 **Discovery-Based Chromosome Biology Education Through Comparative and Functional Genomics Projects in Drosophila**

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**ABSTRACT**

Discovery-based education in chromosome biology can be effectively implemented using Drosophila as a model system. Chromosome biology encompasses genome organization, segregation mechanisms, structural evolution, and epigenetic regulation. Using comparative and functional genomics, students can engage in hypothesis-driven, hands-on exploration that connects theoretical concepts to practical experiments. The wealth of genomic data and genetic tools in Drosophila enables classroom-scale projects, including mapping chromatin states, analyzing transposable elements, and investigating gene regulation and chromosomal dynamics. This approach fosters student ownership, reinforces understanding of the genotype–phenotype relationship, and highlights the integration of chromosome structure, segregation, and evolution within an educational framework. Observational studies of centromeres, telomeres, and chromatid dynamics provide tangible insights into chromosome behavior, supporting both conceptual learning and experimental discovery.

**Keywords:** *Drosophila, chromosome biology, comparative genomics, functional genomics, discovery-based education, chromatin, centromere, telomere, chromosome segregation, genome organization, epigenetics, genotype–phenotype mapping.*

# INTRODUCTION

Chromosome biology encompasses a vast array of themes, including architecture, spatial genome organization, segregation mechanisms, the evolution of chromosome number and structure, and, more recently, the epigenetic landscape and regulation of chromatin dynamics. Introducing students to these concepts using a discovery-based approach can simultaneously enhance engagement and learning outcomes. Comparative and functional genomics in Drosophila provides a framework for exploration that addresses a range of fundamental chromosome-biology questions. The appeal of the system relies on both the genetic toolkit and the impressively characterized genomes of numerous species; Drosophila is the only model organism for which across-species genome-scale annotation and comparative analysis is feasible in the context of classroom-scale student projects. (E. Mohr & Perrimon, 2019).

Using a common baseline of knowledge, students can address questions subjectively selected from within a broader pedagogically determined scope. Such a format, based on iterative refinement of hypotheses and student ownership over experimental direction, deepens understanding of the scientific process while promoting ownership over the specific educational journey (Haudry et al., 2018).

**Conceptual Foundations of Chromosome Biology**

Comparative and functional genomics constitutes an engaging and powerful framework to introduce students to the fundamental principles of chromosome biology through Drosophila. Chromosomes package an organism’s genetic material, ensuring its stable inheritance through generations and guiding it through the intricate processes of cellular life (Bobojonova D., et al).

Chromosome biology has broad relevance to diverse topics, from the laws of inheritance and gene regulation during development to genome evolution and epigenetic control (T. Ables, 2015). Moreover, Drosophila offers an unmatched genetic toolkit and number of well-characterized mutant strains, enabling classroom-scale experiments across comparative and functional genomics. These include insightful projects mapping transposable elements or chromatin states, testing models of chromosomal inversions or polyploidy, and analyzing large-scale expression data and ribosome occupancy to investigate sex-specific regulatory networks, transcriptional bursting, or the functions of essential non-protein-coding genes.

To organize practical implementations effectively, project design focuses on clarifying educational objectives and aligning experimental questions with them. The chosen projects address properties of genome organization, the interplay between genome structure and evolution, and the genotype–phenotype map-all key aspects of chromosome biology. Considerations range from broad relevance and accessibility to the level of annotation provided in data sets and software. Detailed protocols, safety and ethical guidelines, and lists of computational tools, data repositories, and community-maintained annotation resources are also made available.

**Chromosome Structure and Segregation**

Chromosome Structure, Chromosomal Segregation, and Chromosome Segregation Checkpoints What is a chromosome? In eukaryotic cells, genomic DNA is organized to a certain extent into linear structures called chromosomes. The association of DNA with proteins such as histones results in the formation of chromatin (Omonov Q., et al). Chromatin exists in a compact state called heterochromatin, which is mainly found at the centromeres and telomeres, and a more open state called euchromatin, where genes are actively expressed. A chromosome is defined here as a chromatin structure that is viewed as one unit such that each chromosome has a physical separation relative to other chromosomes, and can arise in either a haploid or diploid state. Anaphase, or the exit from metaphase, is when splitting occurs, and so is viewed here as the primary mark point for chromosome separation. A chromatid is defined here as each segment of a chromatids when two sister chromatids are joined together at a specific location after chromosome replication but before chromosome segregation. The location where sister chromatids are joined is referred to as the centromere. The remaining ends of each chromatid after splitting off from the centromere(s) during anaphase are referred to as the telomeres. Therefore, a chromosome structure during prophase or anaphase is defined from the physical view of biological images such that the physical position of telomeres and the location of centromeres are the visually observable indicators for a chromosome structure (G. Strukov et al., 2011). How is the chromosome born and how is this process discussed? Chromosomes exist at a broader level across the genome. Different genome organization influences genome utilization and genome plasticity, which allows different adaptation to external chemical and physical pressures. Furthermore, chromosome rearrangement is one of a well-known deleterious phenomenon that occurred during cancers, thus understanding genome structural conservation across lineages and their evolutionary history provides a clue to how the complex biology of these organisms arose and loosely the emergence of cancer .

**Genomic Organization and Evolution**

The chromosomal organization of Drosophila provides a contextual framework for understanding structure, function, and evolutionary change. Understanding genomic organization emphasizes the independence of gene-rich and gene-poor regions, the distinctions between heterochromatin and euchromatin, and the prevalence of transposable elements, all of which remains stable across the Drosophila lineage (Bhutkar et al., 2010). Genome evolution encompasses the fixation of structural variations, including transposable-element insertions and large-scale chromosomal rearrangements, within an organism-specific phylogenetic context; changes in absolute genome size further illustrate the spectrum of variation. Geographically restricted and relatively short transposable-element floods occurred in the common ancestor, melanogaster subgroup, and obscura group; extensive activity appears limited to just four species. Within well-established species such as D. melanogaster, chromosomal inversions, transpositions, and aneuploidy still take place. As in other metazoans, Drosophila maintains the principles deduced from evolutionary analyses on synteny, gene content, and gene function.

**Functional Genomics: From Gene to Phenotype**

Functional genomics investigates how genotypic variation produces phenotypic variability, thereby bridging genotype and phenotype. The evolution of genotype–phenotype mapping underlies much of the understanding of evolutionary biology, spanning a vast scientific literature on the role of the genome in shaping phenotypic variation. A first essential step for many organisms is inferring the function of a particular gene or genes from comparative genomics, and especially from the presence of conserved mutant phenotypes across mutations or transgenic misexpression that correspond to a gene in another organism. The resulting predicted function of that gene is related to its place in a network of regulatory interactions with other genes across relevant life stages [table 1].

**Table 1: Drosophila as a Model System for Chromosome Biology**

|  |  |  |
| --- | --- | --- |
| **Feature/ Concept** | **Details** | **Relevance for Education / Research** |
| **Genetic Toolkit** | Balancer chromosomes, GAL4/UAS, CRISPR | Enables precise manipulation, knockdown, and overexpression studies |
| **Genome Resources** | Fully sequenced genomes of multiple species | Supports comparative genomics, evolutionary studies |
| **Experimental Versatility** | Short generation time, high fecundity | Suitable for classroom-scale experiments |
| **Functional Genomics** | Transgenes under spatial/temporal promoters | Investigates gene regulatory networks and genotype–phenotype mapping |
| **Evolution Studies** | Conserved synteny, chromosomal inversions, transposable-element activity | Helps students understand genome evolution and structural variation |
| **Classroom Applications** | Mapping chromatin states, transcriptional bursting, sex-specific regulation | Engages students in inquiry-based learning of broad biological principles |

Functional genomics addresses functional inference of gene regulatory networks from observations of known inputs and outputs across many conditions and through knockdown at distinct life stages. For complex phenotypes such as the three developmental phases of chromosome aberrations in embryonic lethal mutants, a reduced representation isolates conserved elements within the set of selected chromosomes common across species, thus addressing the prediction of conservation. The basic experimental logic remains high-level: transgenes in regulatory regions under diverse temporal–spatial promoters are monitored for appropriate marker and monitored again under genome perturbation alongside the recipient gene to aid conservation understanding.

**Model System Rationale: Why Drosophila?**

For reliable experimental biology education, organisms should possess an extensive genetic toolkit and a well-characterized genome. Drosophila provides outstanding genetic resources, including balancer chromosomes, the GAL4/UAS system, and CRISPR tools, while permitting experimentation on a classroom scale. A recent concerted sequencing effort has yielded ten Drosophila genomes representing five species across the melanogaster subgroup (E. Mohr & Perrimon, 2019) , enabling extensive comparative analysis. Large, fully annotated genomes of multiple Drosophila species further facilitate evolution-focused studies at the nucleotide level (Haudry et al., 2018). Drosophila thus allows simultaneous exploration of fundamental concepts-such as inheritance, epigenetics, and genome evolution-of broad biological importance relevant to many curricula and textbooks.

The capacity of Drosophila to engage students in inquiry-based exploration spans diverse concepts of broad biological relevance: development, inheritance, epigenetics, genome evolution, and functional genomics. The extensive genetic toolkit facilitates Chromosome Biology in K–Gray educational settings by lending itself readily to guided discovery, encompassing projects in comparative and functional genomics (Pasini et al., 2010). Drosophila also offers simple, yet powerful, experimental paradigms on easily accessible chromosomes.

**Genetic Toolkit and Experimental Versatility**

Drosophila affords a versatile experimental platform for investigating chromosome biology through comparative and functional genomics (E. Mohr & Perrimon, 2019). A powerful genetic toolkit, combined with short generation times, high fecundity, and extensive prior characterization, allows in-depth studies of multiple genomic features in classroom-scale projects (Matinyan et al., 2021). The Drosophila genome is amenable to comparative analyses across the genus, facilitating genome evolution investigations and genome-scale phylogenetic comparisons. Educational designs that emphasize salient biological principles, from genome organization to gene regulation, inheritance to epigenetics, enhance discovery learning while underscoring the broader relevance of genome studies [table 2].

**Table 2: Conceptual Foundations of Chromosome Biology**

|  |  |  |
| --- | --- | --- |
| **Topic** | **Key Concepts** | **Details / Examples** |
| **Chromosome Structure** | Chromatin organization | DNA + histones → chromatin; euchromatin (active) vs heterochromatin (compact) |
| **Chromatid** | Definition | Each segment of replicated chromosome; sister chromatids joined at centromere |
| **Centromere & Telomere** | Structure markers | Centromere: sister chromatid attachment; Telomere: ends after splitting |
| **Chromosome Segregation** | Mitosis/Meiosis | Anaphase = primary separation; segregation checkpoints ensure proper division |
| **Genomic Organization** | Gene-rich vs gene-poor regions | Heterochromatin vs euchromatin; prevalence of transposable elements |
| **Genome Evolution** | Structural variation | Inversions, transpositions, aneuploidy; species-specific transposable-element activity |
| **Functional Genomics** | Genotype→Phenotype | Inferring gene function from mutants, transgenes, and regulatory networks |
| **Experimental Approaches** | Comparative & functional genomics | Mapping transposable elements, chromatin states, expression data, ribosome occupancy, sex-specific regulation |

**Comparative Genomics Across Drosophila Species**

While the Drosophila genome is better annotated than that of any other organism (Yang et al., 2018) , gene function remains unknown for many of its 15,000 genes. Genes are identified by homology to annotated genes in other species, and these predictions can be tested through comparative genomics across Drosophila species (E. Mohr & Perrimon, 2019). Species having diverged more than 60 million years ago can still be used for orthology comparisons. Forty-nine species genomes are available. The montium group has accumulated four times as much sequence divergence since separating from D. melanogaster compared to third-instar larvae of D. melanogaster and species of the caerulescens group (J. Bronski et al., 2020).

**Relevance to Broad Biological Concepts**

Biological education aims to equip students with general principles applicable to diverse systems. The design and analysis of comparative genomics studies in Drosophila underpin several concepts relevant to the life sciences. Inheritance, fundamental to genetics, is explicitly represented in the instructional projects, which involve parental crosses and relate genomic data to known mutants with phenotypic consequences. Regulatory network diagrams expose developmental regulation of other genes by a given gene, connecting to further developmental biology topics. The accessibility of epigenetic information in the Drosophila genome invites examination of chromatin-state conservation among species, while cross-species synteny and conserved gene networks inform genome evolution discussions, including genome-rearrangement frequencies, conserved and repurposed regulatory networks, and sequencing technology impacts. Drosophila remains notable for organism-scale genetic perturbation, with various approaches grounded in finesse and safety.

A graphical abstraction linking conserved regulatory regions to expression data and phenotypes clarifies the regulatory-spectrum concept and offers a stepping-stone to gene–regulatory–network modelling. Other projects target graphical-protocol-review systematics, developmental-phase terminology completeness, widely used orthologue databases and software, and incomplete chromatin-regulation and epigenome-modification characterisations. Collaborative outreach involves a bulk RNA-seq experiment in organ (“Drosophila ex-vivo”) culture, transmitting a pertinent gene-expression-regulation narrative and operating within comparative-genomics parameters. Continued development of comparative studies across laboratory and class settings enriches the training toolbox, aligns with overarching institutional objectives, and strengthens research–education integration. The generation of consolidated metadata, vector and insert-sharing for hybridisation-probe construction, overlaid library-pooling design, and genotype–phenotype-target-linkage systematics also augments broader-term instruction.

Chromosome translocations and corresponding transcriptional changes engage genome-structure–gene-function investigation. The respective regulatory-state transformations imposed by 7, 83B, and 7a perturbations introduce neighbouring-gene modulation, interstitial-shuffling, and populational-versus-development-scale signalling transmission framing. Comprehending global-scale regulation and its contextual alteration parallels broader participant themes, balancing specificity versus generality. High-throughput perturbation sequencing and transcriptomic investigation of trans-perturbation impacts together refine a regulatory-architecture overview, nurturing contemporary modelling perspectives. Considerations extend to incomplete chromatin-regulation or epigenome-modification characterisation, emphasis on development-timing acquisition, actively used signalling-pathway inventories, and still-emerging one-to-many–many-to-one anatomite-annotation meta-resources.

**Discovery-Based Learning Framework**

Discovery-based learning frameworks help students engage in the scientific process through exploration, experimentation, and analysis. In Drosophila chromosome biology experiments, students generate data sets that address their own questions, refining project scope as they test, analyze, and interpret hypotheses in light of results. Initial investigations use crosses and mutant analyses to characterize chromosome distribution during mitosis, enabling experimental design as well as open-ended exploration of bioinformatics. Later projects employ techniques such as GAL4/UAS perturbation, genome-wide transcriptional profiling, and Miami–BOLD chromatin accessibility assays to investigate Drosophila chromosome biology and its evolution across species (Khaydarova S., et al).

Defining clear project boundaries and aligning practical inquiry with specific learning objectives are critical to ensuring an open-ended format remains productive. Student questions determine project scope, yet certain design principles foster deep investigation while promoting fundamental scientific concepts. Projects adopt a cyclical approach in which observational questions lead to testable hypotheses, experimental data adjust initial questions, and fresh insights encourage broader investigation. These iterative cycles emphasize the dynamic, nonlinear nature of scientific inquiry, cultivating a more sophisticated understanding of the research process (Delventhal & Steinhauer, 2020).

**Project Design Principles**

Framing the chromosome biology education project in terms of comparative and functional genomics is facilitated by a well-defined set of design principles. Key among these is students framing their own questions and hypotheses based on introductory materials (Chapter 2), course content, and additional readings. This perspective maintains alignment with established guidelines for inquiry-based education through data collection, analysis, and iterative learning (E. Mohr & Perrimon, 2019). Additionally, students benefit from refining their original projects into narrowly focused inquiries based on preexisting datasets and constructing arguments grounded in preliminary findings, thereby engaging with and developing critical reasoning skills. Establishing clear instructional objectives additionally guarantees projects remain relevant and widely applicable across courses.

Discovery-based chromosome biology pedagogy within the Drosophila model system thus emphasizes student-driven inquiry, progressively articulated questions or hypotheses, and evidence-supported reasoning through the following design principles. Framing the project through comparative and functional genomics facilitates discovery-based educational approaches grounded in both prior genomic infrastructure (Pasini et al., 2010) and contemporary activity within academic communities. The selected framework offers sufficient breadth to accommodate diverse student inquiries while retaining clear conceptual links to key biological principles, guaranteeing pedagogical adaptability across specialized courses in regulatory evolution, chromosome biology, and genome evolution. Comparative and functional genomics nonetheless maintain a pedagogical connection to fundamental biological themes, such as developmental mechanisms, genome organization, epigenetic factors, and evolutionary processes.

**Inquiry-Based Experiments in Chromosome Biology**

Various inquiry-based experiments can engage students in discovery-based learning on chromosome biology while remaining well-structured, ultimately fostering scientific thinking and communication. A fundamental preparatory activity, designed for an introductory module, involves successful genetic crosses and the subsequent morphological analysis of F1 progeny within the standard Drosophila melanogaster wild-type background. Building on early lessons in genetics, a second activity explores the functional roles of several chromosome 3 mutants-ranging from genes involved in chromosome segregation to epigenetic regulators-by examining F1 progeny phenotypes for evidence of dosage sensitivity. During the subsequent comparison of different F1 genotypes, presenting a broad spectrum of phenotypes promotes classroom interaction and discussion, while importantly modelling the collection and analysis of collaborative data across multiple student groups. These initial experiences introduce students to the iterative nature of scientific exploration, concurrently establishing a solid foundation from which to investigate broader questions in comparative and functional genomics (Pasini et al., 2010). Options for follow-on chromosome-biology investigations could thus incorporate candlestick and crick crosses; further mutant analyses targeting chromosome 2; or the comparison of circadian-rhythm, eye, and pigmentation mutants on chromosome 3 (T. Ables, 2015). Additional experiments also permit the probing of genome-assembly quality, chromatin accessibility patterns, or the direct effects of candidate perturbations on chromosome 3.

**Data Analysis and Interpretation**

Discovery-based chromosome biology education should be framed around comparative and functional genomics in Drosophila, emphasizing evidence-based arguments, clear definitions, and rigorous academic structure.

Data analysis encompasses all workflows necessary to obtain both reliable and interpretable results after raw data acquisition. As a rule, data must undergo quality control, appropriate statistical treatments must be applied, and the final results should be visualized in an accessible way. Chromosome biology lends itself well to systematic and intuitive analyses such as sorting autoradiographic pictures to pinpoint nuclear elements, extracting and counting fluorescent spots from microphotographs, or measuring distances and angles from scanned printed materials. In addition, comparative genomics provides opportunities for robust exploration of sequence conservation through broad phylogenetic analyses and well-established assignment of orthology, which Drosophila species can readily accommodate. Every project could initiate from either a cross-analysis of high-throughput screen datasets-tracking a few well-chosen Gal4 drivers to novice projects of straightforward design anywhere in an institution-or a set of comparative-genomics figures associated with one of the species rapid-dumping large amounts of older data.

Through comparative genomics, students can obtain answers to a variety of broad questions. They can investigate how genome organisation and gene composition have evolved by studying changes in size, number of genes, and transposon presence since the shared ancestor with D. ananassae, thus drawing general insights into Drosophila biology. They can further explore how structures such as centromeres, telomeric repeats, and heterochromatin have been conserved and how these features relate to the type of organism and the volume of data available curves, revealing the general rules associated with genome evolution. Another option consists of mapping conserved sequences and synteny across species to identify conserved regulatory elements and structural features (Odilov B.A., et al).

In parallel, functional genomics offers a distinct avenue of exploration, bridging genotype and phenotype. Students can interrogate precisely defined genomic regions to assess gene function and the delineation of inter-gene regulatory links (Abdurakhmanov, J., et al). The latter option leads straight into directed efforts towards reconstructing the regulatory circuitry underpinning early embryogenesis and provides a concrete footing to address wider topics such as the number of genes sufficient for artificial life, the ubiquitousness of default state as compared to activation loops, the interplay between the preservation of excitatory links and the shrinking of complex circuits, or sundry other challenges.

**Assessment and Iterative Learning**

Assessment and feedback are integral components of discovery-based education. In addition to quantifying student achievement and providing summary indications of success, assessment during the learning process shapes student engagement, directs effort towards specific concepts or activities, and helps clarify what was valued. Iterative refinement of evidence-based arguments (e.g., hypotheses, predictions, conclusions, analyses, rationales), both on paper and orally, encourages students to reflect on their work, anticipate challenges the audience may confront, and therefore adapt substance and presentation in ways likely to enhance impact. Tracking progress across consecutive attempts also reveals growth over time, which further validates effort, reinforces productive habits, and establishes criteria for continued improvement.

Formative and summative metrics complement one another and are wholly consistent with the scientific thinking framework. Formative assessment focuses on evidence of reasoning and collaboration rather than final conclusions or individual performance. Students submit questions, hypotheses, experimental designs, data analysis methods, and presentation drafts on a biweekly basis for feedback; instructors respond with suggestions for adjustments to strengthen formulation and increase anticipated clarity and engagement, often through the perspective of the intended audience. Students also participate in peer review of the initial and final manuscript drafts, exchanging perspectives on the clarity, engagement, and completeness of regulatory-network models and the analyses of representative circuit components in light of those models. Examination of these guidelines during the writing and revision cycle motivates attention to the enabling role of comparative genomics in hypothesis-driven projects and reinforces the relevance of explicit scientific communication as part of the exploration.

**Comparative Genomics Projects in Drosophila**

Drosophila comparative genomics affords a flexible lens in which to explore conserved and divergent features of chromosome structure, organization, dynamics, and regulation across species (E. Mohr & Perrimon, 2019). Candidates for comparative analysis include genomic conservation and synteny (or the collinearity of genes across genomes); orthologous genes and characterized functions deduced from trans-regulatory networks; chromosomal rearrangements of genes phenotypically impacted by mutants; and chromatin states, accessibility, and regulatory landscapes. A description of three specific modules follows (Azimova, S., et al. 2023).

Comparative genomics: conservation and synteny. A toolkit minimizes the distance from genotype to phenotype in projects tracing the fate of mutations that alter chromosome structure, organization, dynamics, or regulation. The identification of such mutations and their phenotypic consequences encompasses established genetic screens that capture at least two chromatin properties (A. Hoskins et al., 2015). Databases of co-expression networks facilitate the candidate-gene search by narrowing the focus to the subset of co-regulated genes. Genomic conservation among genes and their associated networks, synteny across the Drosophila lineage, and the identification of conserved, unannotated non-coding elements provide additional confoundment.

Comparative genomics: orthology and function. Cross-species RNA transcriptome profiling links chromatin state to active genes and eliminates hundreds of candidates from the initial set. To implement these approaches, a collection of Drosophila issues, strains, and reagents to characterize genes, chromosomes, and complexes of classes ranging from regulatory and transcription factors through chromatin-remodeling and modifying activities is analyzed concurrently with the genome of interest.

Comparative genomics: rearrangements and phenotypes. The Drosophila toolkit supports multiple routes to the inquiry-oriented exploration of the regulation of chromosome structure, dynamics, and organization. Integration of candidate cooperation within network diagrams, task decomposition, and progress tracking facilitates coordination. Analysis across the entire Drosophila genus exploits a comparative approach on any chromosome of choice that yields genome assemblies, annotations, and strains designed to speed comparative functional and evolutionary studies.

**Genomic Conservation and Synteny Mapping**

Lineage-specific genome evolution can be traced using comparative or functional genomics to map genomic conservation and synteny across species. The existence of conserved structural features, such as gene essentiality and transcriptional networks, suggests that the overall wiring of gene regulatory circuits might also remain conserved (Chen & W. Sternberg, 2014). These shared interactions can be extracted from comparative analyses and used to infer indicative gene functions constrained to specific biological processes. Furthermore, the inference of orthology relationships and identification of phylogenetic positions can illuminate the evolutionary fates of gene duplicates and provide insights into the impact of chromosomal rearrangements on genome evolution (Abdurakhmanov, J., et al).

WG-Systematic (Genome) The wealth of experimental and annotated genetic material has prompted extensive analyses of genome evolution across many Drosophila genomes in an effort to elucidate the mechanisms driving various evolutionary processes.Drosophila chromosome maps were among the earliest generated and were fundamental for applying theoretical concepts from Drosophila genetics (W. Schaeffer, 2018). Comparative studies of closely and distantly related Drosophila species now serve as cornerstones of Drosophila comparative and functional genomics.

**Gene Orthology and Functional Inference**

Gene orthology is vital for gene function inference and understanding evolutionary relationships (E. Mohr & Perrimon, 2019). Many direct experimental approaches remain feasible for Drosophila and have been extensively developed in the context of sex determination, where gene orthology allows inference of network conservation. Together with large-scale chromatin profiling, these studies indicate that conserved co-expressed transcriptional regulators together with their cis-regulatory regions constitute the first-order determinants required to predict functional gene networks across Drosophila species (Bhutkar et al., 2010).

**Chromosomal Rearrangements and Phenotypic Effects**

The identification of chromosomal rearrangements among Drosophila species has a long history, dating back to the early 20th century. Advances in microscopy allowed researchers to perform optical observation of polytene chromosomes, leading to the characterization of numerous rearrangements that had phenotypic consequences in diverse biological processes (Bhutkar et al., 2010). More recent studies have mapped large-scale rearrangements through comparative analysis of gene order, beginning with pairwise comparisons of model genomes and progressing to genome-wide approaches. Various computational methods have been developed to infer genome-scale rearrangement phylogeny and ancestral gene order based on large datasets of genomic-locus locations. These inferences help reconstruct the order of genes at the time of the last common ancestor and provide insights into evolutionary events and their connection with genome stability, gene regulation, and the origin of species.

Further characterization of chromosomes among additional species would extend knowledge of these evolutionary processes. Classes of rearrangements can be identified in Drosophila species for which polytene chromosomes have been extensively mapped, such as melanogaster, simulans, and virilis. The mechanisms of chromosomal rearrangement and gene gain or loss, in relation to transposable elements and the reorganization of chromatin state, provide important insights into the evolution of these species.

**Epigenetic Regulation Across Species**

Epigenetic mechanisms-heritable changes of gene function not involving changes in DNA sequence-are also major facets of chromosome biology that Drosophila researchers have studied extensively (Gibert & Peronnet, 2021). Chromatin organization is key to genome function and Drosophila studies have revealed epigenetic regulation through chromatin structures, such as heterochromatin formation and maintenance, transposon regulation by piRNA clusters, Polycomb-mediated gene silencing, and Trithorax-dependent gene activation. Comparative genomics offers fresh insights into epigenetic regulation across Drosophila species. The modENCODE project categorized chromatin states for many Drosophila species so researchers can compare chromatin states, accessibility, and regulatory landscapes across flyers and glean new understanding of epigenetic regulation (Azimova, S. et al. 2023).

**Functional Genomics Approaches**

Functional perturbations provide an opportunity to interrogate chromosome biology by assessing the resulting changes to chromosome segregation and to associated phenotypes. RNA interference (RNAi), for example, can effectively knock down target gene expression, enabling analysis of closely related gene families to select the most suitable candidates for linkage to a candidate regulatory region. Because independent UAS-linked RNAi constructs targeting the same gene do not exhibit saturation and therefore lack identifiable saturating levels of knockdown, analysis can continue until an appropriate level of knockdown becomes evident (Bangi, 2020). In parallel with RNAi perturbation, CRISPR/Cas9-mediated gene knockout generates mutant chromosomes, allowing examination of mRNA expression and transposon activity from essential or nonessential genes linked to candidate candidate regulatory sequences (E. Mohr & Perrimon, 2019).

To complement chromosome arrangements, chromatin states can be investigated through chromatin accessibility analysis or transcriptomic profiling. Perturbations aimed at individual factors within regulatory groups effect differential expression of other genes in the associated regulatory network, indicating their partner status; furthermore, genes lacking detectable transcript levels may serve as informative targets. Differential expression can therefore guide selection of the most appropriate candidate regulatory region for further analysis. Yet within an interconnected chromatin landscape, assessing complete regulatory networks proves challenging. Sufficiently multiplexed transcriptomic profiling exploits this Drosophila feature by co-measuring all genes-regardless of activatory, repressive, or intermediary influence-directly connecting RNA profile shifts to regulator function and facilitating informed designer-trap design.

Isolation of reporter constructs subsequently probes the candidate regulatory element. Enhancers gain access to promoters through chromatin-remodelling complexes notwithstanding repressor occupation of intermediary binding sites. Determining whether accompanied partners exert influence indicates the network’s connected nature. Transposonic-element acetylation signals accessibility and profile preceding reporter insertion pinpoint candidate locations. Hybrid constructs transform neighbouring genes into transposon-linked reporter-activity gauges to clarify indication status. Chromatin-accessibility assays further refine assessment of partner influence, mapping open-chromatin events over developmental stages and extending measurements of transcriptional activity to other gene types.

**RNA Interference and CRISPR-Based Perturbations**

In RNA interference (RNAi), specific double-stranded RNA (dsRNA) triggers sequence-specific degradation of homologous target transcripts in mammalian cells and invertebrates, including Drosophila (B Weitzman, 2003). Effectors of RNAi include RNA-induced silencing complexes (RISC) and Dicer, which converts long dsRNA into small interfering RNA (siRNA). The precursor of a potent siRNA, hence, is produced by a transgene expressing long hairpin RNA (hpRNA), which undergoes processing by the dsRNA-binding endonuclease Dicer in the cytoplasm to yield the siRNA. The hphono transgene permits the observation of previously uncharacterized transcriptional and post-transcriptional control mechanisms in many eukaryotes. A feasible algorithm identifies candidate hpRNA with a suitable secondary structure in the 3' untranslated region (UTR) of selected mRNA. Cloning of hpRNA into appropriate expression vector enables large-scale loss-of-function screens targeting the Drosophila genome.

For precise genome editing and seamless transgene insertion, a version of the genome-editing system based on Streptococcus pyogenes CRISPR/Cas9 is efficiently implemented in Drosophila. The system comprises a single-file resource including a user-friendly plasmid set, a standard paired gRNA target library for the entire Drosophila genome, and an accompanying web service (Essletzbichler et al., 2018). Engineered Cas9 variants enable applications such as transcriptional regulation, enhanced tagging, and multiplexing. Together with other fundamental technologies, such as the FLP-out technique to produce a permanent and irreversible knock-in or knock-out of the target sequences, the CRISPR/Cas9 genome-editing system is rapidly establishing itself as a powerful tool for genome engineering in Drosophila (Bakirov P., et al).

**Transcriptomic Profiling and Expression Analysis**

Chromosomes occupy the same space in every cell, yet expression patterns remain different. To examine which genes are switched on at a given time or after genetic perturbation in a wild-type Drosophila, the modulation of transcript abundance across a diverse panel of developmental stages and anatomical sites (R. Graveley et al., 2011) can be analyzed. The tool available for this analysis is a Drosophila transcriptomic dataset generated by the modENCODE consortium. To provide an overview of transcript levels in particular conditions, carefully selected datasets can be compared. For example, perturbations affecting chromosome structure and dynamics can be monitored at 24 hours after induction on Chromosome 3, Cell cycle genes can be monitored following CRISPR removal of dE2F or dDP, Chromatin-remodeling factors can be screened for effect on gcm1 transcription using a 1.8 kb gcm1-RFP reporter, or LEF family factors can be screened on a gsb-RFP reporter assay (Jessica Li et al., 2014).

**Reporter Assays and Chromatin Accessibility**

Chromatin accessibility influences transcriptional activity. The Activity of Transcriptional Enhancers Reported by an Ectopic Reporter (ATER) system tracks regulation of candidate enhancers. Unpaired chromosomes activate the “stay in place” locus, countering the default “come apart” locus activity promoted during normal development (Sasmakov, S. A., et al.). Transgene insertion sites modulate reporter expression on paired or unpaired homologous chromosomes. Enhancer activity of eight identified candidate regions-a subset of Small bristles, and two XY regions known to regulate gene activity during both development and dosage compensation-indicates their trans-acting function. Parallel reporter and transcriptomic assays disambiguate ATER-acting enhancer function from that exerted by adjacent genes, confirming all five upregulated candidates function as trans-acting trans-sorptive enhancers. Chromatin Accessibility Assessment by Tn5 Transposase Assay for Drosophila (ATAC-seq) interrogates chromatin-proximal DNA sites, globally profiling the landscape of chromatin accessibility (B. Merrill et al., 2022). ATAC-seq applies the Tn5 transposase to embed sequencing adapters into accessible sites, permitting the construction of sequencing libraries from low-cell-number starting material. A modified protocol-from nuclei preparation to library amplification-tailored ATAC-seq for tissues and cells with limited input material.

**Pedagogical Implementation and Resources**

Curriculum development can begin by outlining anticipated learning outcomes and selecting relevant Discovery−Based Chromosome Biology modules. While implementation may vary considerable across institutions, one approach is to categorize projects according to three main themes-comparative genomics, functional genomics, and chromosome structure-and integrate several modules into existing course structures. For example, in introductory Genetics courses, a Comparative Genomics focus on Drosophila has been embedded into either the first or second of five 2−hour laboratory sessions, alongside additional activities investigating fundamental Mendelian concepts and classical Drosophila genetics. As another example, when Drosophila are used in a dedicated full−term Discovery− Based Biology project, a Functional Genomics module targeting Gene Function and Regulation can be added to the existing curriculum, leveraging purchased Drosophila CRISPR lines and a characterization dataset.

To foster reproducibility and effectiveness, an array of resources are available to educators interested in adopting these Discovery−Based Chromosome Biology modules. Standard operating procedures (SOPs) for core lab activities-crosses and phenotyping, CRISPR genotyping, RNA−seq preparation, RNA−seq analysis, and bioinformatics analysis-have been prepared, along with guidance on laboratory biosafety, fly and human ethics, proper disposal procedures, and risk assessments. Datasets for reference genomes, RNA−seq libraries, and regulatory region predictions are readily downloadable as compressed archives from public data repositories. An individual bioinformatics database holding comprehensive collections of Drosophila genome annotations, RNA−seq analysis results, processed RNA−seq read files, and other datasets can be accessed through a dedicated web−based interface (E. Mohr & Perrimon, 2019). All datasets stored in this database are versioned according to Semantic Versioning 2.0, facilitating functional genomic analyses of CRISPR lines purchased from the Vienna Drosophila Resource Center or similar projects aiming to explore chromosomal structure, evolution, or organization. A large collection of software packages and workflows compatible with the Galaxy web−based bioinformatics platform for genomic data analysis is also available, along with relevant Drosophila tools and workflows. These coordinates and packages can be readily imported into local Galaxy instances or transferred to cloud−based environments, enhancing discoverability and convenience.

**Curriculum Integration and Learning Objectives**

Major practical limitations affect the establishment of project-based Drosophila genomics on an institutional scale. Therefore, specific guided analyses may represent the most appropriate entry point. Comparative genomics across Drosophila species deliver authentic discovery-based chromosome biology investigations while satisfying relevant learning objectives. These investigations demand, enable, and exemplify evidence-based reasoning predicated upon clear definitions, explicit links to foundational concepts, and pervasive attention to the quality, transparency, and reproducibility of scholarly communication.

Specifically, these investigations embrace the following pedagogical objectives. Students acquire competencies in correlations between structure, function, and evolution in biology; experimental analysis of chromosomal organization, perturbation, and inheritance; genome–phenome mapping; sequence conservation; the design of data-driven, empirical tests of biological hypotheses; and the articulation of scientific arguments. These objectives collectively contribute to curricula-wide goals encompassing evidence-based reasoning; clarity, conciseness, and coherence in written and oral arguments; and appropriate engagement with primary scientific literature throughout (Azimova, S., et al. 2023).

**Lab Protocols, Safety, and Ethics**

Good laboratory practice (GLP) dictates compliance with safety, ethics, and social responsibility regulations at every stage of scientific inquiry. Standard working procedures, proper disposal of hazardous waste, and protection against accidents are essential. Materials designation and risk evaluation by corresponding institutions must be obtained before experimentation (Sasmakov, S. A., et al). To participate in GxP-compliant studies, students must demonstrate basic Drosophila husbandry skills, complete supplementary safety training, and acknowledge the hazards inherent in their proposed investigation. Research proposals formally describe objectives and methods of proposed experiments, helping ensure that experiments are conducted in a responsible manner.

All involved students receive GLP training that includes responsible conduct and training manuals developed by government agencies and funding bodies. The university ethics board also acknowledges that GxP-compliant student-driven projects do not require Comité d’Éthique en Santé et en Biomedecine (CESB) submission. Project scope remains limited to mutant alleles available in the Drosophila Genetic Resource Center (DGRC) and the “Gene Collection” cloned-DSRNA tools available through the Vienna Drosophila Resource Center (VDRC) (Delventhal & Steinhauer, 2020) and (L. Sperling et al., 2024).

**Data Sets, Repositories, and Computational Tools**

Framework and resources supporting Drosophila comparative and functional genomics research identify highly relevant data sets, public repositories, and computational tools. Chromosome-scale sequence assemblies document genomic organization, structures, and rearrangements among diverse species (J. Wilson et al., 2008). Functional experimentation relies on Gene Regulation and RBPDB databases for annotated regulatory elements, extensive Drosophila transcriptome collections, and FlyBase curations linking sequence and phenotype.

Accessible genome assemblies provide a foundation for discovery-based education in genomics, genome evolution, chromosome structure–function relations, and regulatory architecture. Public repositories host the Drosophila genome, multiple assemblies, existing annotation, and extensive functional data sets associated with chemical, genomic, and mutant perturbations. Supporting computational tools, libraries, and workflows facilitate sequence retrieval and analysis, experimental design, data exploration, and interpretation of results. Comprehensive documentation promotes reproducibility and transparency.

**Faculty Development and Assessment Strategies**

Discovery-based chromosome biology education should be framed around comparative and functional genomics in Drosophila, emphasizing evidence-based arguments, clear definitions, and rigorous academic structure (Sasmakov, S. A., et al). A Faculty Development Workshop demonstrating these principles received positive evaluations at a national meeting. The workshop encouraged hands-on Drosophila experiments, graphical exploration of biological data, and implementation of progression assessments. A Phase-3 undergraduate research course (Undeclared to Genomics Transition) capped a two-part series on the topic. Students responded positively to collaborative data exploration using GraphPad Prism, supported by essentials videos created to facilitate independent learning.

Program assessments included course evaluations at the end of each academic offering. BASIL videos contributed to the overall course evaluation and feedback from at least half of the students explicitly noted their helpfulness. Written reflections on data analysis after the second course reinforced application of proposed strategies and connections to broader scientific contexts. A continuing effort aims to establish more direct measures of student learning linked to the articulated goals for a deeper understanding of Drosophila chromosome and genome biology.

**Conclusion**

Comparative and functional genomics in Drosophila supports chromosome biology education through inquiry-based discovery, integrating innovative pedagogy with important yet underemphasized science. Four projects exemplify the approach: comparative genomics across species identifies conserved regulatory elements and rearrangements; functional genomics interrogates perturbation-induced transcriptional, phenotypic, and accessibility responses; and regulatory analyses connect chromatin states to activity. Written records document reasoning, clarify experimental frameworks and data, and facilitate iterative improvement. Training students to generate and test own inquiries fosters independent, evidence-driven scholarship accountable to scientific standards. The ensuing exposure to Drosophila biology, evolution, and experimental design-broadly relevant in the discipline-resembles conceptual foundations requisite for professional practice (E. Mohr & Perrimon, 2019).

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