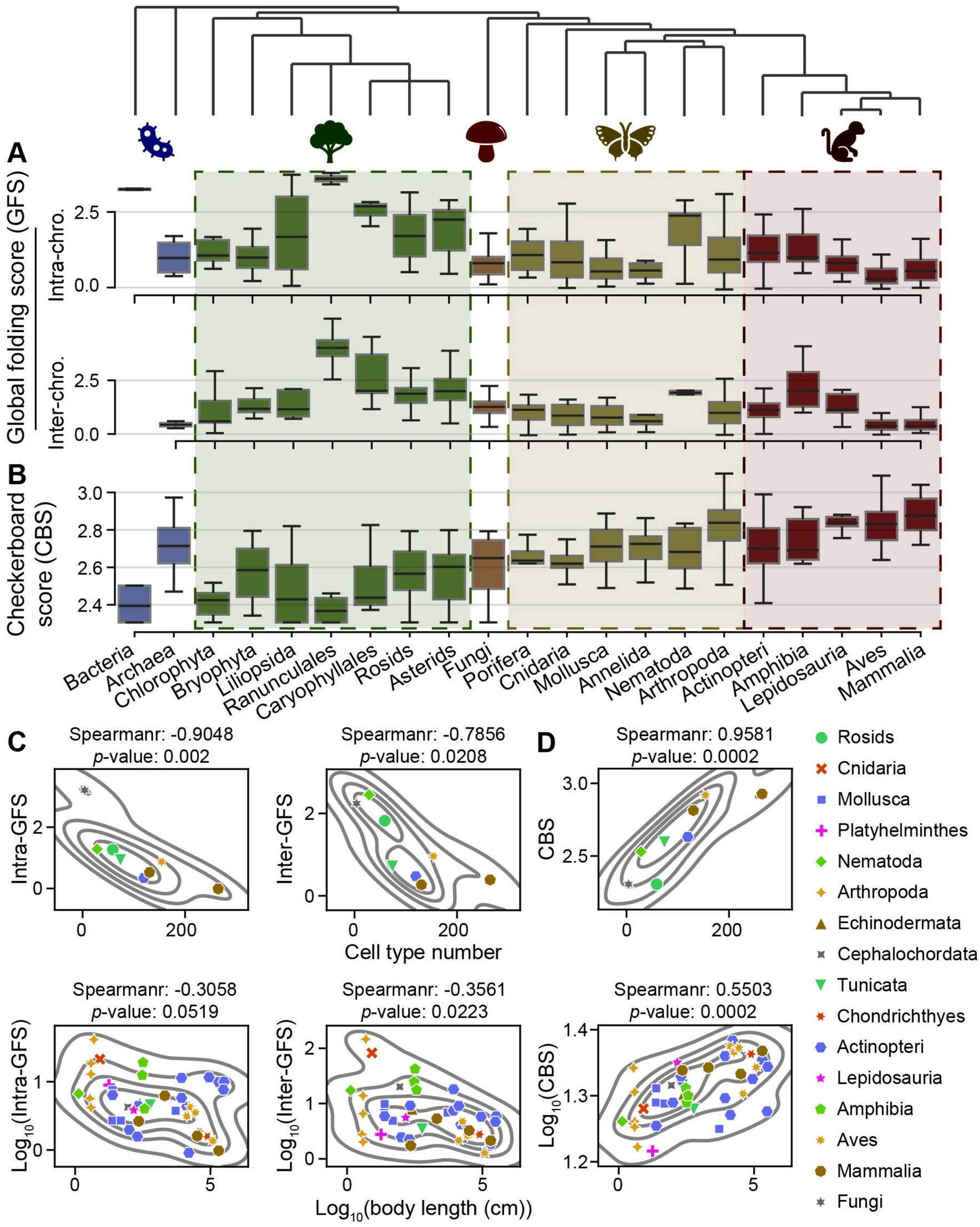
$$CBS = average(CBS_d)$$

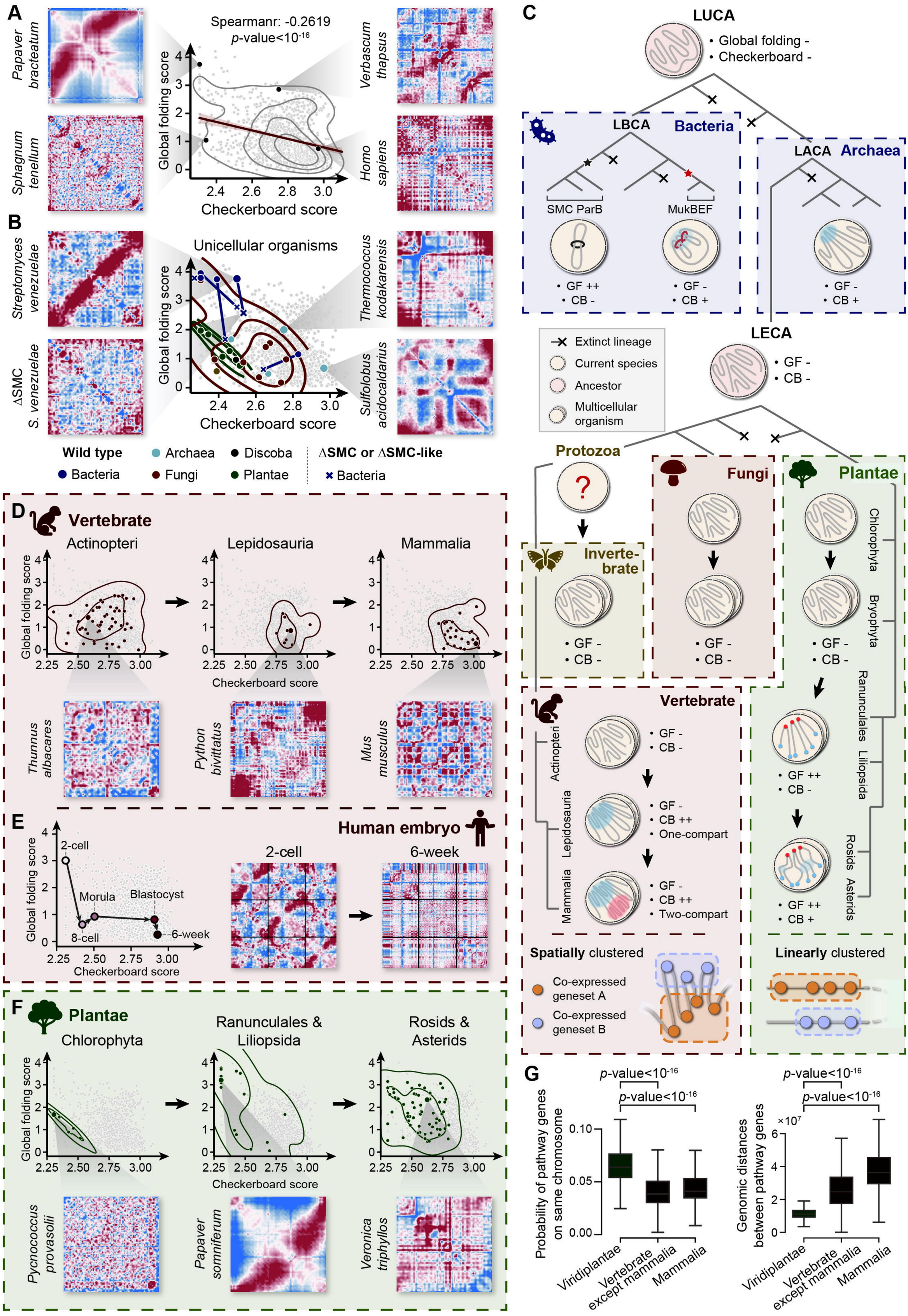
where CBS_d are the distance-stratified entropies. Given the distance-dependence of this analysis, we omitted long-range interactions to avoid distortion from diminishing diagonal lengths. 15% chromosome length is set as the threshold for this long-distance removal. This threshold does not affect the entropy or subsequent results, as consistent results are obtained for thresholds of 10% or 20% (Figure S25). CBSs for 1,025 species are documented in Supplementary Table 4.


Development
(partition of cellular functions)
Human
embryo
Evolution
(Increasing species complexity)


Hi-C dataset of 1,025 species
Prokaryotes
Bacteria 5
Archaea 4
Eukaryotes
Fungi 22
Other 2
Plantae
Chlorophyta 5
Bryophyta 10
Ranunculales 4
Liliopsida 11
Caryophyllales 10
Rosids 39
Asterids 24
Other 3
Invertebrate
Porifera 17
Cnidaria 13
Mollusca 25
Annelida 13
Nematoda 11
Arthropoda 676
Other 12
Vertebrate
Actinopteri 51
Amphibia 6
Lepidosauria 10
Aves 26
Mammalia 23
Other 3
NormDis: Normalization to remove Distance-dependent bias
Counts = f(distance)
Hi-C counts
Genomic distance (× 100Kb)
Normalize
Counts ≠ f(distance)
Normalized counts
Genomic distance (× 100Kb)
Global Folding (GF)
Intra-chro.
Inter-chro.
Centralize & Symmetrize
Processed maps
Flattened
Codebook U
Sparse repre. V
Sparse dimension reduction
T-SNE + DBSCAN
Intra-chro. folding types
Inter-chro. folding types
Intra-chro. type scores
Inter-chro. type scores
Types
Sample 1 0.15 1.52
Sample 2 1.35 0.21
GF score = max(type scores)
Checkerboard (CB)
Strong
Weak
vs.
High CB score
Low CB score
Percentage
Distance


Global Folding (GF)
A Intra-chro.
• 3,973 for clustering
• 19,904 in total
Center n=2,527
Large-center n=2,638
Center-end-axis n=2,468
Center-whole n=2,134
End-whole n=3,809
Inter-chro.
• 24,219 for clustering
• 485,102 in total
Center-center n=40,687
End-end n=58,385
Center-end-axis n=3,066
Center-whole n=47,754
End-whole n=62,156
B
Intra-chromosome
Inter-chromosome
Prokaryote
Fungi
Plantae
Invertebrate
Vertebrate
Normalized folding score
Center
Large-center
Center-end-axis
Center-whole
End-whole
Center-center
End-end
C
Intra-chro.
Inter-chro.
Spearman coefficient
D
Clonorchis sinensis
Macro-chromosomes
Micro-
Center-end-axis
Center-whole
No obvious pattern
Checkerboard (CB)
E
Species count
Checkerboard score (CBS)
I
Interior contact score
p-val: 0.018
p-val: 0.034
Lepidosauria
Aves
Mammalia
Compartment 1
Compartment 2
F
Weak
CBS: 2.39
Aedes aegypti
CBS: 2.53
Caenorhabditis elegans
G
Median
CBS: 2.67
Xenopus laevis
CBS: 2.75
Biston stratarius
H
Strong checkerboard
CBS: 2.90
Fringilla coelebs
CBS: 2.97
Homo sapiens
Hierarchy
Clustered maps
Com. 1
Com. 2


A
Global folding score (GFS)
Intra-chro.
Inter-chro.
B
Checkerboard score (CBS)
Bacteria
Archaea
Chlorophyta
Bryophyta
Liliopsida
Ranunculales
Caryophyllales
Rosids
Asterids
Fungi
Porifera
Cnidaria
Mollusca
Annelida
Nematoda
Arthropoda
Actinopteri
Amphibia
Lepidosauria
Aves
Mammalia
C
Spearmanr: -0.9048
p-value: 0.002
Intra-GFS
Spearmanr: -0.7856
p-value: 0.0208
Inter-GFS
Cell type number
D
Spearmanr: 0.9581
p-value: 0.0002
CBS
Spearmanr: -0.3058
p-value: 0.0519
Log10(Intra-GFS)
Spearmanr: -0.3561
p-value: 0.0223
Log10(Inter-GFS)
Spearmanr: 0.5503
p-value: 0.0002
Log10(CBS)
Log10(body length (cm))
Rosids
Cnidaria
Mollusca
Platyhelminthes
Nematoda
Arthropoda
Echinodermata
Cephalochordata
Tunicata
Chondrichthyes
Actinopteri
Lepidosauria
Amphibia
Aves
Mammalia
Fungi


A
Papaver bracteatum
Sphagnum tenellum
Spearmanr: -0.2619
p-value<10-16
Global folding score
Checkerboard score
Verbascum thapsus
Homo sapiens
B
Streptomyces venezuelae
ΔSMC S. venezuelae
Unicellular organisms
Thermococcus kodakarensis
Sulfolobus acidocaldarius
Wild type
Bacteria
Archaea
Fungi
Discoba
Plantae
ΔSMC or ΔSMC-like
Bacteria
C
LUCA
• Global folding -
• Checkerboard -
LBCA
Bacteria
LACA
Archaea
SMC ParB
MukBEF
• GF ++
• CB -
• GF -
• CB +
• GF -
• CB +
LECA
• GF -
• CB -
Extinct lineage
Current species
Ancestor
Multicellular organism
Protozoa
Fungi
Plantae
Inverte-brate
• GF -
• CB -
Chlorophyta
Bryophyta
Ranunculales
Liliopsida
Rosids
Asterids
• GF ++
• CB -
• GF ++
• CB +
Vertebrate
Actinopteri
Lepidosauria
Mammalia
• GF -
• CB -
• GF -
• CB ++
• One-compart
• GF -
• CB ++
• Two-compart
Spatially clustered
Co-expressed geneset A
Co-expressed geneset B
Linearly clustered
D
Vertebrate
Actinopteri
Lepidosauria
Mammalia
Thunnus albacares
Python bivittatus
Mus musculus
E
Human embryo
2-cell
Morula
Blastocyst
8-cell
6-week
F
Plantae
Chlorophyta
Ranunculales & Liliopsida
Rosids & Asterids
Pycnococcus provasolii
Papaver somniferum
Veronica triphyllos
G
p-value<10-16
Probability of pathway genes on same chromosome
Genomic distances between pathway genes
Viridiplantae
Vertebrate except mammalia
Mammalia