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PhD Thesis

Analysis of Complex Biological Systems Using Hybrid Modeling Approach

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CONTENTS

1. GLOBAL INTRODUCTION

In the post-genomic era of biology, modeling of biological systems becomes especially relevant, because of obtained big data and insights of the regulation of such processes as growth and development of organisms. However, we are still far from a comprehensive understanding of how specific properties of the genotype and genes influence the phenotype of plants, animals, and microorganisms. For instance, it is still not possible to create a digital copy of a living eukaryotic cell, due to the high computational complexity and the huge number of configurations that replace each other in its development process. Therefore, the biological community needs powerful approach for identifying individual subsystems and their key parameters of homeostasis and functioning. One of these approaches is modeling. Firstly, the modeling process itself involves testing many hypotheses about parameters involved in a particular process (for example, gene regulation and/or environmental conditions). Secondly, calibrated models could make a precise prediction of the behavior of biological system in different conditions. Also, many ecological processes not possible to study directly, for example, processes of evolution of ecological communities on the scale of thousands of years, impact of future climate changes on the growth processes of plants, etc. Modeling also could be used for better understanding of existing interaction in complex biological systems. Therefore, the extensive creation of models of biological systems has enormous potential for the development. It allows us to better understand the mechanisms of functioning of living systems, predict their behavior and develop more précised experiments (Kohl P. et al., 2010).

However, the complexity of the biological systems is one of the main limitations for make an adequate true-scale models. Starting with thousands of genes tightly interconnected in their expression and regulations, millions of cells and ending with complex ecosystems, biological systems are truly unique in their properties. In addition, biological processes can be very sensitive to initial conditions and parameters, which also complicates the creation of accurate mathematical models. Besides, the functions of many genes and the mechanisms of adaptation of organisms and their interactions are still unclear. Another limitation is the lack of accurate measurements and data. Modelers are always forced to work with a modest dataset when developing and testing their hypotheses *in silico*. These limitations could be the main sources of error and incorrect conclusions. Also, mathematical models are subjective and unambiguous interpreting of their results is not a trivial task. Finally, it is very difficult to collect and systematize all accumulated biological models, and to link them into a holistic picture of the world.

The hybrid approach of modeling biological systems is one of the most promising for overcoming these limitations. In general terms, “hybrid” property of something relates to it mixed

composition. In the context of modeling, it related to the mixed mathematical formalism used in implementation of models, usually includes continuous process coupled with discrete events. There are three main types of these models: “independent” or decoupled, “adjacent” or coupled and “intricated” (Stephanou and Volpert, 2016). The advantage of using hybrid approach for complex biological systems is much less computational cost then classical discrete models, as well as a huge potential of hybrid models for reducing the number of experiments, according to the guidance received from analysis of its behavior (Anvari S., et al., 2021). Hybrid modeling approach is directly connected with systems biology methods since the natural systems are multilevel and always characterized by spatio-temporal dynamics. This approach could be combined with a data obtained from high-throughput technologies of sequencing (Bardini R. et al., 2017). However, effective application of hybrid modeling approach directly to the omics data is very limited, since the large complexity of genetic systems involved in processes of onthogenesis. For instance, the integrative omics approach could improve our knowledge in the field of developmental biology (Rajasundaram D. & Selbig J, 2016), which eventually could help to present the initial big data as set of rules for the hybrid models.

Advantages of using hybrid models over recently discovered machine learning models (MLM) is that hybrid models can clearly indicate the key components of the studied systems while MLM is a black-box system and do not explain any internal logics inside (J. Li et al., 2021). Also, effective linking and solving of multiscale hybrid models could be a key to further understanding biological phenomena on system level (Cilfone N. A. et al., 2015). In recent years, researchers developed new frameworks for multiscale agent-based modeling in the field of cellular and tissue biology (Letort G. et al 2019; Bergman D. et al., 2022). Also, for modeling of pharmaceutical processes, hybrid approach seems to be a most relevant tool (Tsopanoglou, A., & del Val, I. J. 2021).

The major drawbacks and challenges which prevents the broad usage of hybrid approach in biological field relate to emergent behavior of this models (Norton K. A. et al., 2019), and difficulties of interpretations of their outputs as well as lacking available data and parameters. There is a lot of tools and standardized protocols for cell-based hybrid models, for instance, VirtualLeaf (Merks et al., 2011), CellModeller (Dupuy et al., 2010), but it is difficult to make a universal tool for implementation of hybrid models. Therefore, there are many unresolved global issues in the field of modeling complex biological systems.

The main purpose of this thesis is to give a general idea of the possibilities of mathematical modeling of biological systems using an agent-based and hybrid approach with implementation on general-purpose Python language. This goal was set to demonstrate the capabilities of a general language for implementing the ecological models. This thesis is consisting of three main chapters.

The first chapter (Section №2) is described the state-of-art of tools for cell-based computational modeling. Also, this chapter summarizes new opportunities for advanced plant morphogenesis models, which become possible thanks to single-cell transcriptome data. Together with deeply developed cell-resolution imaging techniques, this achievement opens new horizons for studying the complex mechanisms of plant tissue architecture formation. While the opportunities for integrating data from transcriptomic to morphogenetic levels in a unified system still present several difficulties, plant tissues have some additional peculiarities in structure. Besides, it was show that the microscopy and cell-resolution imaging techniques could solve several spatial problems in single-cell transcriptomic data analysis and enhance the hybrid modeling framework opportunities. At the end of this chapter was proposed a general framework for modeling plant morphogenetic processes based on various biological data. This kind of model should include two main data sources: single-cell RNA sequencing and tissue imaging data.

The second chapter (Section №3) is described the implementation of two-dimensional spatial model which describes plant-soil negative feedback (NF) phenomena as well as obtained simulation results. NF is a well-established phenomenon that preventing the dominance of a single species and allows species coexistence and promotes the maintenance of biodiversity. At community scale, localized NF may cause the formation of exclusion zones under adult conspecifics leading to Janzen-Connell (JC) distribution. Implemented model described the connection between adult density, either conspecifics or heterospecifics, on the probability of occurrence of JC distributions. Using an individual-based modelling approach, was simulated the formation of exclusion zones due to the build-up of NF in proximity of conspecific adult plants and assessed the frequency of JC distribution in relation to conspecifics and heterospecific density ranging from isolated trees to closed forest stands. Overall, the model shows that a plant suffering from strong NF in monospecific stands can rarely exhibit a recruitment pattern fitting the JC model. These results would provide the means to reconcile the well-established NF framework with part the forest ecologists' community that is still skeptical towards the JC model.

The third chapter (Section №4) is dedicated to describing the created hybrid model of multi-cellular cambial growth of conifers, which is a direct descendant of the single-cell model of xylogenesis developed by Cartenì et al., 2018. It is well-known that the main factors influencing processes of cambial growth are climate conditions, such as temperature and precipitation. Constructed model able to answer how the combination of environmental conditions affects cambial growth processes of conifers. Provided modeling framework could be used to extend other models in the field of developmental biology to multicellular level. Our model was tested on data for three species of conifers: *Larix decidua, Pinus cembra, Picea abies* with various characteristics of xylogenetic processes. It was clearly shown that new model can reproduce the precised annual

cellular dynamic of xylem cells. However, there are some limitations in synchronizing both aspects of simulations (cellular dynamics and geometrical properties) at multicellular scales.

Taken together, obtained results are heterogeneous in structure and cover different areas of hybrid modeling of biological systems, as well as agent-based modeling. Python, as a general-purpose language, was suitable for developing the described models. The author notes the special need in specialized libraries suitable for hybrid framework development for effective modeling of environmental processes on the time-spatial scale.

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Chapter 1

A Sight on Single-Cell Transcriptomics in Plants Through the Prism of Cell-Based Computational Modeling Approaches: Benefits and Challenges for Data Analysis

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2. A SIGHT ON SINGLE-CELL TRANSCRIPTOMICS IN PLANTS THROUGH THE PRISM OF CELL-BASED COMPUTATIONAL MODELING APPROACHES: BENEFITS AND CHALLENGES FOR DATA ANALYSIS

2.1 INTRODUCTION

Modern biology is going through the era of big data and omics technologies. Single-cell sequencing (SCS) is one of the breakthroughs and rapidly developing technologies. This technology's value is difficult to overestimate since it allows one to describe with high accuracy the trajectories of cell development and characterize individual cell types (Trapnell, 2015). A targeted study of isolated cells is of particular importance in the context of systems biology, as demonstrated on root hair cells (Hossain et al., 2015). The main steps of SC analysis include cellular dissociation, single-cell RNA sequencing (scRNA-seq), dimensionality reduction, clustering, and reconstruction of the developmental trajectories. McFaline-Figueroa et al. (2020) provide currently available techniques for such kind of analysis. However, such a data-driven approach provides only a partial understanding of the developmental processes for different cell types since it includes only the molecular level.

Thus, a combination of microscopy methods (Li et al., 2014) and imaging techniques (Omari et al., 2020) could provide a new level of understanding the developmental processes. In turn, the combination of high-precision SCS approaches with high-quality microscopic data can be integrated into mathematical models describing morphogenesis. Therefore, we believe that current methods for processing SC data should be coupled with morphological data on a tissue level and computational frameworks describing tissue development. Such a systemic-biological cycle will allow researchers to find out the essential spatiotemporal regulators of morphogenetic processes and provide an *in silico - in vivo* verification of emerging hypotheses.

The relationship between growth characteristics of individual cells and organogenesis was noted in the work of Hong et al. (2018). It was shown that growth rate and growth direction significantly affect organ developmental processes, and, therefore, could determine the invariant organ formations. Consequently, it is essential to study cells' individual characteristics to create a holistic picture of morphogenetic processes at the tissue and organ levels. The main drivers of morphogenesis are shown schematically below, in Figure 1. Stem cells can divide, either symmetrically or with precise daughter-cell size ratio, the so-called formative divisions, which are fundamental determinants in the processes of morphogenesis Smolarkiewicz and Dhonukshe (2013).

Also, the emergence of cellular patterns forming tissues significantly depends on the anisotropic cell growth biomechanics, which occurs in tip-growing cells (Rounds and Bezanilla, 2013).

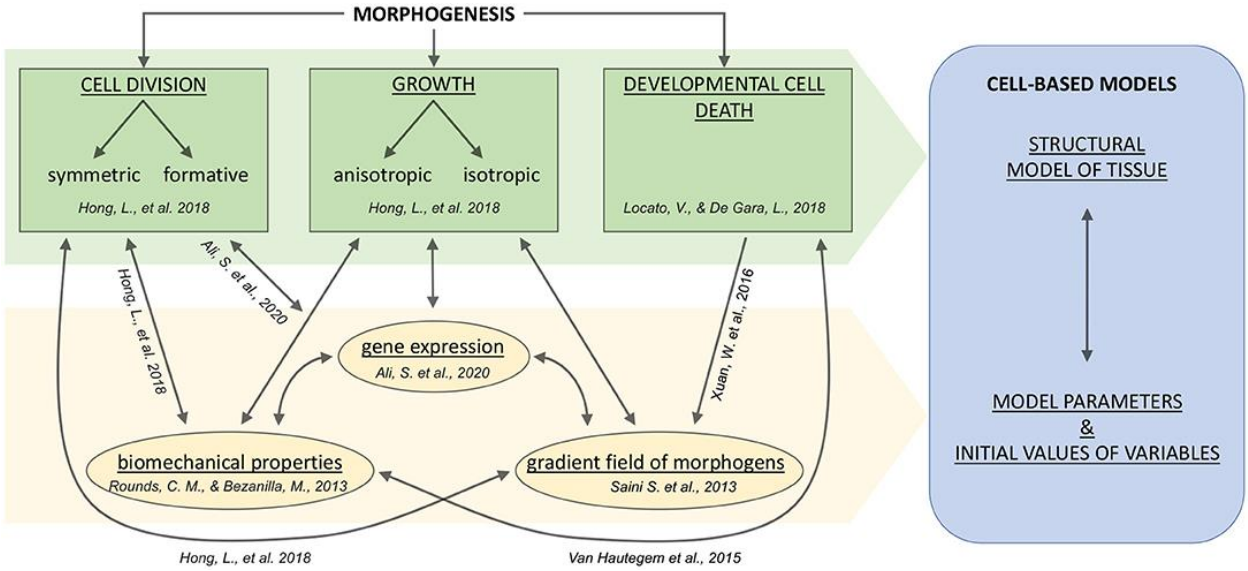

Figure 1. A general scheme for systems biological and modeling concepts of plant tissue morphogenesis including cell growth and division, and developmental PCD (plant cell death). Arrows indicate the relationships between fundamental cell fate and intracellular processes. The cell fate processes are indicated in green; the intracellular processes or properties are indicated in yellow. The blue box indicates the significant components of the cell-based modeling approach. References correspond to theoretical articles briefly explained in the text.

2.2 EXISTING APPROACHES TO THE ANALYSIS OF SINGLE-CELL DATA AND THEIR POTENTIAL FOR CELL-BASED MODELS

Characterizing the plant cell fate and ontogenesis using SC technologies is a novel and promising approach for getting high-resolution genomic data that reveals new facts about various cell types. The first SC transcriptomic experiments have been carried out for the model plant *A. thaliana* in 2019. For *A. thaliana*, most of SC studies were conducted on root cells (Denyer et al., 2019; Jean-Baptiste et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Turco et al., 2019; Zhang et al., 2019; Farmer et al., 2021). Whereas there are only two studies conducted on leaf tissues (Kim et al., 2021; Lopez-Anido et al., 2021). Thus, for all the main cell types of roots and leaves, the developmental trajectories were revealed. Also, *Zea mays*, being a representative of C4-photosynthetic cereals, is a promising object for SC experiments due to the large size its cells, which allows to easily isolate specific cells, for example, from the shoot apical meristem. To date, there are studies based on the single-cell analysis for corn tissues carried out on a shoot apex (Satterlee et al., 2020), phloem (Bezrutczyk et al., 2021), and ears (Xu et al., 2021). The first and so far, only scRNA-seq on rice roots (Liu et al., 2021) revealed significant differences in the characteristics of individual cell types in comparison to the cell types of *A. thaliana*, which indicates the presence of significant species-specific differences at the cellular level. A summary of the currently existing Sc-experiments is given in Table 1.

There are several fundamental questions about the limitations and capabilities of the SC method (Rich-Griffin et al., 2020): How realistic is it to recreate a cell atlas using such data? Can we apply the technology to cells of any type? How to identify the main gene regulators and gene networks of development?

The problem of combining SC data from different plant species is of particular interest since the successful application of this approach can be used to create a unified developmental atlas. However, it is necessary to consider the species-specific features of tissue development and organization, which imposes certain restrictions on the joint interpretation of the exact SC data.

TABLE 1. Summary of scRNA-seq datasets obtained for plants.

Publication date	References	Drop-Seq platform	Illumina platform	Organism	Plant organ
March 2019	Denyer et al., 2019	NanoDrop	NextSeq	*A. thaliana*	Root
April 2019	Ryu et al., 2019	10X Genomics	HiSeq 4000	*A. thaliana*	Root
May 2019	Zhang et al., 2019	10X Genomics	NovaSeq	*A. thaliana*	Root
May 2019	Shulse et al., 2019	Drop-seq v. 3.1	HiSeq 2500, HiSeq 4000, NextSeq	*A. thaliana*	Root
May 2019	Jean-Baptiste et al., 2019	10X Genomics	NextSeq 500	*A. thaliana*	Root
July 2019	Turco et al., 2019	Drop-seq v. 3.1	NextSeq	*A. thaliana*	Root
April 2021	Lopez-Anido et al., 2021	10X Genomics	NextSeq500, HiSeq4000	*A. thaliana*	Leaf
December 2020	Satterlee et al., 2020	Droplet microfluidics	NextSeq 500	*Zea mays*	Shoot
January 2021	Kim et al., 2021	10X Genomics	HiSeq 2500	*A. thaliana*	Leaf
January 2021	Farmer et al., 2021	10X Genomics	HiSeq	*A. thaliana*	Root
January 2021	Bezrutczyk et al., 2021	10X Genomics	HiSeq	*Zea mays*	Phloem
February 2021	Xu et al., 2021	10x Genomics	NextSeq 500	*Zea mays*	Ears
March 2021	Liu et al., 2021	10x Genomics	HiSeq 2000	*Oryza sativa*	Roots

There is an acute lack of SC data of leaf and shoot stem cells except for *A. thaliana*. The small amount of existing SC transcriptome data is partly due to the complexity and length of the required experimentation and data analysis. In a recent overview of SC methods for plants (Lähnemann et al., 2020; Shaw et al., 2020), the authors highlight the major challenges and drawbacks of single-cell approaches: (i) gene expressing bias caused by the protoplasting procedure, (ii) unequal efficiency for extraction of different types of cells, (iii) difficulties for the reverse reconstruction of the cell atlas based on transcriptomic data, (iv) lack of data. We also want to point out that there are fuzzy boundaries between cell populations due to their connectivity and the presence of transport processes between them. Therefore, there are still several limitations to the biological interpretation of the SC data.

Thus, the classification of cell types and reverse spatial reconstruction are critical stages of SC transcriptome data analysis. This task is rather complex and requires using the original dimension of the expression data. SC data generally represent a filtered and normalized array with dimension $M \times N$, where M is the number of cells with enough reads, N is the number of genes with a non-zero expression. The first component that can facilitate this problem is certain developmental trajectories caused by intracellular factors that limit the space of developmental possibilities and cause their partial determinism. Such factors have a different nature: the

concentration of substances and energy substrates in the cell, the concentration of hormones and morphogens, the mechanical characteristics of cells (e.g., turgor pressure, tension, and thickness of the cell wall). Unfortunately, it is currently impossible to estimate the effect of these factors and their contribution to genes' expression. However, their presence makes it possible to identify the main differentiation genes. In general, this fact allows to carry out the procedure for reducing the dimensions of data. Depending on the data set's complexity, it is proposed to select from 1,000 to 5,000 highly variable genes for clustering and cell classification (Luecken and Theis, 2019).

A variety of available methods and tips for single-cell data dimensionality reduction and clustering are presented in the work of Nguyen and Holmes (2019). In most cases, researchers choose t-SNE and UMAP algorithms. The large computational complexity of the t-SNE method on big datasets was eliminated by adding fast Fourier transforms (https://github.com/KlugerLab/FIt-SNE, Linderman et al., 2019). Comparison of t-SNE and UMAP methods revealed that UMAP outperforms even an optimized t-SNE in the computation time; also, clustering by UMAP is the most meaningful for distinguishing between cell types (Becht et al., 2019). Before the widespread use of t-SNE and UMAP, there was a probabilistic modeling method using Bayesian mixture of factor analyzers (MFA) (Campbell and Yau, 2017), based on the assumption that changes in gene expression are a linear function of time, which allows performing the Gibbs sampling procedure. This method's stability is inversely proportional to the number of genes with non-linear transient behavior, and its threshold was estimated in 40% of the total sample; if this threshold is exceeded, the authors recommend using the Diffusion Pseudotime (DPT) method (Haghverdi et al., 2016).

Also, machine learning demonstrates its consistency and efficiency in the analysis of SC transcriptomic data. For example, single-cell interpretation via multi-kernel learning algorithm (SIMLR) can perform dimension reduction, clustering, and visualization; this algorithm is characterized by enhanced performance and better visualization and interpretability compared to t-SNE, PCA, and zero-inflated factor analysis (ZIFA) methods (Wang et al., 2017). There are additional packages and algorithms for analyzing single-cell data, from preprocessing to data visualization; for example, on the Bioconductor platform (Amezquita et al., 2020), or the Python-based scalable toolkit SCANPY (Wolf et al., 2018).

Modeling the dynamics of gene networks is a promising approach for extracting biological facts from single-cell transcriptomics. When reconstructing such networks, it is possible to identify both transcriptional regulators and their targets. For example, a high-performance TENET protocol is based on the calculation of transfer entropy and can predict large-scale gene regulatory cascades and relationships in single-cell data (Kim et al., 2020). Also, there is SCENIC, a fast calculation Python algorithm that reconstructs the regulons (Van de Sande et al., 2020). Comparing the

accuracy of calculations of gene networks by different algorithms showed that successful methods on artificial data sets are characterized by low accuracy on real data (Pratapa et al., 2020). The authors have selected three promising methods with high computational accuracy on real data: partial information decomposition and context (PIDC) (Chan et al., 2017), gene network inference with the ensemble of trees (GENIE3) (Irrthum et al., 2010), and GRNBoost2 (Moerman et al., 2019).

Elaboration of specific algorithms for using SC transcriptomic data to reconstruct developmental gene networks and identify new regulators remains a challenging issue. Databases and genetic interactions can serve as an additional source for expanding genetic networks and their verification. For example, STRING database (Szklarczyk et al., 2019) includes information about protein-protein interactions and allows to perform network reconstruction, visualization and functional enrichment analysis. Cytoscape is a suitable environment for further network visualization and addition of meta-information (Shannon et al., 2003). The functionality of this application has been significantly expanded due to the many available plugins. For example, the GeneMANIA plugin (Warde-Farley et al., 2010) allows to predict additional network elements and new connections, whereas the plugin *yFiles* (Wiese et al., 2004) provides additional tools for network layout.

Another ambitious challenge is the integration of multi-omics SC data. Ma et al. (2020) examines the capabilities of 10 SC integration tools and tests the functionality of the four most relevant ones (Giotto, MOFA, LIGER, Seurat3). It should be noted that the existing problems in the analysis and interpretation of data give rise to the rapid development of various methods and approaches to their processing. The available collection of various methods and tools for analyzing SC data is presented in this online repository. Also, pipelines and statistical methods useful for analyzing SC data are presented in the work by Petegrosso et al. (2020).

Although obtaining high-quality SC transcriptomic data for plants is a routine, standardized procedure, cell extraction processes, meaningful interpretation and verification of data are essential and non-trivial stages for the development of this technology. An important step in data validation and interpretation is the construction of mathematical cell-based models, which combines the data about concentration of morphogens and expression of genetic regulators inside the cells and “rules,” which determine intercellular communications, cellular mechanics, transport processes as well as the transition between cellular states. However, with current technology, we cannot directly use the entire array of transcriptome data to create mathematical models of morphogenesis due to the large number of dimensions. Therefore, it is important when comparing different cell types to identify the main genetic and metabolic differences and take them into account in models.

There are a few methods, which can potentially allow researchers to use scRNA-seq data for building the cell-based models (see Figure 2):

1. Identifying crucial genes (main effect genes) and regulators which explain a lot of variance/differences between cell types.

2. Searching for novel regulatory genes, which have a spatial distribution of expression between cells of different types.

3. Reconstructing Boolean gene networks using transcriptomic data.

4. Estimation of differences in integral characteristics (such as biomass, wall thickness, concentration of metabolites).

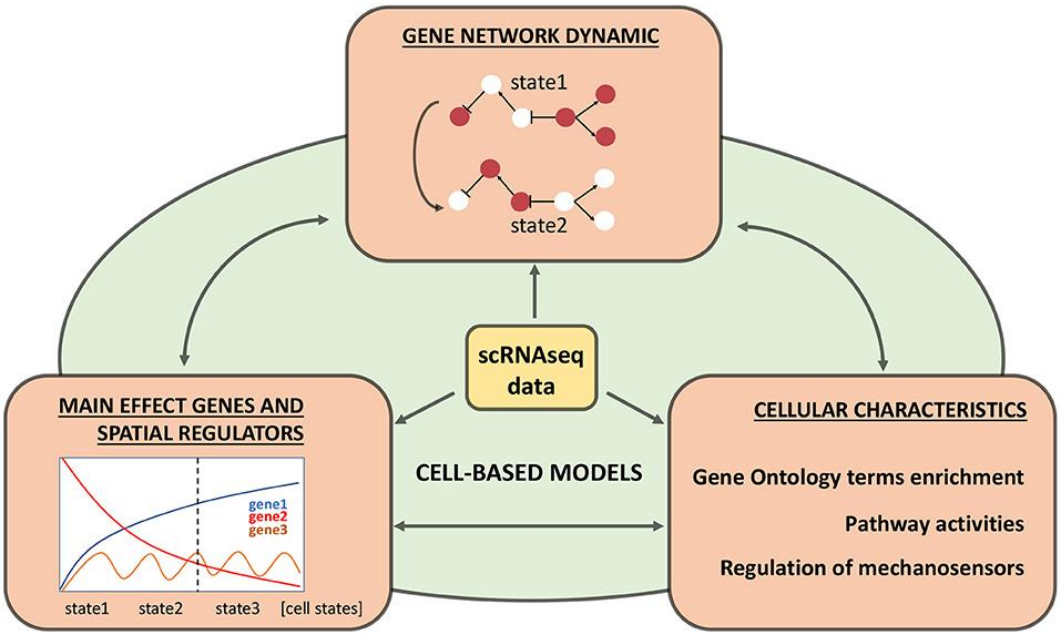

Figure 2. Relevant information from single-cell transcriptomics experiments for cell-based models. Three types of information are highlighted in orange blocks, their integration into the cell-based model is shown in green, and double-headed arrows indicate each block's comparison. The central yellow block indicates original processed single-cell RNA sequencing (scRNA-seq) data.

For example, SC transcriptome data could provide some indirect estimations of the cell wall's mechanical properties. The main mechanosensing genes are described in Du and Jiao (2020): receptor-like kinase FERONIA (FER), Leucine-rich repeat extensins (LRXs), DEFECTIVE KERNEL 1 (DEK1), and their targets of cell wall integrity pathways. Therefore, assessing these genes' expression levels in different cell types can potentially describe their mechanosensitivity and cell wall stiffness. Thus, SC data allows the definition of cell types of molecular characteristics, identifies regulatory subnetworks, and assesses their dynamics. These data can potentially be considered as parameters in cell-oriented models.

2.3 MODERN IMAGING TECHNOLOGIES FOR OBTAINING DATA ON PLANT TISSUES WITH A SINGLE-CELL RESOLUTION

Spatial organization plays a significant role in each cell's fate, affects transport, the direction of division, apoptosis, and the cells' structural peculiarities. Therefore, this information is the basis for a systemic integrative study of the processes of morphogenesis.

The cells of vascular plants form a shared symplast through the cell walls, which determine the fixed position of the cells in the tissue (Vaahtera et al., 2019). In plants, cell migration is almost absent, but in some cases, cells can shift their positions relative to each other: part of the plant cell remains in its original place, while other parts of the cell grow to the new locations, moving significantly relative to other cells (Lev-Yadun, 2015).

There are various specialized approaches for phenotyping (Figure 3): visible light, spectroscopy, infrared, fluorescence, 3D, and tomographic methods for getting plant images (Li et al., 2014). The imaging techniques for plant quantification are broadly used due to their inexpensive cost, simplicity of operation, and maintenance (Omari et al., 2020).

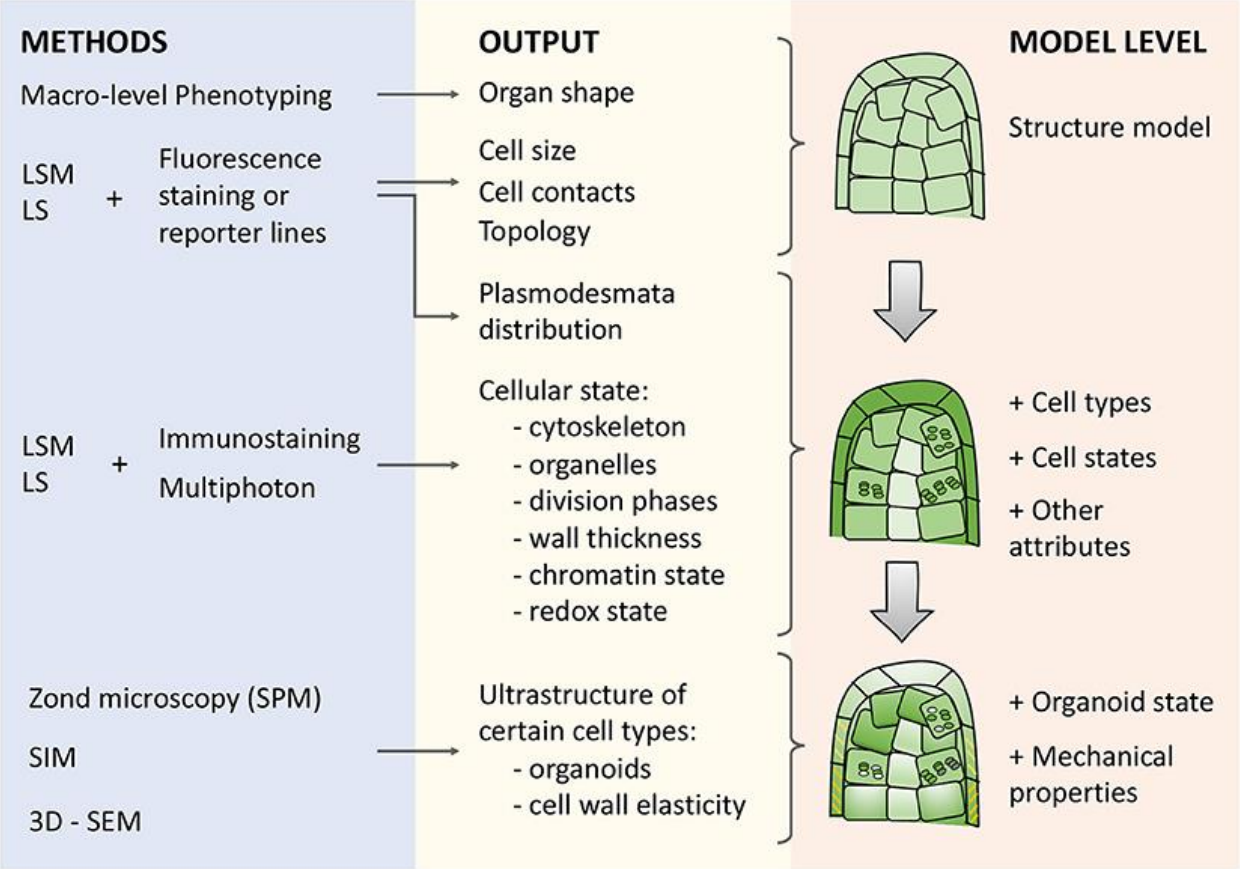

Figure 3. Types of microscopy techniques, their outputs, and meanings for describing morphogenetic processes in cell-based models. There are three blocks in the scheme: (i) methods (blue box), (ii) corresponding outputs (yellow box), and (iii) model levels (orange box) from structural to organoid resolution. Abbreviations used: LSM (Laser Scanning Microscopy), LS (Light-Sheet microscopy), SPM (Scanning probe microscopy), SIM (Structured Illumination Microscopy), 3D-SEM (3-Dimensional Scanning Electron Microscopy).

Reconstruction of plant architecture in terms of shape, size, and topology of cell connections (Figure 3) is an essential component to reach an integrative systemic understanding of aspects of the functioning of both individual cells and tissue as a whole (Fricker, 2016; Zubairova et al., 2019; Kerstens et al., 2020). A variety of optical tissue imaging techniques (Figure 3) currently allow access to such cellular characteristics (optical and fluorescent microscopy, laser scanning approaches, and structured lighting microscopy). Since higher plants' organs are multilayered and volumetric, imaging techniques based on 3D analysis of a fluorescent signal, such as laser scanning microscopy, are currently among the most widespread visualization methods of cellular architecture. It allows to reconstruct the architecture of tissue and organ fragments consisting of thousands of cells (Zubairova et al., 2019) and to analyze *in vivo* large time-series for reconstructing the dynamics of development (Goh, 2019; Seerangan et al., 2020).

Together with modern image analysis methods, they provide a reliable decomposition of cell layers and assessment of cell morphological parameters (Legland et al., 2016; Erguvan et al., 2019; Zubairova et al., 2019). The number of cells reconstructed by ImageJ-plugins LSM-W^2 (Zubairova et al., 2019), SurfCut (Erguvan et al., 2019), as well as MorphoGraphX instruments (Kerstens et al., 2020) is limited by the computer performance and technical capabilities of the microscope. They allow working on a local computer with arrays from thousands of cells, which is of a comparable order to scRNA-seq methods. The most comprehensive range of methods makes it possible to segment cells, measure cell shape parameters, and reveal the topology of cells' connection with each other (Jackson et al., 2017).

Over the past few years, the possibility to study many entire organs through complete reconstruction at the cellular level became a significant breakthrough (Wolny et al., 2020). The root tip of *A. thaliana* is the most abundant target for scRNA-seq in plants. At the same time there are many reconstructions and 3D atlases for it (Dolan et al., 1993; Bowman, 2012; Mai et al., 2014) and even specialized software that allows displaying the various cellular characteristics into cellular ensembles, for example, the iRoCS Toolbox (Schmidt et al., 2014). *In vivo*laser scanning microscopy techniques coupled with mathematical modeling allowed describing the processes of morphogenesis for the arabidopsis root apical meristems (Mironova et al., 2012). The dynamics of the development of *A. thaliana*lateral roots are also available for visualization at the cellular level from the earliest stages of their establishment (Goh, 2019). Using confocal and multiphoton microscopy approaches, apexes and leaf primordia can also be completely reconstructed (Kiss et al., 2017; Wolny et al., 2020), as well as adult leaves (Wuyts et al., 2010) and sepals (Tauriello et al., 2015).

3D reconstruction of *A. thaliana* ovule coupled with transcriptome sequencing provides incredibly detailed data about developmental processes of this organ (Vijayan et al., 2021), which can serve as a set of reference points for further integration of future single-cell data on this organ. Simultaneously, the methods of visualization and analysis of images also allow working with plants with larger organs, for example, with *Nicotiana tabacum* roots (Pasternak et al., 2017).

Light-sheet imaging techniques allow to increase the scan depth and improve the quality of the reconstruction. These technologies, coupled with mathematical modeling, gave insights into the geometrical organization of divisions during the formation of the lateral root of *A. thaliana* (von Wangenheim et al., 2016). In particular, the first division of the cell-founders is always asymmetric and determines the formation of a layered structure, while the pattern of further cell division forms thanks to a regular change in the orientation of the division plane. Also, the technique of optical cleaning of plant tissues allows for getting deep 3D imaging and is compatible with fluorescence-based microscopy (Warner et al., 2014). The measurements of morphological characteristics of cells and their mutual arrangement allowed researchers to form a structural model of the studied organ and identify cell types (Kerstens et al., 2020).

The current opinion about coordination of growth processes and divisions (Sablowski, 2016) stressed the role of individual cell characteristics and intercellular interactions in these processes. Optical microscopy is a valuable method for obtaining the structural characteristics on the subcellular resolution. For example, this approach allows studying the ultrastructural features of the cell wall (Yarbrough et al., 2009), which enables us to assess cellular biomechanics indirectly. The combination of large-scale annotated image datasets and deep learning approaches is a promising technique for annotating physical, morphological, and tissue grading cellular properties (Fricker, 2016; Biswas and Barma, 2020).

The cell wall's mechanical parameters deserve special attention since they determine features of the growth process (Bidhendi and Geitmann, 2016), and therefore is incredibly important for modeling plant morphogenetic systems. In addition to assessing the thickness of the cell wall (Krzesłowska et al., 2019), modern approaches make it possible to evaluate its composition and mechanical parameters. For example, probe microscopy can assess the spatial composition of polysaccharide filaments on the surface of living tissues (Zhang et al., 2016), and Raman microscopy can produce data on the composition and ultrastructure of the cell wall on sections of organs in the usual (Zeise et al., 2018) and confocal modes (Gierlinger et al., 2012). The ultrastructure of cell walls as well as tissues and organs can be studied with a 3D electron microscope (Kremer et al., 2015). All these methods make it possible to assess biomechanical parameters within organs and serve as the basis to improve the simulation modeling of growth processes.

Therefore, the next important step is integrating the structure model with the cell parameters that mark the individual and group characteristics of cells (Figure 3). Many characteristics of the nucleus, organelles, and cell walls can be identified at the scale of an entire organ using approaches of protein immunolocalization, expression of reporter constructs that mark certain cellular features, as well as using methods to increase the resolution of microscopy (Figure 3).

The data on the frequency of mitoses along the root (Pasternak et al., 2017; Lavrekha et al., 2020) provides insight into the dynamics of replenishment of cell files and the size zones, where cell divisions occur. Also, cells in S-phase can be identified by incorporating labeled nucleotide analogs (Pasternak et al., 2017). The passage of the cell cycle phases is closely associated with the cell fate specification (Roeder et al., 2012). The state of chromatin in cells of various types can be identified using immunolocalization (She et al., 2018) and shed light on cell activity. Visualization of the cytoskeleton can be done both by immunolocalization, staining with phalloidin, and, *in vivo*, using reporter genetic constructs (Zhang et al., 2020). These cells' characteristics can be related to changes in gene groups' expression in cells and are suitable for improving the integration of the structural model with single-cell transcriptomic data.

The distribution of various proteins in plant organ cells can also be determined (Sauer and Friml, 2010) and used for integration into a model. Proteins can be transporters that determine the fluxes of substances that deserve special attention; for example, the auxin membrane transporter PIN1 has a significantly uneven distribution over root cells and a polar arrangement on the cell surface (Omelyanchuk et al., 2016). It has also been shown that RNA molecules capable of being transported from tissue to tissue play an essential role in the regulation of biological processes in a plant, and their visualization within an organ is also possible (Luo et al., 2018).

Also, plasmodesmata play a unique role in the processes of intercellular symplastic transport and signaling in plant tissues (for comprehensive review, see Heinlein and Epel, 2004). Plasmodesmata are intercellular channels characterized by various states from open to closed (Crawford and Zambryski, 2001). Plasmodesmata behavior underlies the isolation of groups of cells in the tissue, called symplastic domains (Pfluger and Zambryski, 2001; Lucas and Lee, 2004; Yadav et al., 2014). Stress factors affect the formation of plasmodesmata (Fitzgibbon et al., 2013). The transport of mRNA and metabolites through the plasmodesmata affects the concentration of substances and gene expression levels inside particular cells (Lucas and Lee, 2004). Many non-cell-autonomous transcription factors and small RNAs are known to move through plasmodesmata between cells and regulate their interaction during development (Kragler, 2013; Yadav et al., 2014; Sevilem et al., 2015).

Transmission electron microscopy is the classical method for studying the morphology of plasmodesmata. Combined with light-based microscopy, it allows one to study the structure and

distribution of plasmodesmata between cells of specific cell types (Nicolas et al., 2017). Also, the topology of plasmodesmata of contacting cells at organ scale can be studied using confocal and super-resolution microscopy (Fitzgibbon et al., 2010; Fitzgibbon et al., 2013). In this sense, microscopy allows us to assess the location and topology of plasmodesmata and, therefore, identify the potential of local transport of substances through these transport channels, symplastic domains, and to assess the order of cell division. Thus, the organization and localization of transport channels inside the plant tissues relate to the intracellular characteristics.

On the other hand, intracellular sensing processes contribute to intercellular signaling. For instance, there are special sensory plastids in epidermal and vascular parenchyma cells, which can cause a global systemic stress response in a plant (Beltrán et al., 2018).

The redox state of organelles is also an additional factor associated with developmental processes, ROS signaling, and antioxidant systemic plant cells (Bobrovskikh et al., 2020). In particular, the CellROX fluorescent reagent visualizes the oxidative potential of cells in a tissue (Kováčik and Babula, 2017).

Besides, mass spectrometry imaging and live single–cell mass spectrometry practically corresponds to single-cell metabolomics and makes it possible, for example, to mark the concentrations of secondary metabolites on the whole adult organ (Yamamoto et al., 2019). Such approaches can be combined with SC analysis of the expression of these metabolites' biosynthetic enzymes and transporters. As a result, they provide a basis for modeling the distributed regulation of these processes at the tissue level (Figure 3). The most important polynucleotides, such as RNA, can also be detected at the level of single molecules (Huang et al., 2020), which allows direct integration into the structural model of the organ.

Modern imaging techniques allow access to the structural and physiological characteristics of cells in a whole organ manner. It provides ample opportunities to create, enrich, and verify structural models of plant organs and tissues. An important aspect is that many assessments can be carried out over time. Comparison of temporal dynamics in zones with active morphogenetic events will make it possible to track changes in cellular topology, and thus, to trace the nature of division (symmetric and asymmetric) and growth (isotropic and anisotropic), as well as to detect several mechanical features of the developing tissue (for example, the relative stiffness of different cell zones).

Thus, a large arsenal of available microscopic and imaging techniques allows obtaining high-quality multilevel data integrated into plant morphogenesis models. For example, there is a computational morphodynamics approach that allows formalizing quantitative data from morphometry measurements into a set of rules (Formosa-Jordan et al., 2018):

1. To set ODE, which describes the growth rate of individual cells using data from regulatory networks.

2. To set various rules for the geometry of division (periclinal/tangential divisions with different angles) according to mechanical constraints of intercellular vertex interactions.

3. To use the first two steps to calculate effective growth and final rate equation.

2.4 CELL-BASED MODELING APPROACHES REPRODUCING PLANT TISSUE MORPHOGENETIC PROCESSES

2.4.1. Existing Models and Modeling Approaches

This section will discuss existing mathematical models describing the tissue organization and/or properties of individual cell types. While considering plant growth and developmental processes, researchers often highlight a unique role for the hormone auxin. For instance, in plant roots, auxin triggers cascades of events during development and morphogenesis, while other hormones (cytokinins, brassinosteroids, abscisic acid, gibberellins, and others) interact with auxin (Saini et al., 2013). Auxin is also an important regulator in developing shoot apical meristems in combination with cytokinins, gibberellic acid, and some transcriptional factors: WUSCHEL, ARR7/ARR15, ARF5 (Durbak et al., 2012). Mironova et al. (2012) demonstrated the effectiveness of the reverse fountain and the reflected flow mechanisms of PIN-associated transport in the root apical meristem. Comparison of different complexity models showed that a model that only describes auxin transport processes is insufficient for the reproduction of realistic patterns of morphogenesis but adding an additional layer-specific regulation or layer-driven growth could help solve this problem (De Vos et al., 2014).

Simultaneously, the mechanical characteristics of tissues, which are determined through a complex interplay of genetic and physiological systems, are an essential component for describing the processes of morphogenesis. The feedback effects of mechanical interactions and stresses, which affect the regulation of proliferation patterns, are highlighted in Nelson et al. (2005). The experimental evidence of the mechanical stress approach's consistency for plant tissue development is shown in the work of Uyttewaal et al. (2012). The transition from the linear models of hormonal transport to hybrid multicellular and multiscale models has excellent potential for predicting the emergent properties of the system (Voß et al., 2014). The basis for mechanical models of cell growth is the representation of multicellular tissues in vertex-based graphs with the calculation of the interaction forces between these elements. The equations binding the growth of plant cells with the rate of water absorption and the cell wall's growth were first published in Lockhart's work for the case of constant turgor pressure (Lockhart, 1965). To model growth in a more general case, Lockhart's equations were extended, taking into account the change in turgor pressure as a result of reversible elastic deformation and transpiration processes in the Ortega model (Ortega, 2010). Within the framework of this approach, a linear leaf growth model was proposed (Zubairova et al., 2016). In addition, Newton's First Law and Hooke's Law can be used to describe cell growth and expansion, as was done in the recent work by Retta et al. (2020).

Unfortunately, most available auxin-related models are focused only on the transport processes in the root tissue and poorly explain the overall processes of growth and development (Morales-Tapia and Cruz-Ramırez, 2016). However, several models combine both a mechanical approach and auxin transport processes. For example, there is a dynamic model that describes molecular mechanisms in conjunction with physical tension fields and auxin dynamics (Barrio et al., 2013). This model reproduces emergent patterns of morphogenesis from proliferative to transition and elongation zones. The study combining experimental data on the organization of the extracellular matrix and numerical simulations demonstrated that auxin plays an essential role in altering cells' mechanical properties; this process involves the ABP1 and KATANIN 1 proteins (Sassi et al., 2014). Also, the advanced cell-based mathematical model describes the relationship between the concentration of morphogens and the cellular mechanistic properties in the developing apical shoot meristems (Banwarth-Kuhn et al., 2019).

Thus, the models of plant tissue morphogenesis put at the forefront three biological facts: (i) the dependence on intercellular hormonal signaling, (ii) the importance of the intracellular state and individual cellular characteristics, (iii) the relevance of mechanical stresses in intercellular interactions. Therefore, scRNA-seq technologies, microscopy, imaging techniques, and a range of complementary approaches to measuring cell mechanical properties (Banwarth-Kuhn et al., 2019; Bidhendi and Geitmann, 2019) can provide a complete picture of morphogenetic processes at the cellular level.

2.4.2. Available Software and Tools for Cell-Based Modeling

In general, elaborating mathematical models of morphogenetic processes could base on specialized software, which we discuss in this section. Researchers may also develop and implement their frameworks and algorithms using mathematical packages and general-purpose programming languages (Python, Mathematica, MATLAB). Three formalisms are most often used to build cell-based models: vertex-based, center-based (also called spring-based), and Cellular Potts models. Vertex-based models are often used to simulate plant tissue and make it possible to conveniently describe the dynamics of cell movements in cell ensembles considering mechanical constraints (for example, during morphogenesis). This formalism is implemented in the Cellzilla (Shapiro et al., 2013), VirtualLeaf (Merks et al., 2011) packages. In center-based models, cells are represented as dots with mass, connected by mechanical elements (springs). Banwarth-Kuhn et al. (2019) give an example of this formalism's application to the description of growth processes in the shoot apical meristem. Cellular Potts models are often used to describe the processes occurring in animal tissues and tumor formation processes; this formalism is implemented in CompuCell3D

(Swat et al., 2012). It is also possible to use the Voronoi tessellation formalism for modeling morphogenetic processes, e.g., see Romero-Arias et al. (2017).

Below we discuss available software, while a summary is presented in Table 2.

Table 2. The most popular tools for cell-based plant tissue morphogenesis modeling.

Name	Publication	Link	Formalism used	Spatial scale	Examples
Virtual Cell	Moraru I. I. et al., 2008	vcell.org	Processes (kinetics, diffusion, flow, membrane transport, electrophysiology);	2D / 3D	Gajdanowicz P. et al., 2011 Onal, S. et al., 2020
VirtualLeaf	Merks R. M. et al., 2011	code.google.com/archive/p/virtualleaf/	Vertex dynamics model	2D	Vos D. D., 2014
CellZilla	Shapiro B. E. et al., 2013	cellzilla.info	Vertex dynamics model	2D / 3D	Nikolaev et al., 2013
CellModeller	Dupuy L. et al., 2008	haselofflab.github.io/CellModeller/	Biphasic systems; viscous yielding of the cell walls	2D	Dupuy, L., et al., 2010
LBIBCell	Tanaka S.et al., 2015	bsse.ethz.ch/cobi/Software.html	Lattice Boltzmann method for solving fluid and signalling processes	3D	Stopka et al., 2019
OpenAlea	Pradal C. et al., 2008	openalea.gforge.inria.fr/dokuwiki/doku.php	Functional–structural plant models	2D / 3D	Muraro et al., 2014
CompuCell3D	Swat M. H. et al., 2012	compucell3d.org	Cellular Potts model	2D / 3D	Hester SD et al., 2011 Swat M. H. et al., 2015

Virtual Cell (Cowan et al., 2012; vcell.org) is an environment for modeling, analysis, and simulation of cellular processes, and it includes tools for gene network and for the integration of biological images. This package consists of distinct functional modules: rule-based networks, ODE, PDE and kinematics, stochastic simulations, parameter estimation and has the ability to integrate it into hybrid models. Users can define the model structure and the system automatically builds the code and compiles it. A detailed overview of this tool is given in Moraru et al. (2008). Also, there is a VCell extension for compartmental and spatial rule-based modeling (Blinov et al., 2017). The implemented models using VCell can have a different scale, for example, the model of potassium transport in plant vascular tissues (Gajdanowicz et al., 2011), and model of the paracrine-juxtacrine loop for breast cancer cells and macrophages (Onal et al., 2020).

VirtualLeaf package (code.google.com/archive/p/virtualleaf/, Merks et al., 2011) using a vertex-based approach (Nagai and Honda, 2001); the algorithm includes vertex motions at each step that minimize the Hamiltonian energy by the Monte Carlo algorithm. For each cell, an unstressed area is specified, corresponding to the cell's state when the turgor pressure is balanced

with the external pressure. For each cell wall element, the unstressed length is specified, corresponding to the length of the cell wall segment in the absence of turgor pressure. The balance between turgor pressure and the cell wall's resistance can be described in terms of the generalized potential energy (Hamiltonian) calculated as the sum of all cells and cell wall elements, which is then minimized by the algorithm. The growth models of root were implemented using this framework (De Vos et al., 2014).

Cellzilla uses a vertex dynamics model for describing morphodynamics processes and considers morphogenetic regulation (http://cellzilla.info/, Shapiro et al., 2013). The cellular structure is represented by a list of three elements: a list of vertex coordinates, a list of edges consisting of pairs of vertex numbers, and a list of cells consisting of lists of edge numbers belonging to a cell. The interaction between morphogens and the transport flows in each cell is described in terms of chemical kinetics using the arrow notation of the Cellerator package (Shapiro et al., 2003). This software automatically constructs and solves a system of differential equations describing the dynamics of morphogens' concentration in all tissue cells. Methods for constructing models of plant cell growth in CellZilla are described by Shapiro et al. (2013). Using this system, Nikolaev et al. (2013) constructed a model for *A. thaliana* shoot apical meristem structure maintenance.

CellModeller (https://github.com/cellmodeller/CellModeller; Dupuy et al., 2010) is a software with modular structure for 2-dimensional simulations. It can reproduce the intracellular dynamics of metabolites, intercellular transport processes, as well as cell mechanics using physical laws. This software can be used for modeling plant morphogenetic processes. For example, a simple morphogenetic system for the Coleochaete alga has been developed (Dupuy et al., 2010).

LBIBCell (https://tanakas.bitbucket.io/lbibcell/, Tanaka et al., 2015) was developed specifically to simulate morphogenetic processes in tissues. This tool uses the immersed-boundary concept (which describes cells as viscous fluid with elastic walls), coupled with the Lattice Boltzmann method. The model of biased epithelial lung growth was implemented using this tool (Stopka et al., 2019).

OpenAlea (Pradal et al., 2008) is an integrative platform that combines various computational frameworks. This platform's main goal is the integration and mutual enrichment of experience in different sections of plant process modeling. This system is based on Python language and has a visual programming interface. For example, the OpenAlea package VPlants (https://team.inria.fr/virtualplants/) allows building models of tissue morphogenesis. This package was used in modeling vascular development in *A. thaliana* (Muraro et al., 2014).

CompuCell3D (Swat et al., 2012) is a C++ software for 3D modeling, which includes both graphical user and command-line interfaces. This system uses classical mechanics for describing

cellular behavior according to mechanical constraints. Multicellular systems are described using the Cellular Potts model. The input data include the grid's size, number of cells, cellular interactions, energy functions, and activator concentrations. The protocol for using this program to study cellular morphogenesis parameters is presented in Palm and Merks (2015). Most of the models elaborated with this software describe the development of animal tissues (Hester et al., 2011) and the processes of tumorigenesis (Swat et al., 2015).

Thus, the available software and methods are pretty diverse, and the choice of a particular tool depends on the specifics of the task at hand. Among these tools, it is necessary to highlight Cellzilla and VirtualLeaf as the most specific for describing plant morphogenesis processes. On the other hand, the development of new frameworks and algorithms, which depend on researchers' ability to program, is a promising approach since it significantly expands the functionality and removes several restrictions on applying one or another formalism implemented in existing software.

2.4.3. Our Framework and Model Flowchart

In this section, we propose a general framework for modeling plant morphogenetic processes based on various biological data. This kind of model should include two main data sources: scRNA-seq and tissue imaging data; besides, SC metabolomics and cell wall stiffness studies can serve as additional data sources. For plant organ growth modeling, the accurate description of processes on the cellular level is essential since this level combines molecular regulation with hormonal regulation, cell division, and reproduction processes (De Vos et al., 2012).

Mathematically, events occurring in plant tissues and cells can be classified into continuous and discrete ones. The first ones include the processes of metabolism, growth, transport and development of cells. Discrete events, on the other hand, include processes such as birth (or emergence), division, death, and change of cellular state. Individual cells' metabolic characteristics are influenced by their genotype and developmental stage, which would be described by single-cell transcriptomics approaches. The nature of the proposed framework is hybrid since it combines different mathematical formalisms and modules: (i) ODE/PDE equations for describing the dynamics of substances and morphogens inside the cell and the processes of intercellular transport, (ii) discrete events occurring during the onset of threshold conditions (for example, cell division when a specific cell area is reached, or cell differentiation at a hormone concentration above the threshold), (iii) the biophysical laws of mechanical interactions between cells (such as Ortega's approach Ortega, 2010 or Newton's and Hooke's laws Retta et al., 2020). In this sense, scRNA-seq data helps measure individual characteristics of cell populations (which characterize system dynamics), while microscopy should help to define geometrical patterns and “rules” (e.g., division

geometry or dividing plane orientation). These steps will help to create hybrid models with tissue/cellular resolutions.

The usefulness of such a hybrid approach in describing ecological systems was described in the work of Vincenot et al. (2011). In particular, the combination of discrete and continuous phenomena is a natural property of multicellular systems, and such hybrid frameworks allow researchers to make more realistic simulations *in silico*. Van Liedekerke et al. (2015) described the advantages and disadvantages for different types of agent-based models of tissue mechanics and noted that hybrid models could reproduce spatial resolution, physical aspects of interactions, cell shapes diversity. Osborne et al. (2017) compared different approaches to cell-based modeling using typical cases of the described processes; the authors noted that the vertex-based approach, in contrast to others, allows one to simulate boundary conditions in proliferation processes effectively. This feature allows us to consider this method as the most promising for modeling the root apical meristems, which has more severe mechanical restrictions for growth than leaf and shoot tissues. For modeling leaf and shoot tissues, for example, it is possible to use the Voronoi tessellation or overlapping spheres modeling approach described in Osborne et al. (2017).

Thereby, we assume the use of such a hybrid approach complementary to modern research due to its multilevel nature; it combines SC transcriptomic and microscopy data into a cell-based modeling framework. Below in the text and in Figure 4, we outline the main stages of our framework that must be considered.

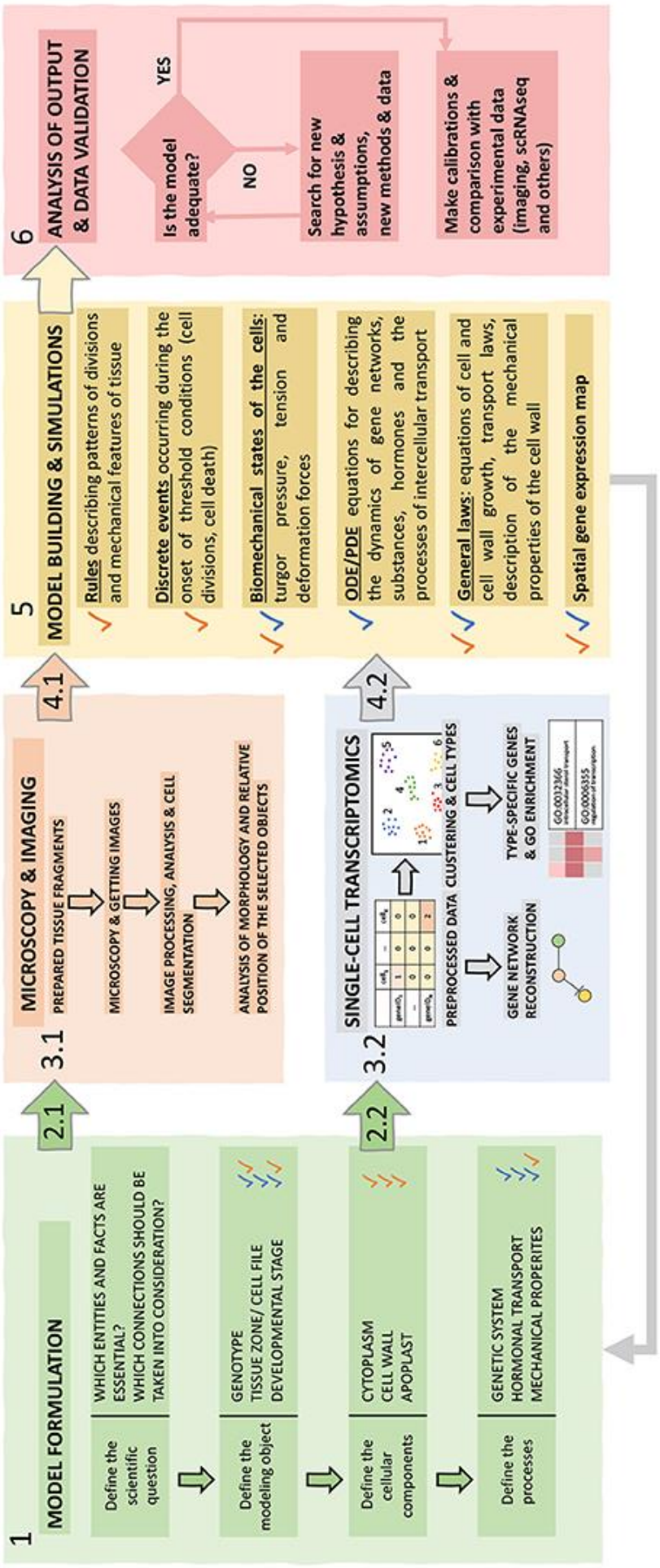

Figure 4. The proposed hybrid framework for cell-based models construction. The framework includes six functional blocks explained in the text. Individual blocks are marked with corresponding colors; colored arrows indicate the transition between blocks. Blue and orange checkmarks indicate information related to single-cell and imaging data, respectively.

1. The posed biological problem determines the structure of the model. A modeler should define a biological system's properties, its elementary subsystems, and connections between these elements, which are significant to reproduce them in the model. Based on these decisions, it is necessary to determine the main properties of the simulated object: genotype, organ, tissue zone, stage of development. Since a cell is a crucial element for describing the processes of plant morphogenesis, the next step is to find out which cellular structures will be reproduced in the model to determine the formalism used to describe them and the equations for growth and the rules of division. Then, it is necessary to decide on the objects at the molecular level to be considered, the genetic systems of interest, to find out whether it is required to consider transport processes for morphogens (for example, hormones), and to decide whether it is necessary to take into account the biomechanics of cells for the modeled system.

2. Designing experiments to obtain imaging (2.1) and scRNA-seq (2.2) data based on the given aim. For imaging (2.1), it is essential to choose a suitable plant portion and microscopy technology and determine whether it is necessary to track the dynamics of development of a given fragment of tissue and for which interval of time. For scRNA-seq (2.2), it is important to make sure that the process of isolation of protoplasts and their analysis will not be limited due to the structure of the tissue and/or organ of the plant, imperfections, and shortcomings of the available methods, otherwise, this technique will have to be worked out and improved to an acceptable level.

3. Perform the experiments and produce data. (3.1) It is necessary to prepare (for example, fix and stain) a target tissue fragment, get images, process, and analyze them (manually or using plugins), and digitize the resulting patterns to build a structural model of the tissue/organ and identify morphogenetic rules for incorporation into a computational model. (3.2) While obtaining and analyzing scRNA-seq data, special care should be taken to ensure that the research aim is as close as possible to the intended modeling goals. Care should be taken to avoid contamination with cells of those classes that are not needed and so that for most of the required cells, it would be possible to analyze the molecular systems required for the model. Besides, scRNA-seq-based approaches for the reconstruction of gene networks of the corresponding processes have high potential.

4. Analyze experimental data. Experimental results at cell and tissue level have to be analyzed in order to derive key parameters to be used in the model formulation in terms of cellular characteristics (4.1) and molecular processes (4.2) for all the considered cell types.

5. Systematic assembly of the hypotheses, available data and mathematical formalization into a single hybrid model, which consists of the following blocks: (1) ODE / PDE equations for describing the dynamics of substances and morphogens inside the cell and the processes of intercellular transport, (2) discrete events occurring at the onset of threshold conditions (for

example, cell division when a specific cell area is reached, or cell differentiation at a hormone concentration above the threshold), (3) biomechanics interactions between cells (4) agent-based rules describing patterns of divisions and mechanical features of the tissue.

6. Validation and verification of models is based on their success in reproducing the behavior of real biological phenomena that can be evaluated experimentally. In this sense, it can be useful to return to the stage of morphometry and compare the dynamics of tissue development with simulations and study in detail the molecular organization of the subsystems described in the model.

In general, the proposed approach is universal for describing any morphogenetic system; however, the pipeline described above may differ in some steps for each specific case, while some of them could be eliminated. Plant tissue morphodynamics is context-dependent due to mechanical interactions inside cell ensembles and the transport of morphogens through plasmodesmata, which is confirmed by numerous studies (Crawford and Zambryski, 2001; Heinlein and Epel, 2004; Lucas and Lee, 2004; Kragler, 2013; Yadav et al., 2014; Sevilem et al., 2015; Luo et al., 2018). At the same time, models for morphodynamics of animal tissues with strong neighborhood structures could include analogous mechanisms modified to consider cell adhesion processes. For example, this approach is applicable to model the processes of animal epithelial or tumor growth (Interian et al., 2017).

2.5. CONCLUSIONS

Post-genomic technologies made it possible to obtain detailed information about processes at genomic and transcriptomic levels using SC and whole tissue RNA sequencing technologies. Besides, the existing abundance of microscopy methods allows high-quality characterization of morphology and physiology at the level of extended fragments of tissues and organs. However, microscopy approaches do not allow to perform quantitative assessments of important intracellular characteristics, such as concentrations of substances and metabolites. SC metabolomics approaches for plants, which are beyond this review's scope, remain overshadowed, although significant developments have been made in mass spectrometry approaches for such kind of analyses (de Souza et al., 2020). Gilmore et al. (2019) discuss the latest advances in mass spectrometry imaging: matrix laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS), which have a high potential for assessment of metabolism at subcellular spatial resolution. The development of these methods will allow metabolomics to achieve the same spatial resolution level as SC transcriptomic. The review of Bidhendi and Geitmann (2019) presents the main features and possibilities of measuring the cell wall's mechanical properties: indentation technique, tensile test, acoustic microscopy, fracture measurements, and microfluidics. The authors emphasize that multiscale *in silico* mechanical modeling has excellent potential for the field and could help obtain a unified understanding of mechanical behavior across different scales.

To date, the methods, and technologies necessary to obtain various experimental data for plant morphogenesis models have reached a balance and are mostly consistent with each other in terms of power, productivity, and spatial resolution. The community of mathematical biologists and programmers faces crucial theoretical challenges and is creating efficient computational frameworks capable of large-scale numerical simulations involving cellular ensembles of several thousands of cells. Such models will provide more accurate resolution and realism in the description of morphogenetic processes. Examples of optimization works are the algorithm of Jeannin-Girardon et al. (2015), and graphics processing units (GPU) accelerated framework for 3D cellular growth and division models (Madhikar et al., 2018). Moreover, declarative modeling perspectives concerning morphogenetic processes are considered (Mjolsness, 2019), which potentially will help formalize mathematical calculations at higher levels compared to general-purpose programming languages.

The widespread development of SC technologies in the future could serve as a driver for other areas of cellular and developmental biology of plants (Libault et al., 2017). However, we have an urgent need for data integration to successfully apply the technology, at tissue level with

its organization's peculiarities as an emerging system. Besides, an increased availability of SC data can stimulate the development of methods and modeling concepts at cellular and tissue levels, which will open the way for the binding of multi-omics characteristics for individual cell types and the observed phenotype.

On the other hand, it is necessary to verify the emerging issues related to the interpretation and analysis of SC data using advanced microscopy and *in silico* biology. In this sense, one of the most urgent problems of SC sequencing is the reverse reconstruction of the spatial position of cells based on corresponding transcriptome expression. Searching for major regulatory genes that characterize certain cell lines will be a critical step to solve this problem. Also, cell-based models of morphogenesis could help interpret and integrate SC and imaging data, making the reasoning more transparent and establishing an understanding of essential parameters and mechanisms for the described systems.

Summarizing all the above, we have found the following key features related to SC-technologies that need to be addressed:

1. Some limitations are still present in the phases of integration, analysis, and interpretation of data.

2. Only a limited set of plant species and organs is suitable for obtaining transcriptome and structural data with cellular resolution.

3. There is a need for a more precise reconstruction of scRNA plant atlases.

The task of elaborating and analyzing *in silico* models of morphogenesis, due to the complexity of the studied systems and computational limitations, are non-trivial. Thus, cell-based models, which use a hybrid formalism, could effectively combine our knowledge on different levels and help tackle the complexity of the system. However, the current problem of the large number of dimensions of the initial SC data should be solved by applying preprocessing and filtering algorithms, as well as for the reconstruction of related gene networks. Thereby, model formulation and numerical experiments *in silico* could be applied using only the essential part of the initial high-dimensional SC data. Such reduction should aim to contain data on gene expression changes and metabolites concentrations, which determine the different cellular states.

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Chapter 2

Adult conspecific density affects Janzen-Connell patterns by modulating the recruitment exclusion zones

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3. ADULT CONSPECIFIC DENSITY AFFECTS JANZEN-CONNELL PATTERNS BY MODULATING THE RECRUITMENT EXCLUSION ZONES

3.1. INTRODUCTION

More than 50 years ago two ecologists, Janzen (1970) and Connell (1971) in Central America and in Australia respectively, independently proposed the hypothesis that seeds and seedlings suffered a distance- and density-dependent mortality. Empirical observations reported that seed-fall obviously concentrated under the parent fruiting trees, whereas seedlings and saplings recruitment didn't match the seed-fall kernel, unexpectedly peaking at a certain distance from the source tree. Their descriptive model consisted of two curves: the first showing seeds dispersal around a mother tree and the second reporting the survival probability of the seedlings as a function of distance from the same tree. Accordingly, a species was found unable to recruit under adult conspecifics because of the formation of an "exclusion area" where the mortality was disproportionately higher and not matching with the large seed availability (Janzen 1970). Such phenomenon, preventing the dominance of a single species, was also recognized to allow species coexistence and to promote the maintenance of biodiversity in the ecosystems (Levi et al. 2019). In other words, field observations showed that a mortality agent operated in a distance, density-dependent, and species-specific way, impairing only the recruitment of the focal tree, with no effects on seeds and seedlings of other species.

Early reports of the Janzen-Connell (JC) recruitment distribution suggested that insects were killing all seeds falling under the canopy of the studied trees (Janzen 1971). However, most later studies assessed only the occurrence of some indirect insect damages, missing the identification of a particular causative pest species (Basset et al. 2019). Other studies blamed the activity of vertebrates and mammals, but in most cases the activity of such animals cannot be related to the distance-dependent effects, considering their mobility over the forest floor (Song et al. 2021). In any case, predation is still considered as the main causal mechanism of the JC effect also in theoretical studies (Smith, 2022).

The role of soilborne and airborne pathogens has been also considered based on the assumption that pathogens propagules, including spores and sclerotia, may accumulate under the focal tree. For instance, Packer and Clay (2000) reported that the oomycete *Pythium* spp. disproportionately killed the seedlings rooted under the canopy of conspecific trees. Then, plant pathogens have been considered as main key factors in maintaining coexistence by causing local plant-soil Negative Feedback (NF), especially in wet ecosystems where oomycetes and fungi

thrive (Agrios 2005). However, most of the available experimental and field studies only reported pathogens damage, e.g., seeds covered by mould, seedlings wilting, leaf discoloration and spots, often missing the identification of the causal pathogenic agent and always without any assessment of consistency between the spatial distribution of the NF occurrence and the pathogens dispersal behaviour.

Less popular is the hypothesis of the "exclusion zone" being generated by the accumulation of autotoxic chemical compounds. The study by Webb et al. (1967) was one of the first reporting that some unidentified autotoxic compound, killing seedlings of *Grevillea robusta*, could be the cause of the JC patterns reported in Australian forests. Autotoxicity has been reported for hundreds of plant species, mostly crops, and associated with the release of phytotoxic compounds during the decay of leaf and root litter, including a range of different compounds such as aromatic phenols, saponins, coumarins, and organic acids, among others (Singh et al., 1999). However, the autotoxicity hypothesis has suffered from a relevant objective weakness because all the above-mentioned compounds produce a generic phytotoxicity, thus unable to explain the species-specificity of the JC distributions. Moreover, no studies were able to identify and quantify the accumulation of any specific toxin under and around the focal tree. Despite these major problems, the autotoxicity hypothesis has continued to be inconsistently associated to generic allelopathic phytotoxic effects (Inderjit et al., 2021). Differently, autotoxicity found a logical explanation in the discovery that accumulation of fragmented extracellular DNA from decomposing leaf litter did cause extensive seed germination impairment and root damages to a broad range of higher plants, with toxic effects specifically limited to conspecifics (Mazzoleni et al. 2015). The evidence of such inhibitory effect by extracellular self-DNA was then recently confirmed and further investigated by means of whole-plant transcriptome profiling on the model plant *Arabidopsis thaliana* (Chiusano et al. 2021).

Irrespectively of the underlying mechanisms, several studies explored the consequences of the JC hypothesis using simulation models. Hubbell (1980) argued that disproportionately high seed densities under the parent tree would overcome the lower survival, thus resulting in monotonic recruitment patterns independent of distance from the focal plant. Later, Nathan and Casagrandi (2004) made a first systematic modelling exploration of the JC hypothesis. By using a mathematical model of distance and density-dependent seed mortality, the authors demonstrated that the net balance between seed dispersal and recruitment survival could generate all observed recruitment patterns, including both the hump-shaped typical JC distribution and monotonically decreasing (Hubbell) patterns (Figure 1). Later, Vincenot et al. (2017) presented an individual based model, at the scale of one focal plant, reporting that strong NF under a conspecific tree may overtake the seed dispersal kernel, thus creating an "exclusion area". Moreover, the study also

demonstrated that NF could produce a shift outward of the recruitment peak from seedlings to saplings, during a longer assessment of the recruitment process. More recently, Levi et al. (2019) changed the perspective from a single focal plant to ecosystem scale and, using high-performance computing and analytical models, demonstrated that distance-responsive natural enemies can maintain tropical forest diversity nearly indefinitely by favouring rare species. Moreover, the effect of NF at ecosystem level has been modelled, clearly explaining species coexistence (Bonanomi et al. 2005) and its consistent relationship with biodiversity levels in different ecosystems in association with the rates of litter decomposition producing autotoxicity (Mazzoleni et al. 2010).

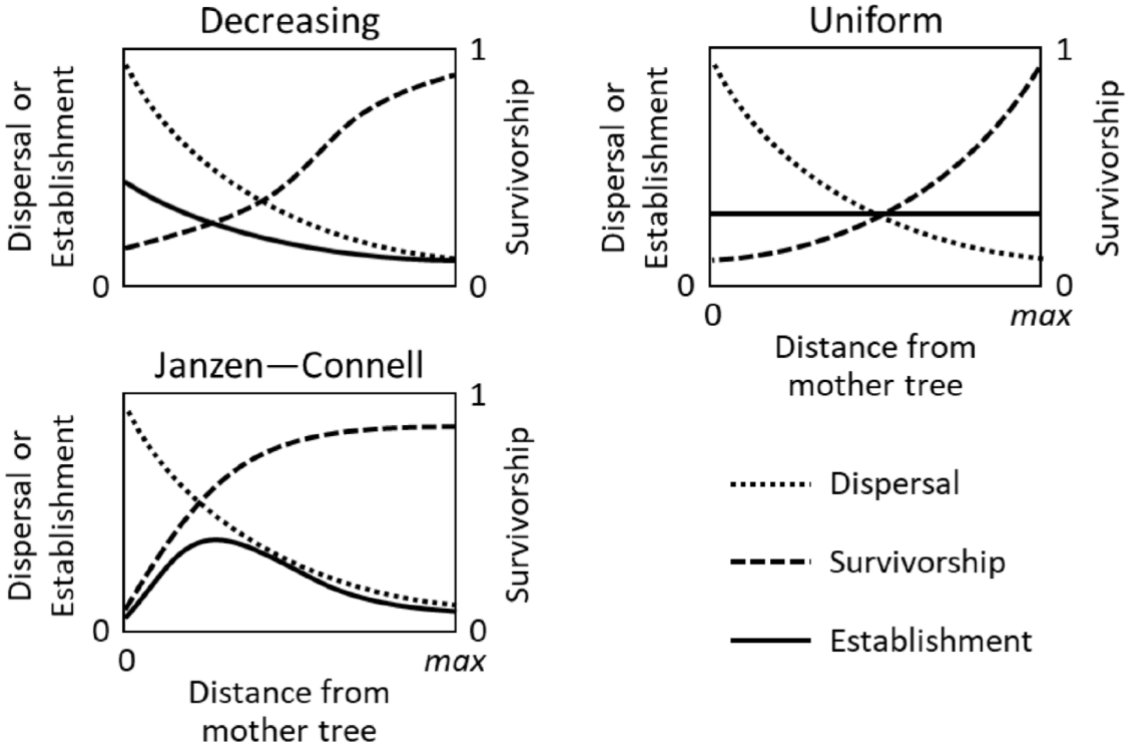

Figure 1. Common types of recruitment patters (adapted from Nathan and Casagrandi, 2004). Each plot shows the seed dispersal (dotted lines), survivorship (dashed lines) and establishment (solid lines) curves for the three most common recruitment patterns: monotonically decreasing (or Hubbell); uniform (or exact compensation); Janzen-Connell.

Besides these robust modelling studies, the importance of the JC recruitment pattern is largely supported by many publications of empirical data from a broad range of ecosystems, including tropical (Mangan et al. 2010; Comita et al. 2014) and temperate forests (Fox 1977; Packer and Clay 2000), as well as shrublands (Bonanomi et al. 2008, Teste et al. 2017) and grasslands (Petermann et al. 2008). However, despite such strong base, scepticism still persists especially in the community of forest ecology (Terborgh 2020). The main doubts about the actual relevance of the JC hypothesis are caused by the variable results observed in multispecies studies reporting JC distributions for some species but Hubbell patterns for others, also named as reverse

JC, and interpreted as a positive distance-dependent process due to accumulation of symbiotic microbiota (Zahra et al. 2021). Although it cannot be denied that JC patterns do exist in many species, their absence in other coexisting species raised a strong debate on the effective generality and magnitude of JC effects (Song et al. 2021). Explaining the reasons of such variability of occurrence of JC distributions would shed light on the relevance of this ecological phenomenon and on its effect on species coexistence and diversity maintenance.

In this regard, a relevant issue is whether the distance-dependent mortality factors, preventing the recruitment near the parent tree, are affected by the surrounding density of neighbouring conspecific adults. This point has been mostly neglected by previous studies that focused only on the focal tree concept, overlooking the possible role of the surrounding landscape of both conspecific and heterospecific trees. Only the recently published work by Smith et al. (2022) recognized that conspecific density may affect JC effects on species coexistence relating this to putative changes of predation levels. Theoretically, the density of conspecific adults may affect the behaviour of invertebrate and vertebrate predators (Janzen 1971), the spread of airborne and soilborne pathogens (Sapoukhina et al. 2010), as well as leaf and root litter distribution and the associated self-inhibitory factors produced during decomposition (Bonanomi et al. 2017). For example, an isolated tree accumulates litter under its canopy, thus creating a pattern associated with the concept of "island of fertility" when interpreted in terms of positive soil conditions for plant growth (Facelli and Brock 2000), but also generating a round shaped exclusion zone around its trunk and within its own crown projection by NF. However, as the surrounding density of adult trees of the same species increases, the spatial distribution of litter progressively overlaps among individuals, generating a complex patchiness in terms of exclusion zones created by the compenetrating conspecific "litter islands".

The aim of this work is to explore the connection between adult density, either conspecifics or heterospecifics, on the probability of occurrence of JC distributions. In detail, using an Individual-Based Modeling (IBM) approach (DeAngelis and Grimm, 2014), we simulated the formation of exclusion zones due to the build-up of NF in proximity of conspecific adult plants. The specific hypotheses of our study were:

(i) The frequency of JC distribution is high in the case of isolated trees;

(ii) the occurrence of JC distributions decreases as adult conspecific density increases due to the progressive overlap of exclusion zones;

(iii) the JC distributions are rare in the case of isolated individuals of a species when immersed in a matrix of heterospecific trees because of a dilution effect on NF conditions.

3.2 MODELING PIPELINE AND MODEL DESCRIPTION

3.2.1 Model rationale

The model presented here was developed to investigate the role of forest stand density and species diversity on the occurrence of exclusion zones produced by localized NF. The model is developed to represent the effect of NF on seed germination and seedling establishment. In this study, the term NF stands for the ecological conditions negatively affecting the establishment of seedlings.

The model is based on three assumptions: i) NF is species-specific i.e., it affects only plants of the same species; ii) NF intensity is proportional to the aboveground tree biomass, and; iii) the presence of heterospecific individuals in the same area decreases the intensity of the NF. The first assumption is based on a very large number of studies demonstrating the species-specificity of this phenomenon (review in Kulmatiski et al. 2008; Van der Putten et al. 2013; Cesarano et al. 2017). The second assumption is reasonable considering the autotoxicity hypothesis (Mazzoleni et al. 2007), with a release of autotoxic factors proportional to the amount of standing litter and its decay rate. Moreover, also the amount of soilborne pathogens inoculum is often proportional to the amount of plant residues left over and incorporated into the soil (Agrios 2005; Bonanomi et al. 2007). The third assumption is based on the hypothesis of a physical dilution of conspecific autotoxic litter in mixed multispecies stands (Mazzoleni et al. 2007, Mazzoleni et al. 2010). Moreover, rare species are indirectly protected by non-host, neighbouring heterospecifics, as predicted by the herd-immunity hypothesis, reducing the probability of contact with propagules of virulent plant enemies (Wills et al. 1997),

In the following sections, the model implementation and the simulation design are described.

3.2.2 Model description and simulation setup

Each simulated experiment is initialized with an area of 140 x 140 m (1.96 ha) and a predefined number of individual adult trees, randomly placed in the domain. The first individual is always placed in the centre of the domain and represents the target (focal tree) of each simulated experiment. For simplicity, every tree is assumed to have a canopy radius of 5.5 m and its biomass distribution is represented by a paraboloid function, with its maximum value at the centre of the tree crown. After this initialization step, a map of biomass distribution for each tree species is calculated as sum of the biomass occurring in every pixel. Following the assumptions defined in the model rationale, i.e., that the *NF* is proportional to the aboveground tree biomass, a map of *NF* for each species is calculated using the biomass map multiplied by a coefficient (i_{NF}) representing

the intensity of the *NF*. Seeds and seedlings are assumed to have no direct contribution to *NF* because of their negligible biomass compared to the litter produced by the adult trees.

In the case of co-occurrence of two or more tree species, in the case of biomasses of different tree species overlapping, a dilution effect was considered due to the presence of heterospecific litter. In detail, the *NF* for each species in each point in space is calculated as follows: $NF_i = i_{NF} \cdot Bi \cdot \frac{B_i}{\sum_{j=1}^{n} B_j}$ (eq.1), where B_i is the biomass of the *i-th* tree species, B_j are the values of biomass of other species and *n* is the number of coexisting tree species. It has to be noted that in the case of monospecific stands (*n*=1), the last equation becomes: $NF_i = i_{NF} \cdot B_i$ (eq.2)

A visual representation of the calculation of the trees' biomass and related *NF* is shown on Figure 2. Examples for an isolated tree (Figure 2A), overlapping trees of the same species (Figure 2B) and overlapping trees of different species (Figure 2C) are provided.

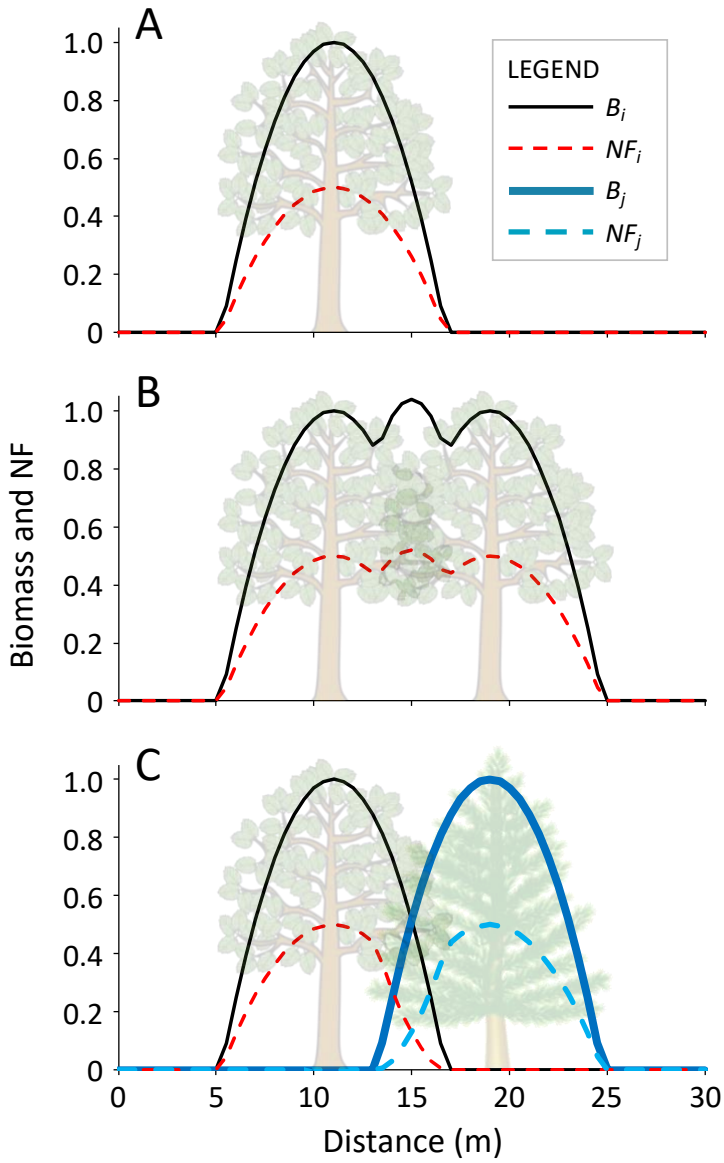

Figure 2. Graphical representation of calculation of tree biomass and NF in monospecific and bi-specific stands: A) single tree; B) two overlapping trees of the same species; C) two overlapping trees of different species. Bi and Bj represent the biomass curves, while NFi and NFj represent the calculated negative feedback for two generic species i and j, respectively.

Using the procedure described above, we performed two sets of simulations, in either mono- or bi-specific stands, to study the effects of *NF* on the resulting seedlings recruitment distributions. In the case of monospecific forests, we carried out numerical experiments using six different levels of adult tree densities (1, 10, 25, 50, 100, and 200 trees in the simulated plots) factorially combined with three levels of NF intensities (i_{NF} = 0.1, 0.5 and 1.0). Overall, 18 different scenarios of monospecific stands were produced.

In the case of bi-specific stands, we carried out the simulations with stands represented at four different adult trees densities (25, 50, 100, and 200 individuals in the simulated plots). For each density level, the concept of species replacement series (Jolliffe et al. 1984) was applied as follows: i) only one individual of species A; ii) 25% of species A and 75% of species B, iii) 50% of both species A and B, iv) 75% of species A and 25% of species B. All simulations were run with three levels of *NF* (i_{NF} = 0.1, 0.5 and 1.0). Overall, 48 scenarios representing bispecific stands were produced. Examples of the biomass, *NF*, and seedlings distribution maps in the simulated scenarios as described above are presented in Figure 3.

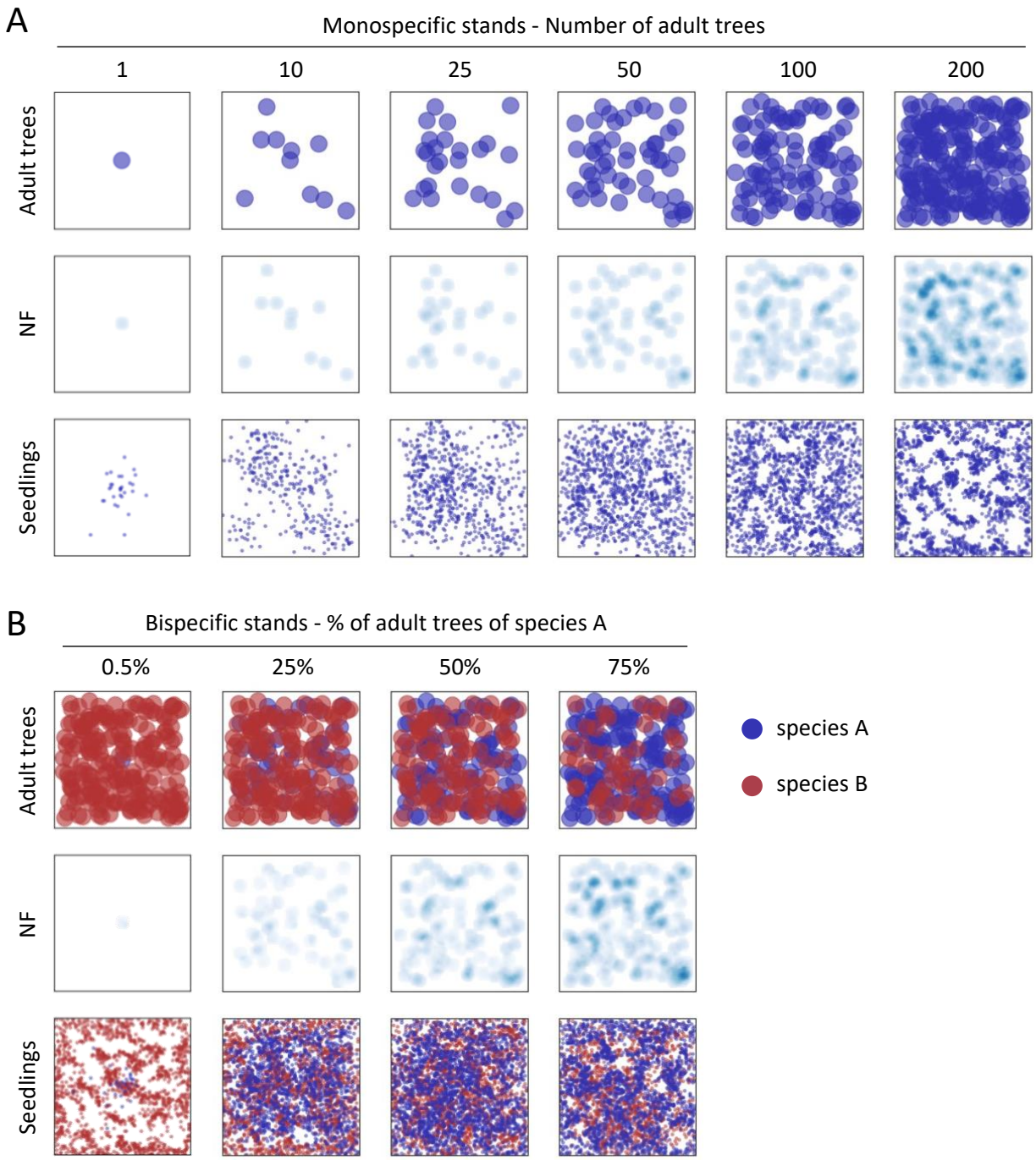

Figure 3. Examples of the biomass, NF, and seedlings maps in the simulated scenarios. A) monospecific stand simulations at increasing tree density; B) examples of bi-specific stand simulations in the case of 200 adult trees at different % of the two different species. All NF maps are represented using iNF=0.5.

Due to the stochastic nature of some of the modelled processes (initial tree distribution, seeds distribution and seedlings establishment), each simulated scenario was run 1000 times by a Montecarlo approach. The presented model was implemented in the Python3 programming language with standard Python libraries: math, numpy, matplotlib, random.

3.2.3 Data analysis

The aim of the analysis of simulation results was to quantify and classify the distribution of established seedlings of a focal tree species. Starting from the centre of the target tree located at the plot centre, the map was divided into concentric circular annuli, i.e., rings, with a 1 m width. In each annulus, the number of established seedlings after every simulation run, has been counted and normalized by the total area of the corresponding annulus. The distance-dependent recruitment patterns were classified using the average density of seedlings of three specific annuli with the following radiuses from the centre of the focal tree: 1) between 1 and 4 m, 2) between 6 and 9 m and 3) between 47 and 50 m. These three areas were selected to represent the seedling density below the crown of the mother focal tree, the area right outside its crown, and an area corresponding to the maximal seed dispersal distance. In particular, plotting the specific seedling density of the three abovementioned areas, we construct the two straight segments connecting each pair of consecutive points. We then calculate the slope (σ) of the two segments and associate each couple of possible values (σ_1 and σ_2) to a seedlings recruitment distribution as follows (Figure 4):

- $\sigma_1<0$ and $\sigma_2\leq0$ = Decreasing distribution;
- $\sigma_1\geq0$ and $\sigma_2<0$ = Janzen-Connell distribution;
- $\sigma_1=0$ and $\sigma_2=0$ = Uniform distribution;
- $\sigma_1>0$ and $\sigma_2\geq0$ = Saturation distribution.

The case $\sigma_1\leq0$ and $\sigma_2>0$ was never observed in any simulation. Finally, the occurrences of each recruitment pattern within each simulated scenario were counted and expressed as relative abundance over the 1000 independent replicates.

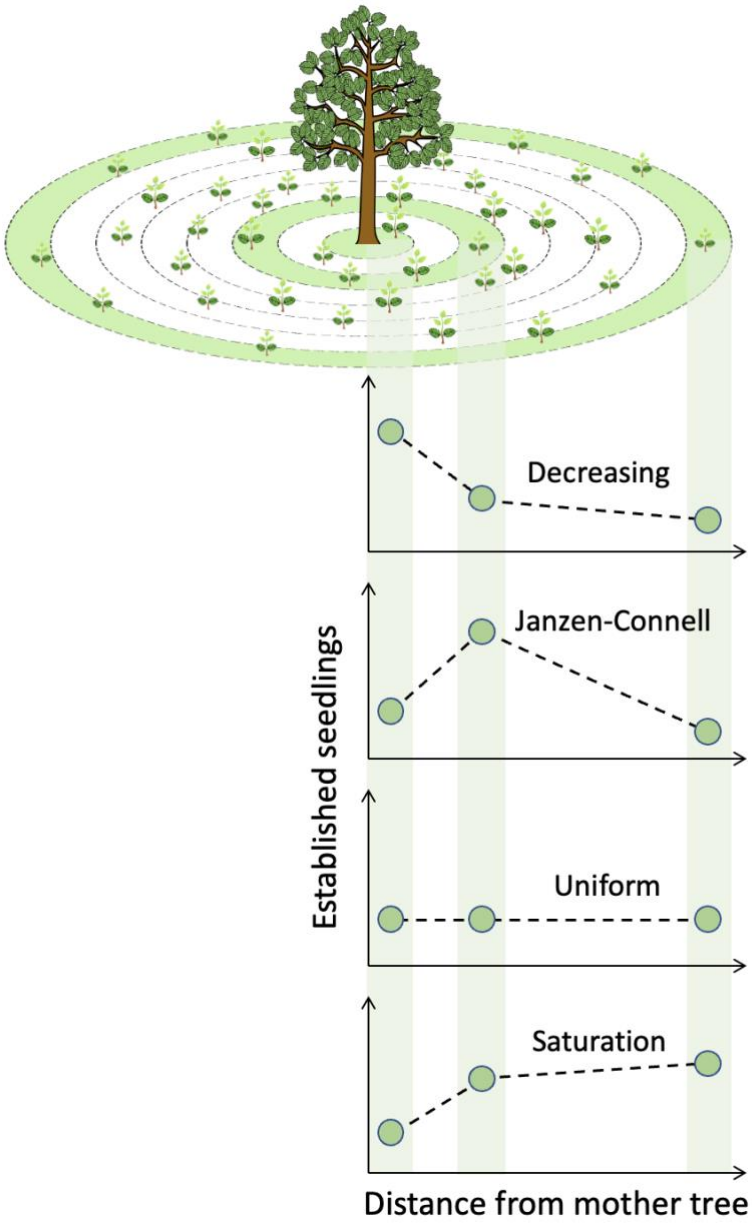

Figure 4. Seedling distribution calculation scheme and types of resulting recruitments patterns. Green areas indicate the three sampling areas where established seedlings were counted. After calculation of the slopes of the segments (dashed lines) between each pair of consecutive points (number of established seedlings), four recruitment patterns were defined: Decreasing, Janzen-Connell, Uniform and Saturation.

3.3 RESULTS OF NUMERICAL SIMULATIONS

3.3.1 Monospecific Forest stand

In monospecific simulations with isolated trees (Figure 5), the JC distribution is frequent only with strong NF (iNF=1), whereas with reduced self-inhibition decreasing distributions increase their frequency becoming dominant at low levels of NF (iNF= 0.1). Noteworthy, in monospecific communities, the occurrence of different recruitment distribution patterns is greatly

affected by tree density. In detail, the probability of observing a decreasing distribution decline with increasing tree density, especially at medium and strong NF levels (iNF= 0.5 and 1). We observed a smooth and gradual reduction in the occurrence of the decreasing distribution at low levels of NF (iNF= 0.1), which was replaced by the JC recruitment pattern. At high NF intensity (iNF= 1), we observed a similar trend, but with the replacement of the decreasing distribution by a saturation distribution proportional to tree density. At high tree density (200 trees), representing a forest with continuous and dense cover (see Figure 3A, last column), the occurrence of the JC distribution decreases with increasing NF, reaching only 19.4% of cases at the highest NF intensity. A uniform distribution was found in very few cases (less than 5% of the simulations) and only in the condition of isolated trees with medium and strong NF levels (Figure 5).

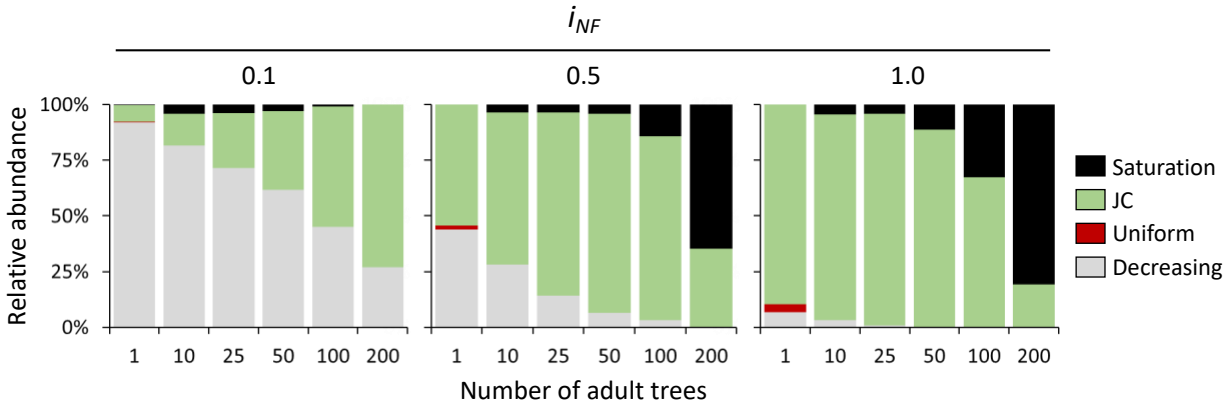

Figure 5. Relative distribution of seedlings recruitment pattern in monospecific stands at different levels of NF (iNF = 0,1, 0.5, and 1) and density of adult tree (1, 10, 25, 50, 100 and 200 individuals per simulated plot).

When all the 1000 permutations were averaged, the results confirmed what was observed in terms of frequencies of establishment patterns. Considering the case of low NF intensity, the average distributions of seedlings assume the shape of a decreasing pattern even when more than 50% of the cases were classified as JC at high tree density (100 and 200 trees in Figure 5). For higher values of NF intensity (iNF= 0.5 and 1), almost all average curves are classified as JC distributions with the only exception of the highest density (200 trees) where the resulting patterns are saturation (i.e., increasing number of established seedlings with distance from the mother tree). In terms of absolute numbers of established seedlings, a clear pattern emerges, i.e., the establishment decreases strongly with the increase of the NF intensity. In all simulations, even at the highest tree density (200 trees), there is presence of bare soil among tree crowns in the plot (Figure 3A). Differently, in the case of simulations performed imposing a full coverage of the plot, the constant accumulation of NF all over the domain, produces the disappearance of any spatial pattern of seedlings establishment and, in particular, the absence of observable JC distribution.

3.3.2 Mixed forest stand

In the case of mixed two-species stands, rare species immersed in a matrix of heterospecifics rarely shows JC distributions with decreasing recruitment pattern predominating, especially at low and medium NF intensity (iNF=0.1 and 0.5), (first column of each bar plot in Figure 6). In the case of a co-dominated community with a forest stand composed by 50% species A and 50% species B, both tree density and NF affect the probability of observing a JC distribution. At low tree density (representing a Savannah ecosystem), with 25 and 50 total trees, the low NF simulation (iNF=0.1) showed that the decreasing distribution is the most frequent and only few cases of occurrence of the saturation pattern. However, as NF intensity increases (iNF=0.5 and 1.0), the JC distribution replaces the decreasing distribution, becoming the most frequent with over 80% of the cases. In co-dominated stands with high cover (either 100 or 200 trees per plot), JC and decreasing distributions are almost equally likely to occur at low NF level (iNF=0.1). Instead, on one hand, when NF is medium or high (iNF=0.5 and 1.0), JC distribution is observed in more than 80% of simulations with few cases with either decreasing or uniform recruitment patterns. On the other hand, in stands with high density (200 trees) and dominated by the target species (75% of cover), the probability of observing JC recruitment is more than 50% regardless of NF intensity, reaching the highest value (71.1%) at medium NF intensity level (Figure 6).

The presented results are confirmed when the average of the 1000 permutations are considered. The most abundant establishment pattern is a decreasing distribution at low NF intensity (iNF=0.1), while the JC pattern gradually appears with increasing NF levels (iNF=0.5 and 1.0). Also in this case, as was observed for the monospecific stands, the absolute density of seedlings decreases strongly with increasing NF intensity.

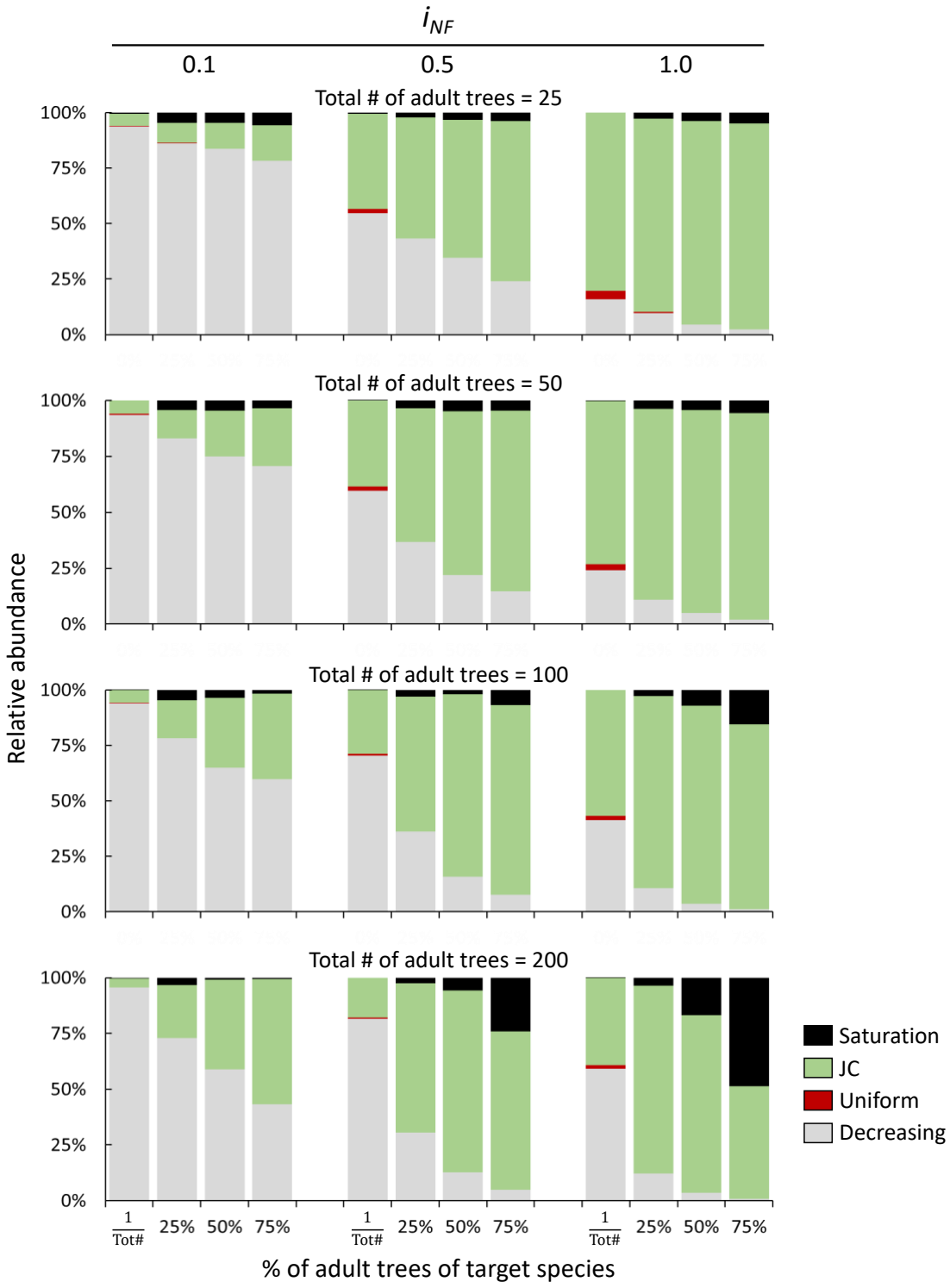

Figure 6. Relative distribution of seedlings recruitment patterns in bi-specific stands at different levels of NF (iNF = 0,1, 0.5, and 1) and density of adult tree (25, 50, 100 and 200). The x-axis shows the species replacement series starting with only one individual (first column) followed by 25%, 50% and 75% of individuals of the target species

3.4 DISCUSSION AND CONCLUSION

Assuming that NF is species-specific, localized under an individual tree, and with limited horizontal diffusion, our simulations show that tree density is critical to understand the observed variability of tree recruitment patterns. Our model highlights the complex interconnection between NF intensity, stand density, and recruitment patterns explaining where and why the JC distribution occurs, and clarifying the relevance of this ecological phenomenon in different plant community frameworks.

Our initial hypothesis that JC distribution is very common in the case of an isolated tree was partially supported by the model simulations. Indeed, we found that JC distribution was very frequent for isolated trees when NF was strong and capable to form an exclusion zone under the parent tree. However, with decreasing NF intensity, both JC and decreasing patterns cooccurred and were recorded with similar frequencies. A prevalence of the decreasing pattern was also observed at very low NF, because under such conditions the inhibitory effect due to NF was unable to overcome the clustering effect of the seed dispersal kernel, with resulting concentrated recruitment under the parent trees. JC distribution in isolated individuals has been previously reported for both shrubs and trees, but in far less cases compared to tropical and temperate forests (reviewed in Bonanomi et al., 2010; Comita et al., 2014; Song et al., 2021). A notable example is the study of Clark & Clark (1981), reporting a clear distance-dependent recruitment limitation for isolated trees of Bursera graveolens in arid ecosystems with discontinuous vegetation. Moreover, a recruitment distribution consistent with the JC model has been reported for woody plants belonging to Fabaceae, a plant family forming fertility islands under individual canopies, associated to the accumulation of organic matter, nitrogen and phosphorus (Facelli & Brock, 2000). Under this scenario, in order to observe a distancedependent inhibition, the generating factors of NF, attributed either to soilborne pathogens or soil autotoxicity, must overwhelm the positive effects of both nutrients and beneficial microbes of the fertility islands under the canopy of woody plants. In this context, it is well established that plants belonging to Fabaceae suffer greatly from NF in both agroecosystems and natural plant communities (Cesarano et al., 2017). Accordingly, several studies reported the presence of intense NF and JC recruitment distributions in woody perennial plants, including Medicago marina in Mediterranean sand dunes (Bonanomi et al., 2008), Medicago sativa in US old field (Jennings & Nelson, 2002), Genista aetnensis over volcanic lavas (Stinca et al., 2015), and several Acacia species in South Africa (Ben-Shahar, 1991).

Regarding the second hypothesis, i.e., the decreasing occurrence probability of JC distribution with high density of conspecifics, our model demonstrated a complex scenario dependent on the intensity of the NF. Indeed, when the NF intensity is low, the JC frequency

increases linearly with the density of adult conspecifics. However, if NF is strong, the peaks in JC frequency are still observed at intermediate stand densities while suddenly decrease in stands with a continuous conspecific cover of 200 trees in the plot. So, counterintuitively, our model shows that a plant suffering from strong NF in monospecific stands can rarely exhibit a recruitment pattern fitting the JC model. This seemingly paradoxical result is due to the progressive expansion of the exclusion zone surrounding all trees in the forest stand. In other words, as individual trees become more clustered and denser, their exclusion zones progressively overlap, leaving no safe place for an effective recruitment in the stand. This is consistent with the lack of JC evidence in many monospecific stands in temperate and boreal forests, including Fagus sylvatica in Mediterranean forests (Rita et al., 2021), as well as monodominant tropical forests (Hart et al., 1989; Richards, 1996). In general terms, our model demonstrates the association between strong NF and lack of distance-dependent inhibition in dense, monospecific stands. This result reconciles NF with forest composition and should reduce the scepticism of many forest ecologists towards the JC model (Terborgh, 2020).

Our third hypothesis supposed that the JC distribution should not be frequent in the case of rare species immersed in a matrix of heterospecific adults. This was largely confirmed by our numerical simulations demonstrating that a species with only 25% stand cover showed lower frequency of JC distribution compared to stands in which the species occurrence was at 50% and 75% cover. This effect was observed in both low and strong NF conditions, and reflected the fact that the abundant presence of heterospecific adult neighbors provides a suitable place for recruitment overlapping with the exclusion zones by conspecifics and thus reducing the NF effect. Rare species are indirectly protected by non-host, neighboring heterospecifics, as predicted by the herd-immunity hypothesis, which reduces the probability of contact with propagules of the virulent plant enemies (Wills et al., 1997). In the case of the autotoxicity theory, the presence of leaf litter and root debris from heterospecifics likely results in the dilution of conspecific plant residues, thus providing soil patches free of NF even near conspecific mother plants.

With respect to forest dynamics, our model simulations are consistent with robust field data on alternative species replacement reported in temperate and boreal forests around the globe, in stands co-dominated by two tree species (Fox, 1977). Notable examples include Fagus grandifolia with Acer saccharum, Picea rubens with Abies fraseri, Fagus grandifolia with Tsuga canadensis, and Picea engelmannii with Abies lasiocarpa (Whittaker & Levin, 1977; Woods, 1979; Runkle, 1981; Waters & Savill, 1992). In all these studies, the recruitment of tree species was found to be significantly more abundant and healthier under heterospecific adults. In this context, on one hand our model shows that localized NF is able to explain species replacement in forest ecosystems. On

the other hand, the observation of decreasing JC recruitment patterns in codominated mixed forests reflects a reduced NF effect related to departure from monospecificity.

Future model simulations can focus on multispecies systems to test the effect of adult density on distance-dependent inhibition also in forest stands with high tree diversity and also assess the fate of rare species having different levels of sensitivity to NF compared to the most abundant plant in the community (Van der Putten et al., 2013). Spatial comparison of numerical simulations with real data obtained from long-term forest censuses for tropical forests such as Barro Colorado (Condit, 1998), other tropical forests (Lewis et al., 2004), and temperate forests (Král et al., 2018) could be particularly useful to this goal.

From a modelling point of view, future work could address the following points: (i) explicit representation of the germination and establishment processes separately to help disentangle the effect of different causal mechanisms due to either chemical autotoxicity or action of soil-borne pathogens. Specifically, the effect of chemical autotoxicity is reported to affect both germination and early seedlings' growth, whereas soil-borne pathogens mostly affect the establishment phase. (ii) In order to provide a more general description of plant-soil interactions, the inclusion of facilitation by heterospecific biomass can be also explicitly considered to evaluate its relevance in the emergence of seedlings' JC patterns. (iii) To test the effect species-specific characteristics like crown shape and seed dispersal strategies on the emergence of seedlings' establishment patterns, different formulations of the biomass distribution and seed dispersal kernels could be implemented. (iv) Moreover, future studies could investigate the impact of the priority effect by simulating different colonization timings, as occurs in ecological succession, and the consequent effect on the recruitment of conspecifics.

Finally, a challenge for future studies will be a spatially explicit definition of the exclusion zone in forests with different tree density and diversity. New-generation sequencing techniques may enable the production of fine-scale metagenomic maps coupled with an assessment of the conspecific extracellular DNA accumulated in the soil where the NF effects are observed. Such in-depth investigations on spatial information associated to tree recruitment distribution will be relevant to support the discussion on the putative mechanisms of the JC effect and to disentangle between the hypotheses of soilborne pathogens and self-DNA inhibitory effects, thus providing a better understanding of the spatial and temporal patterns of this important phenomenon.

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Chapter 3

Predictions of annual xylogenesis of conifers using the hybrid modeling approach

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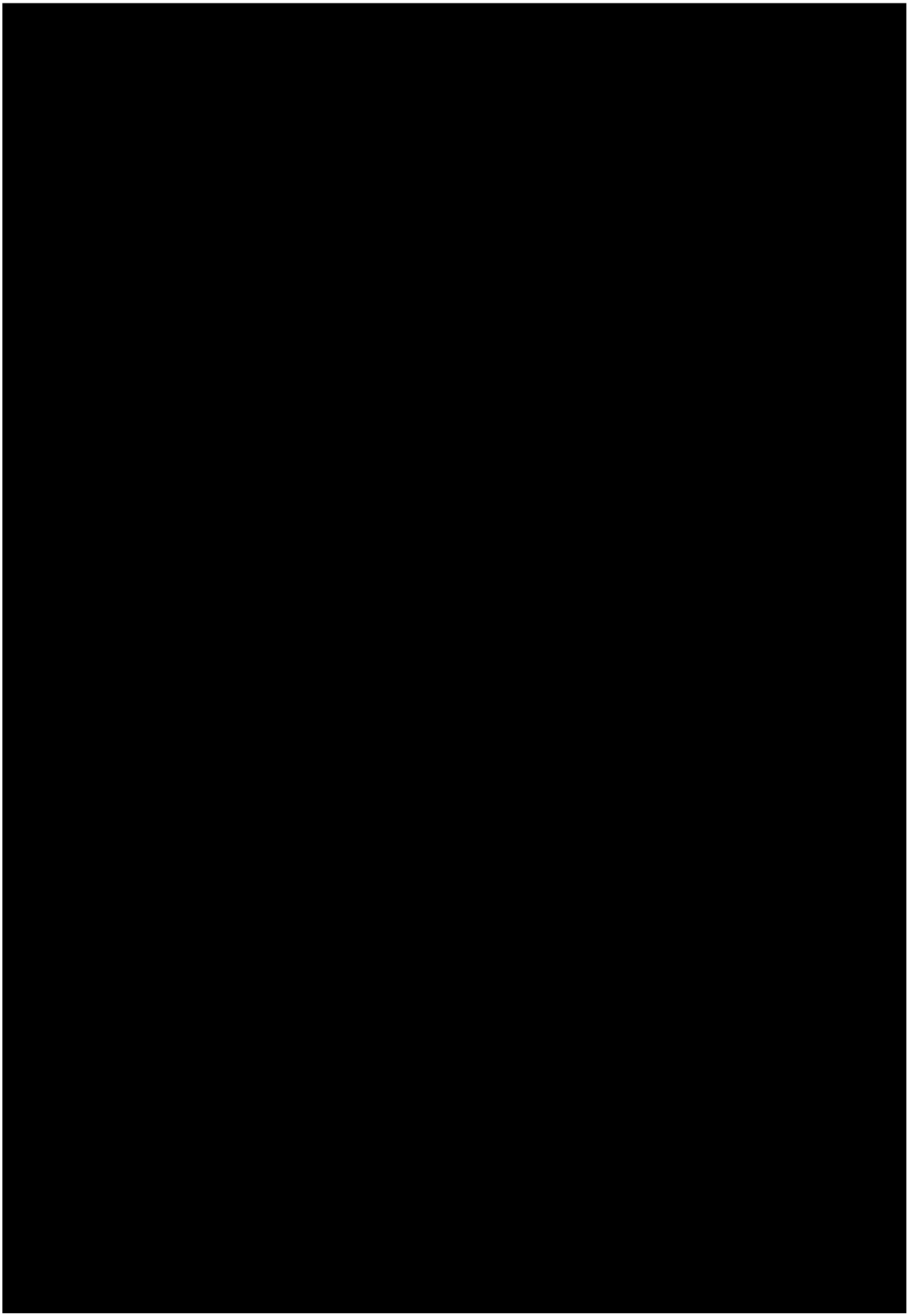

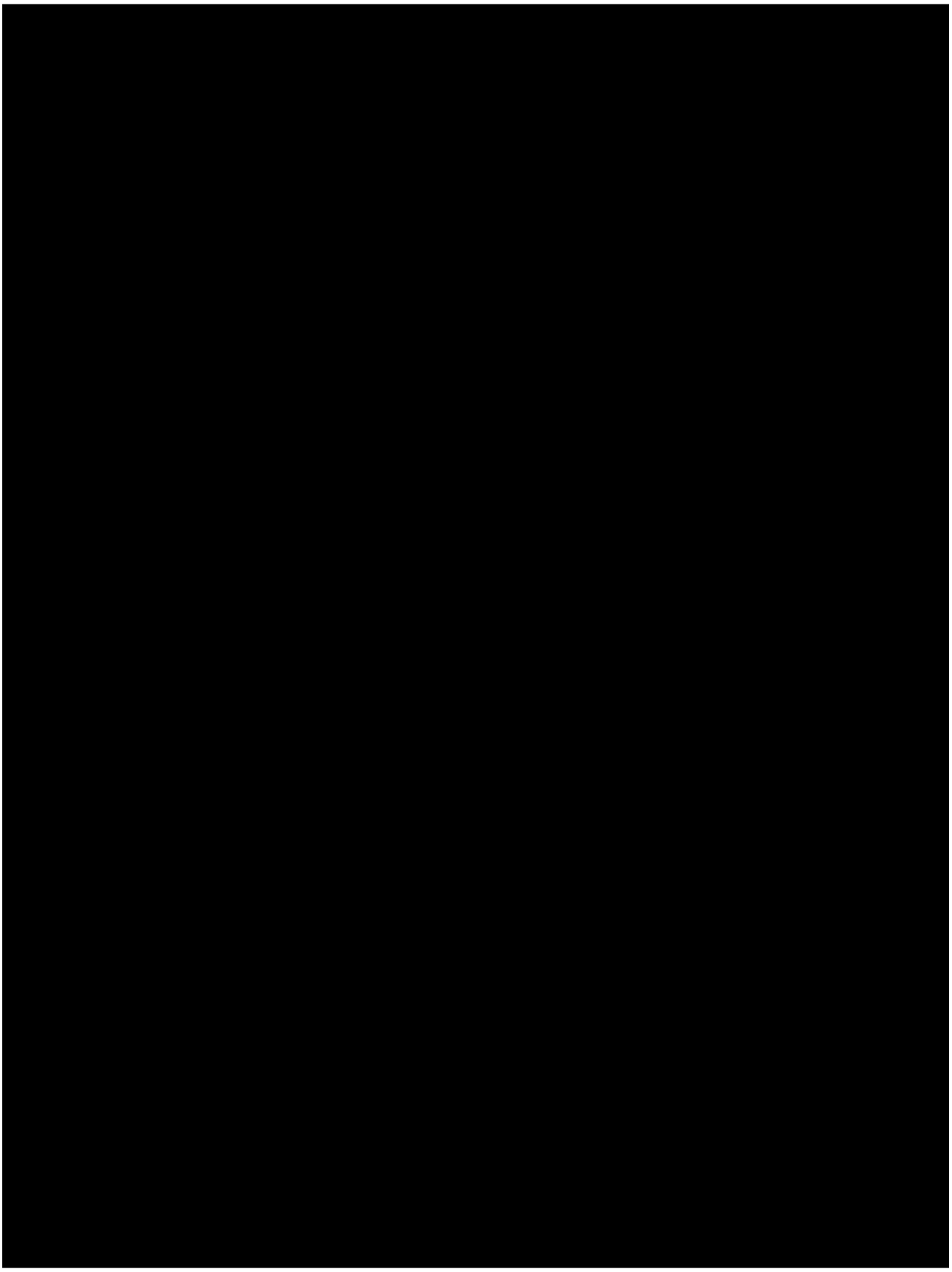

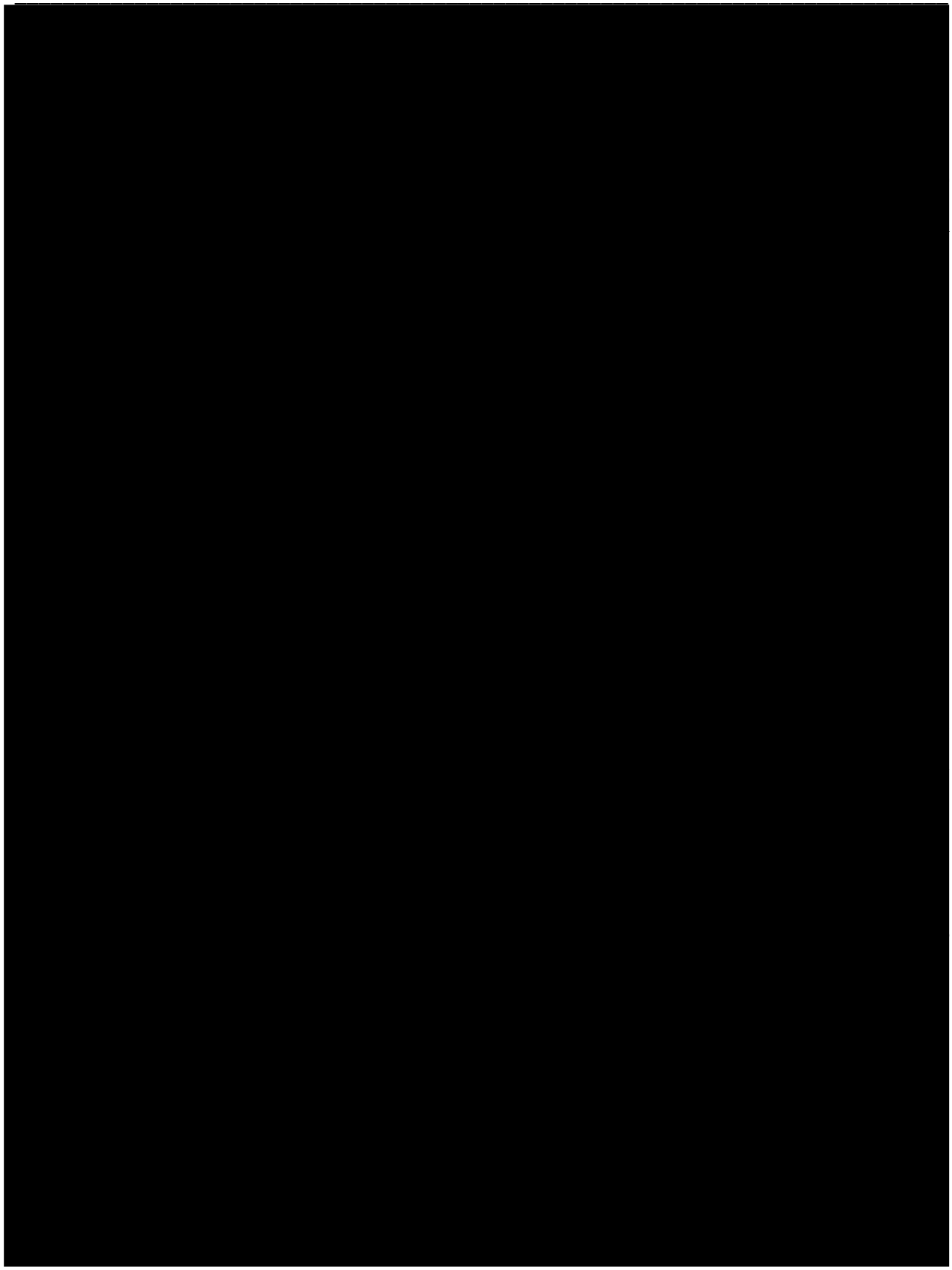

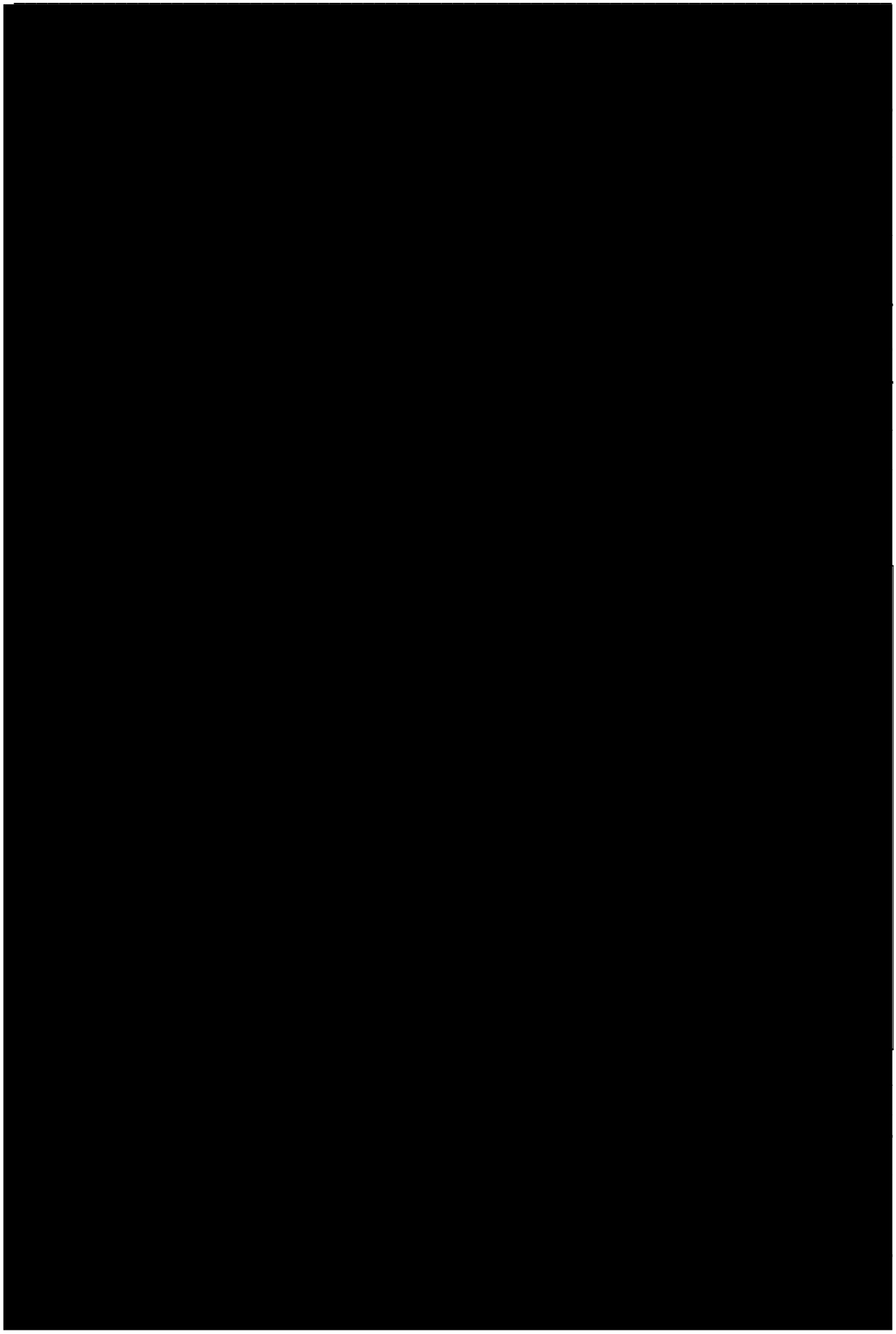

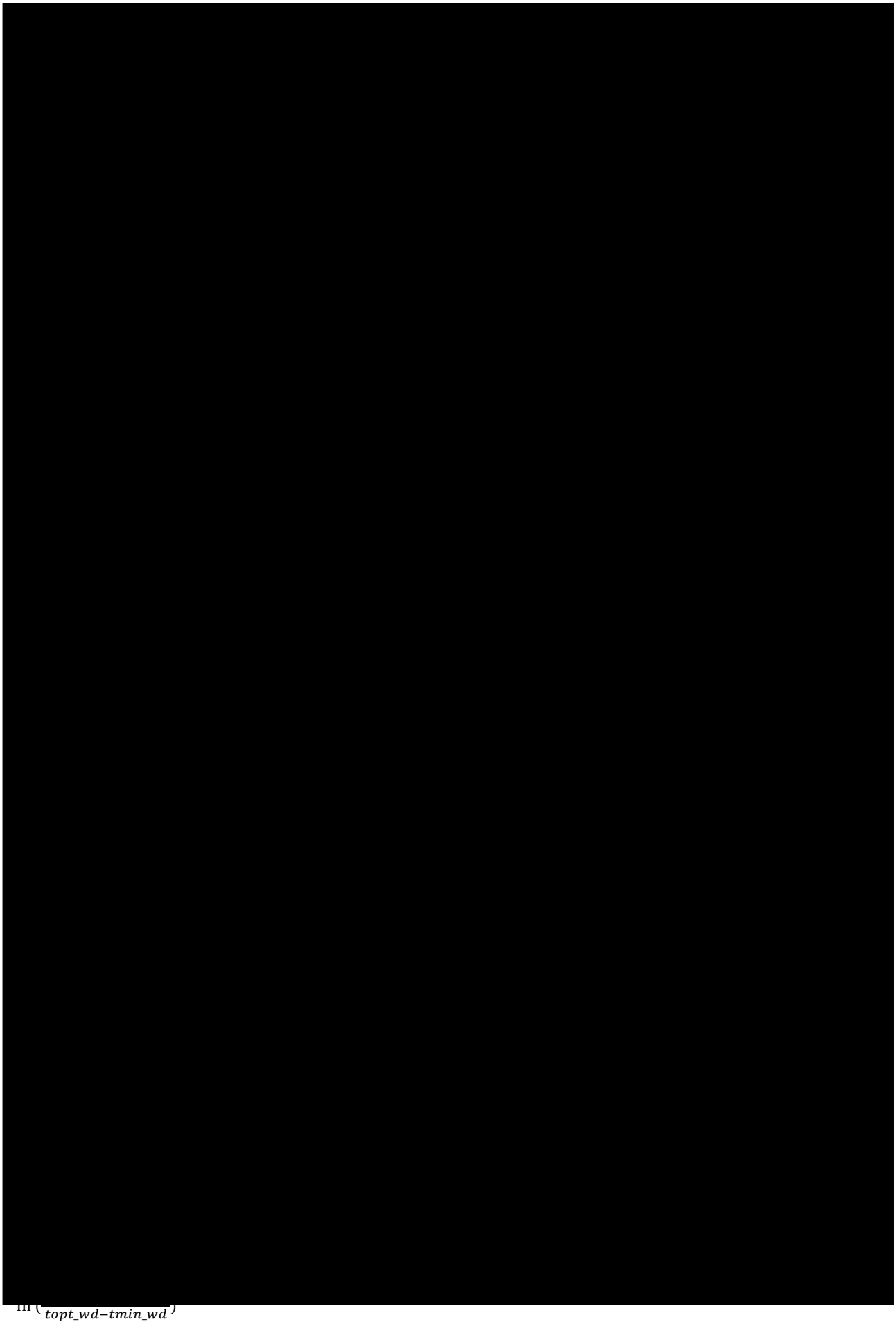

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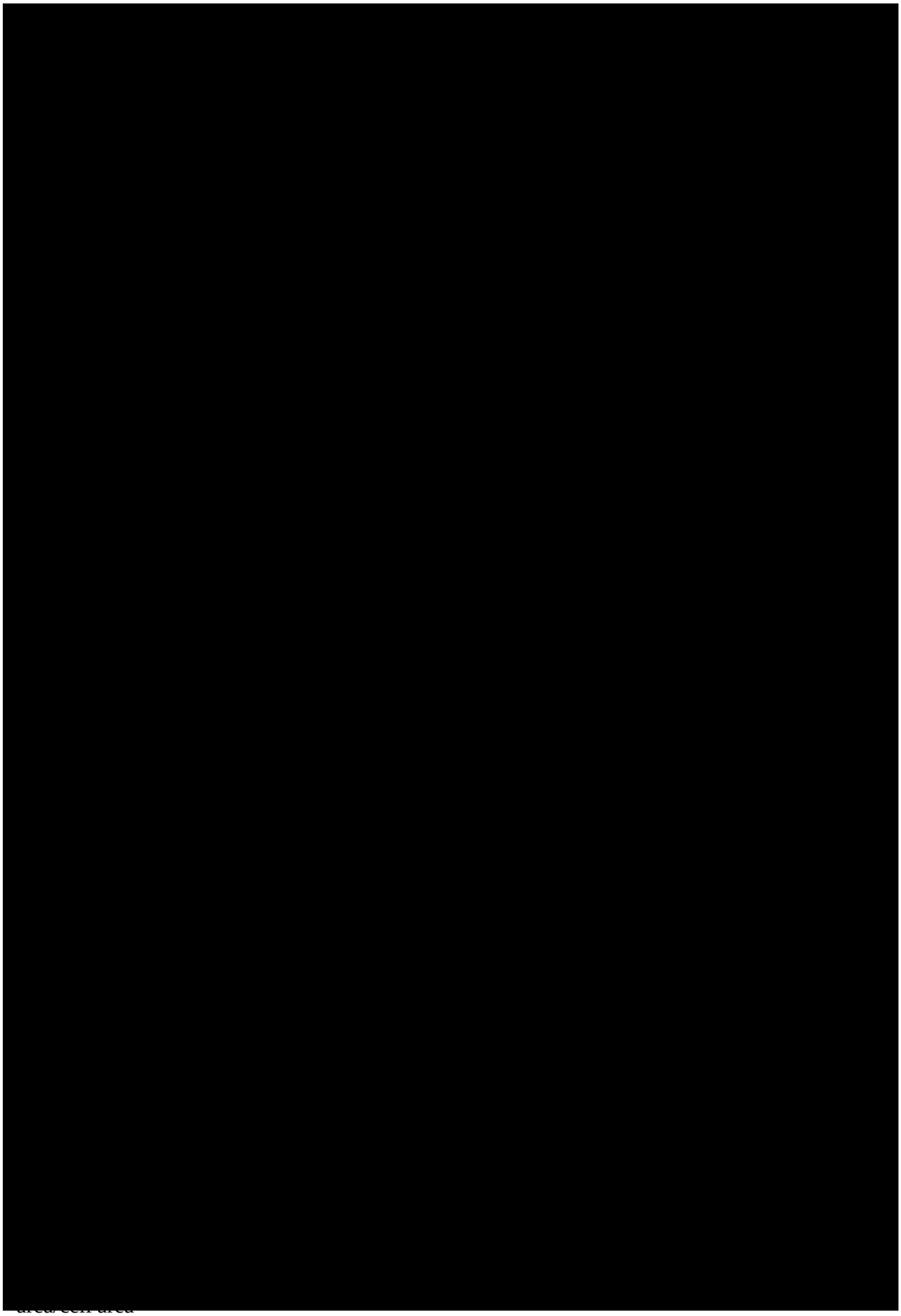

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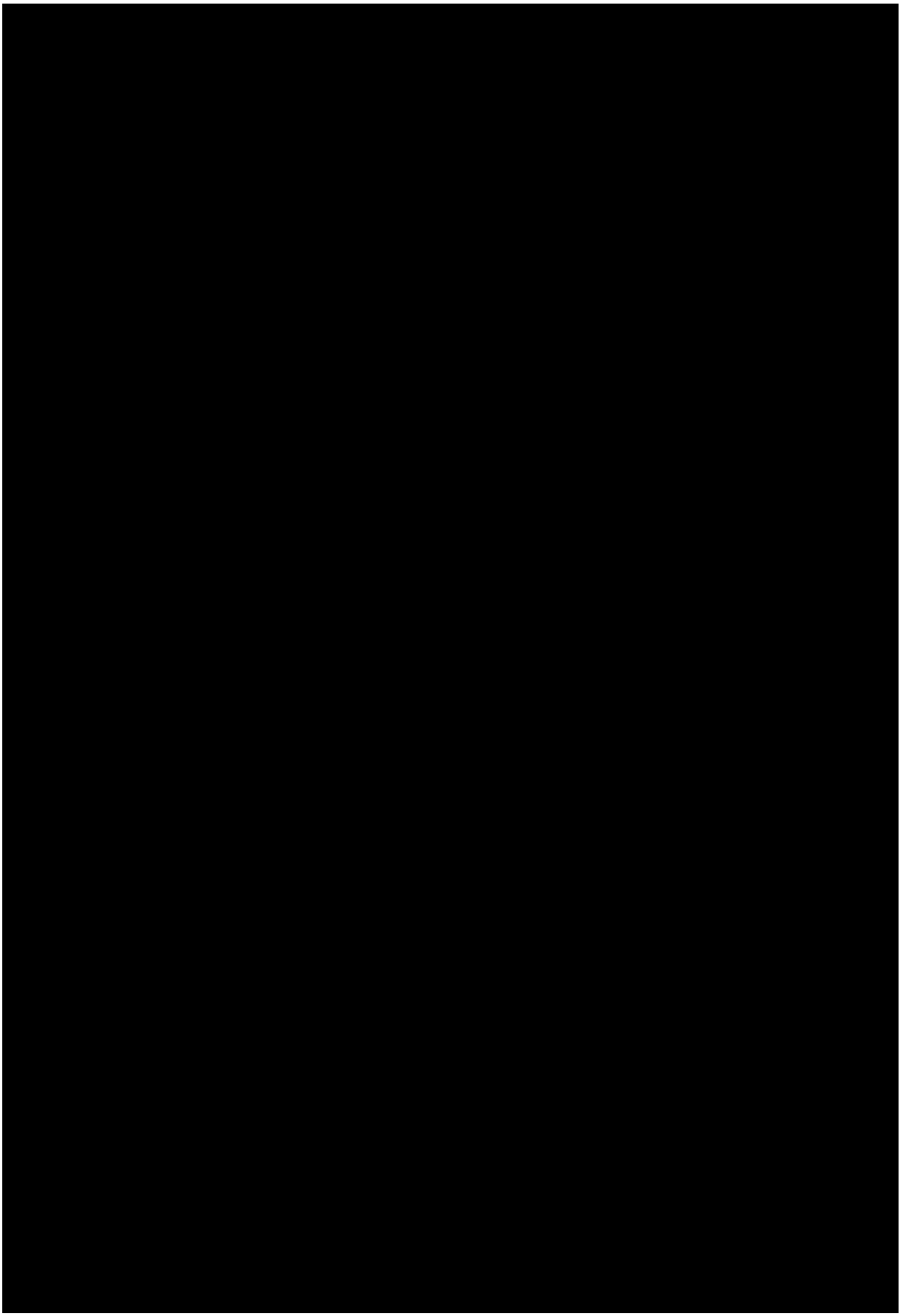

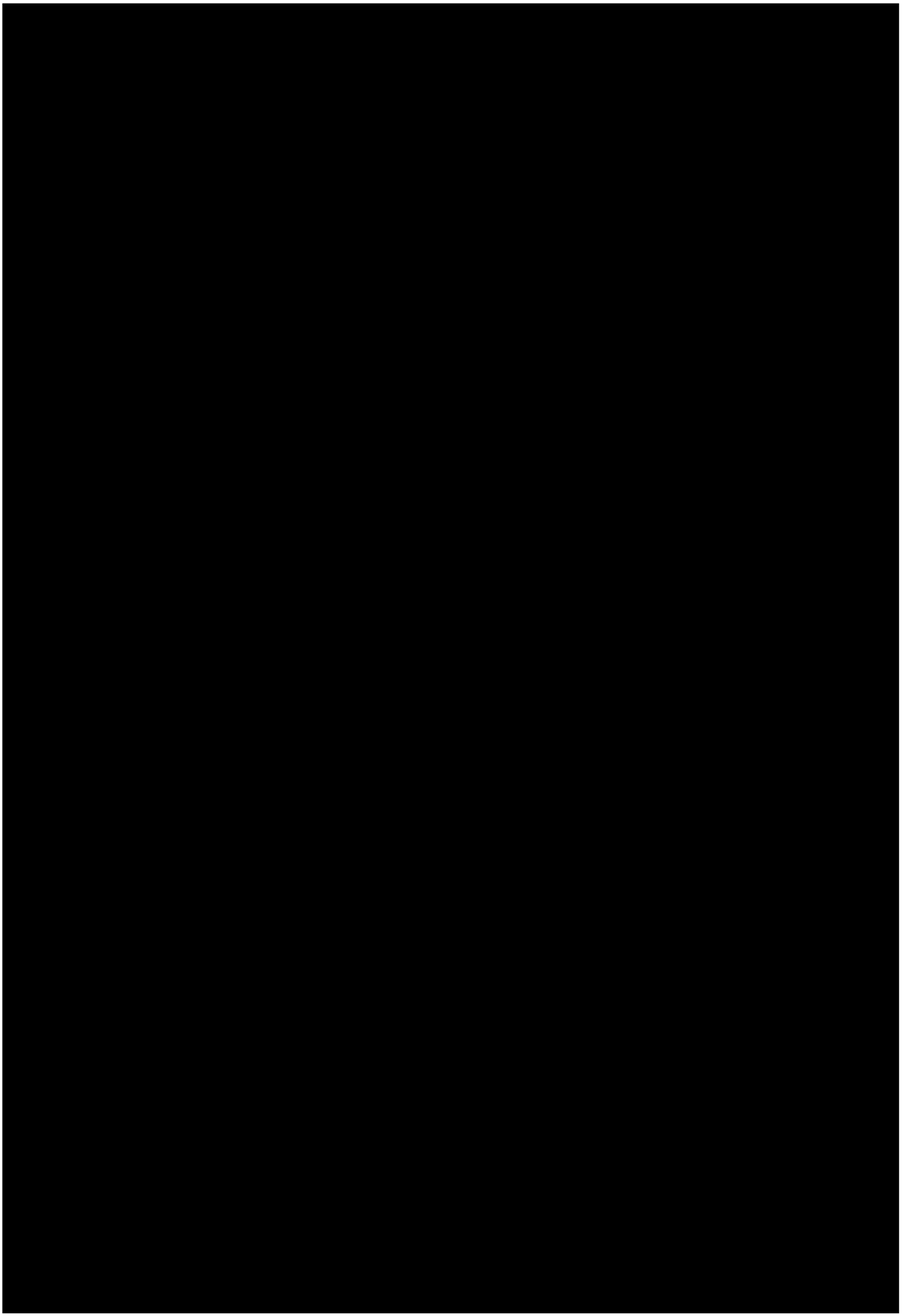

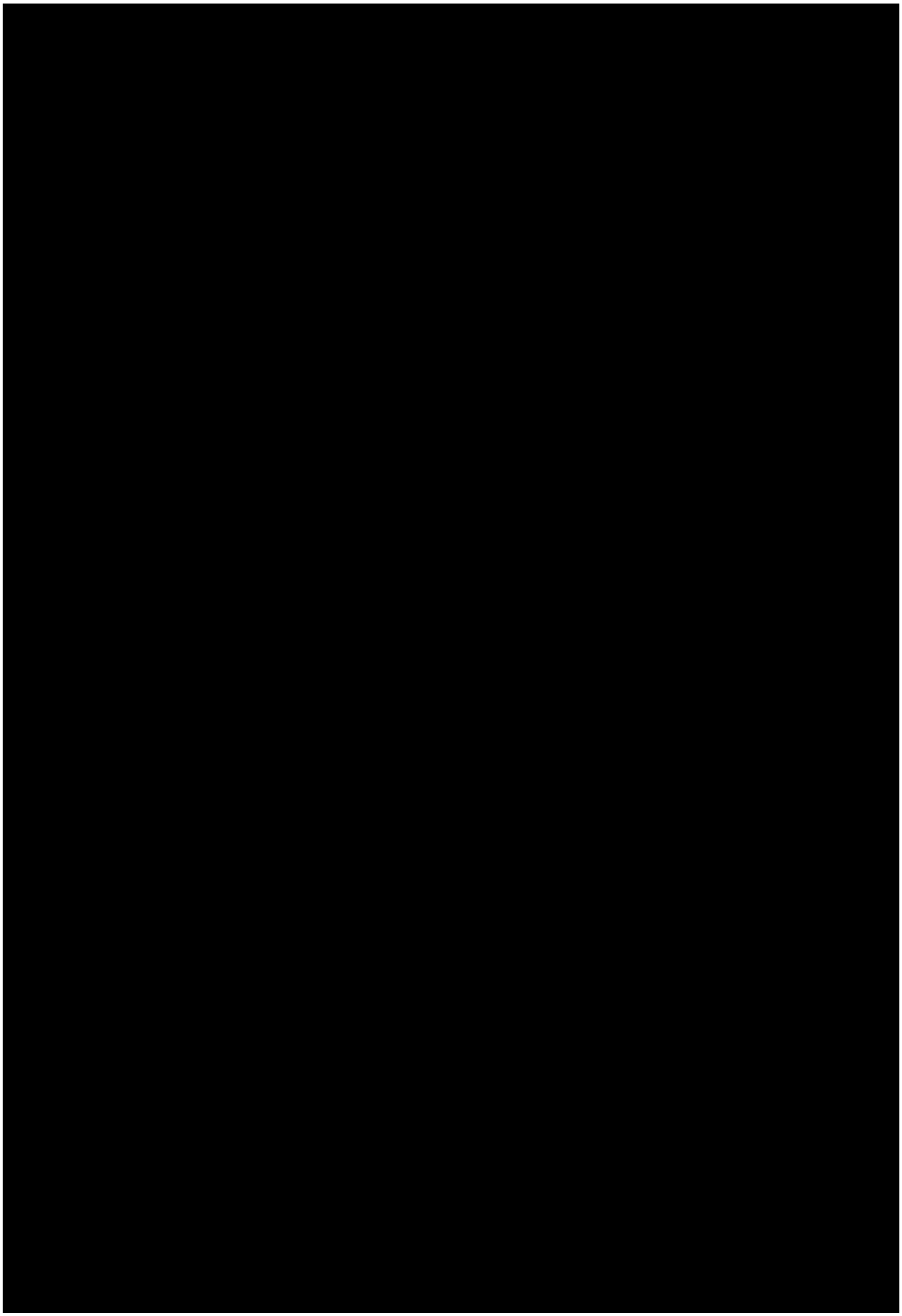

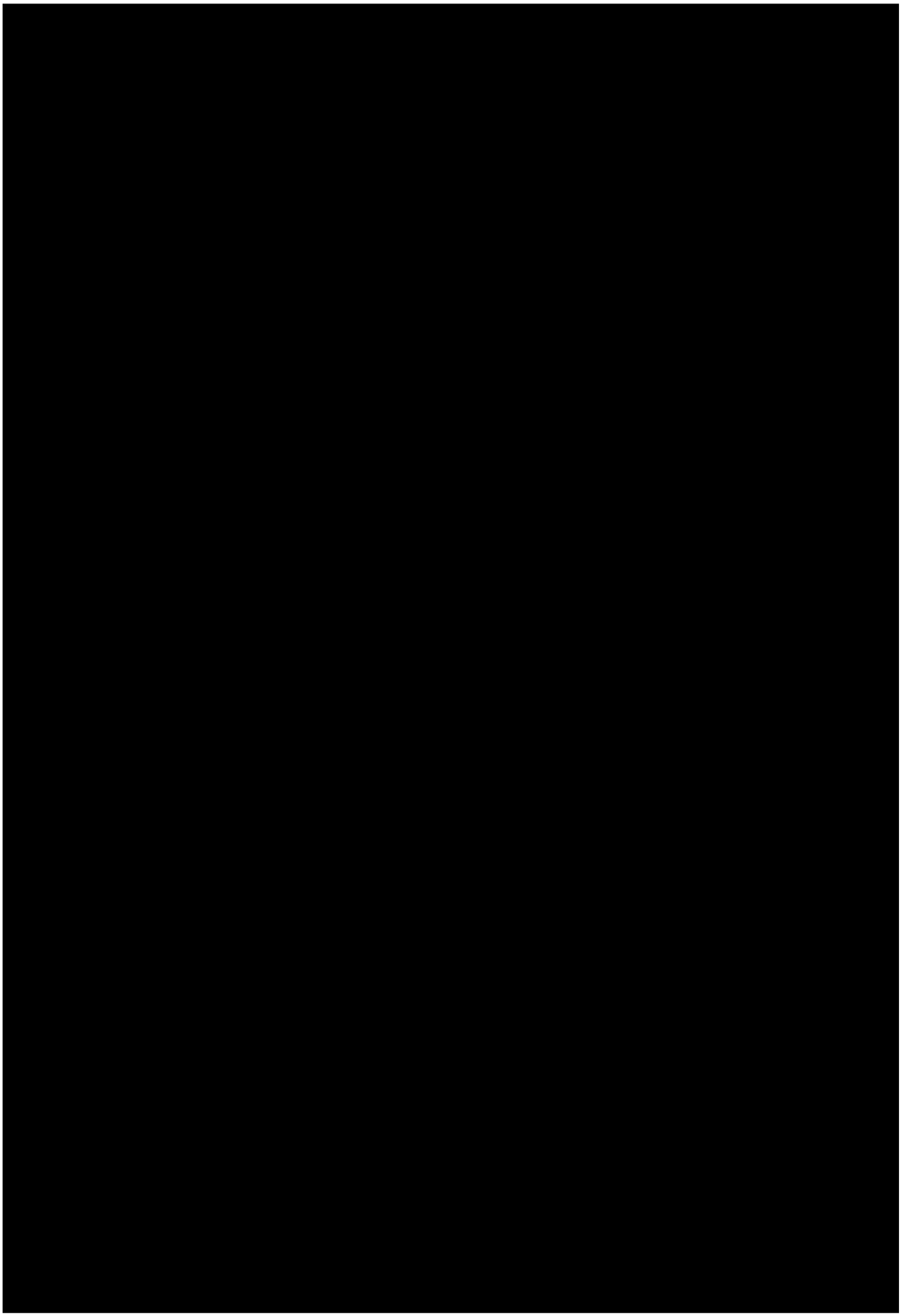

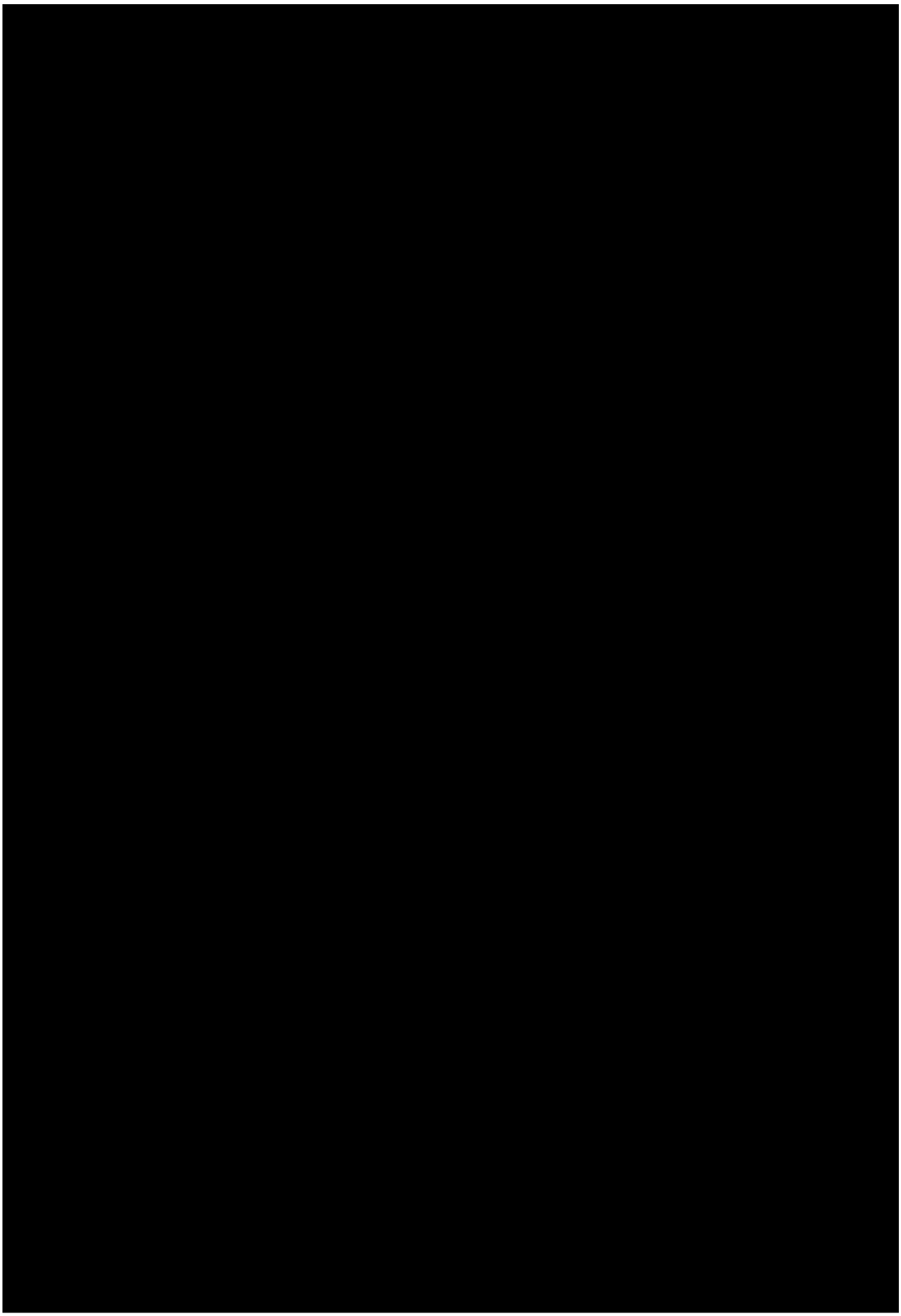

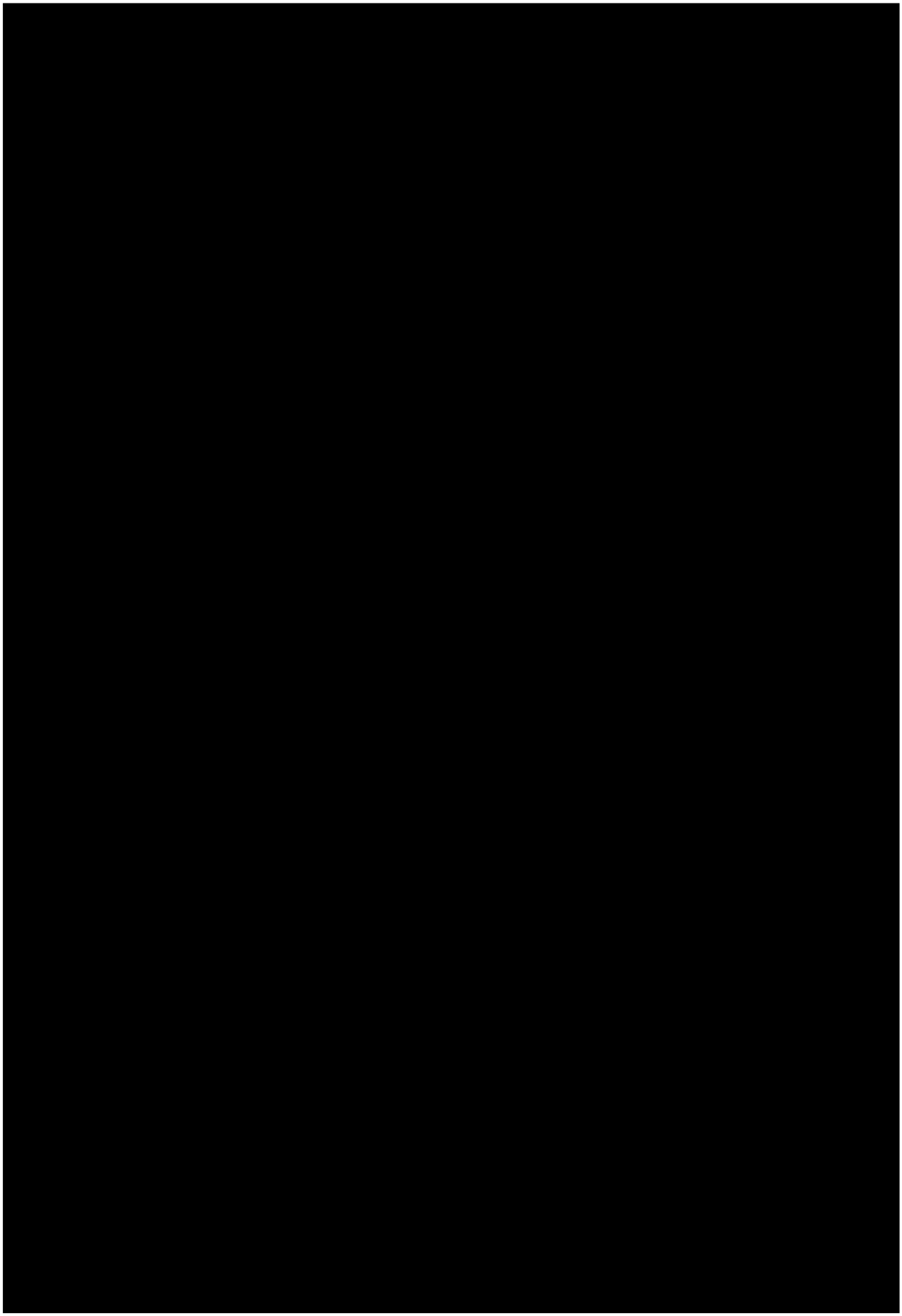

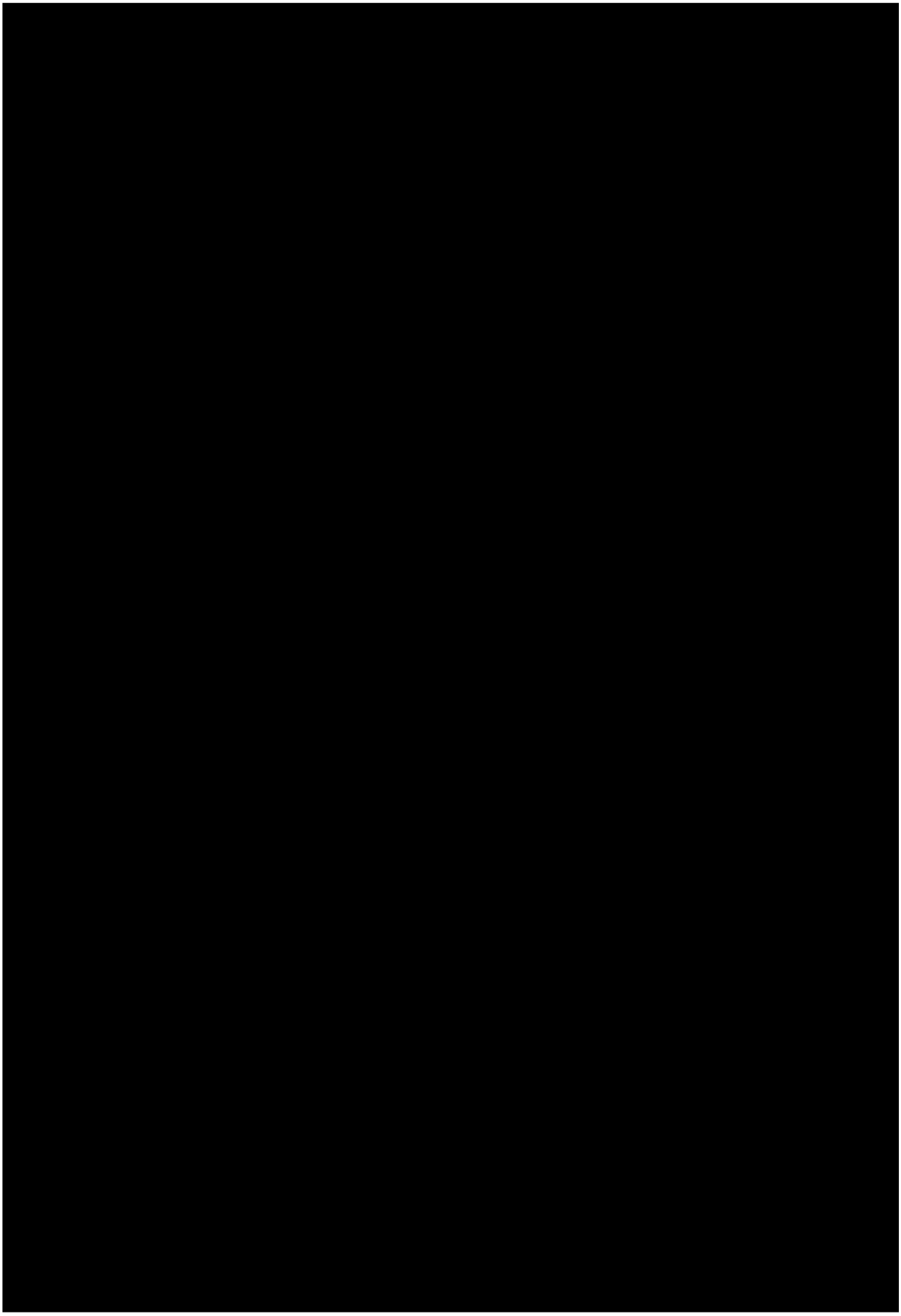

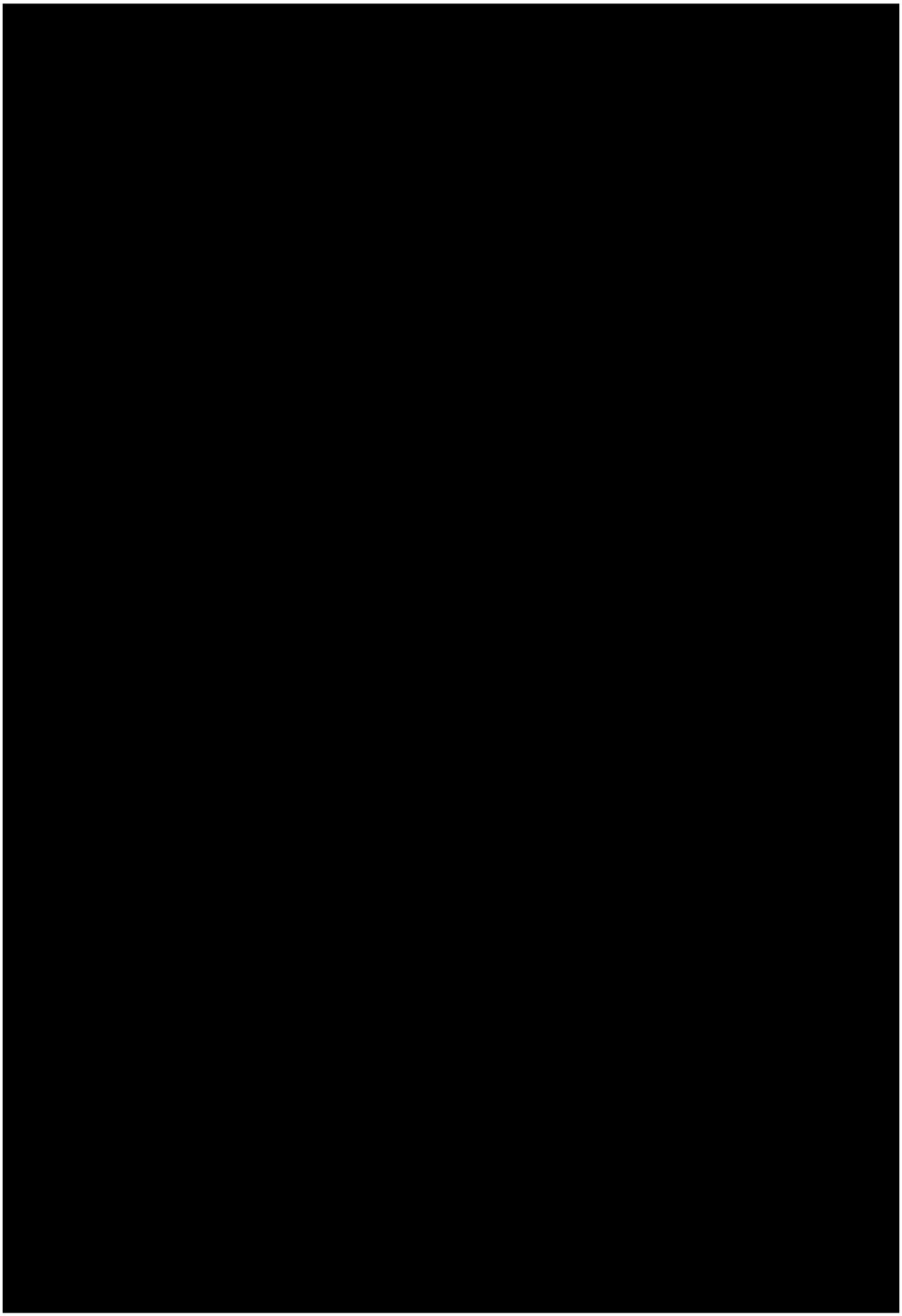

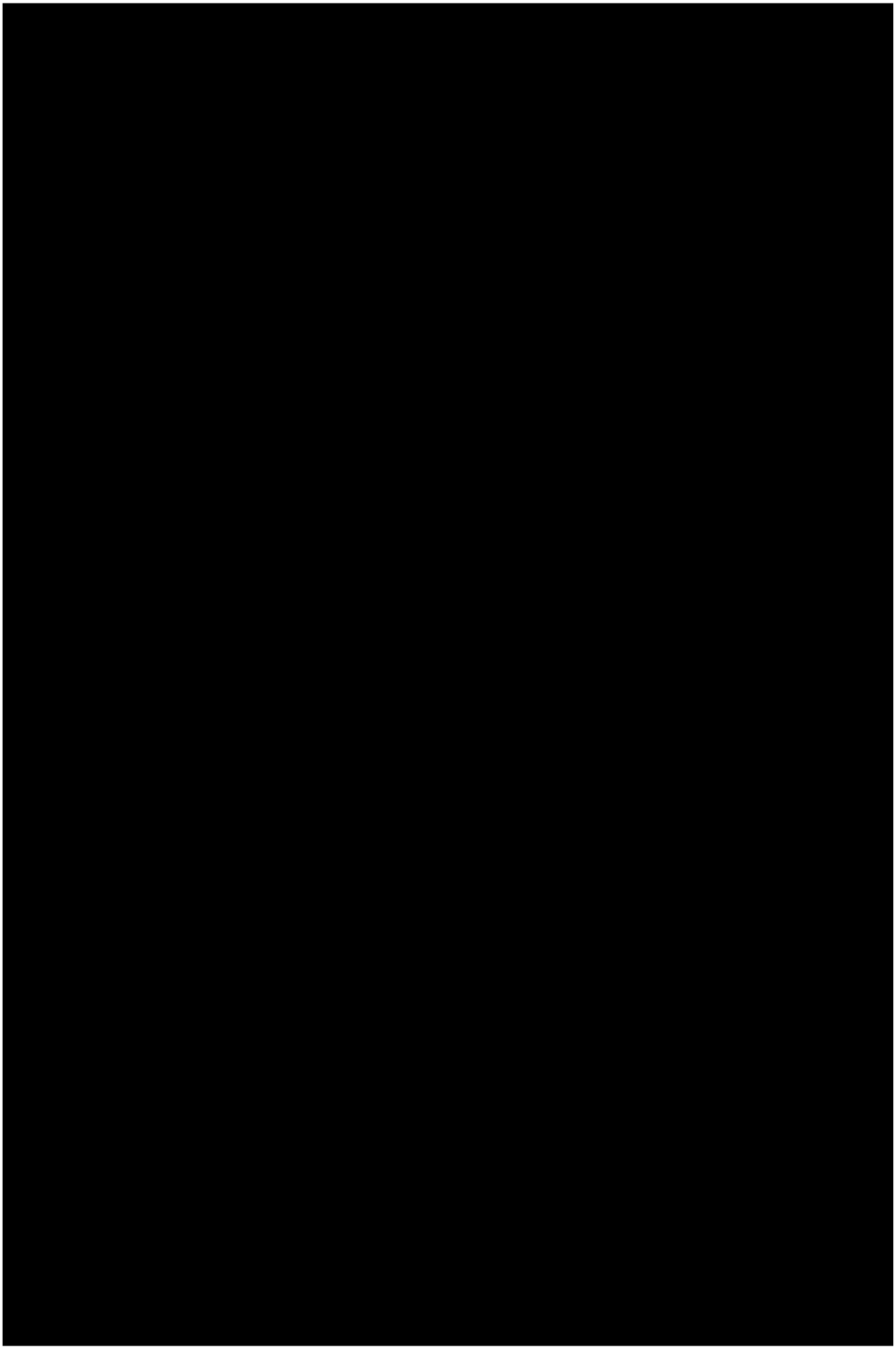

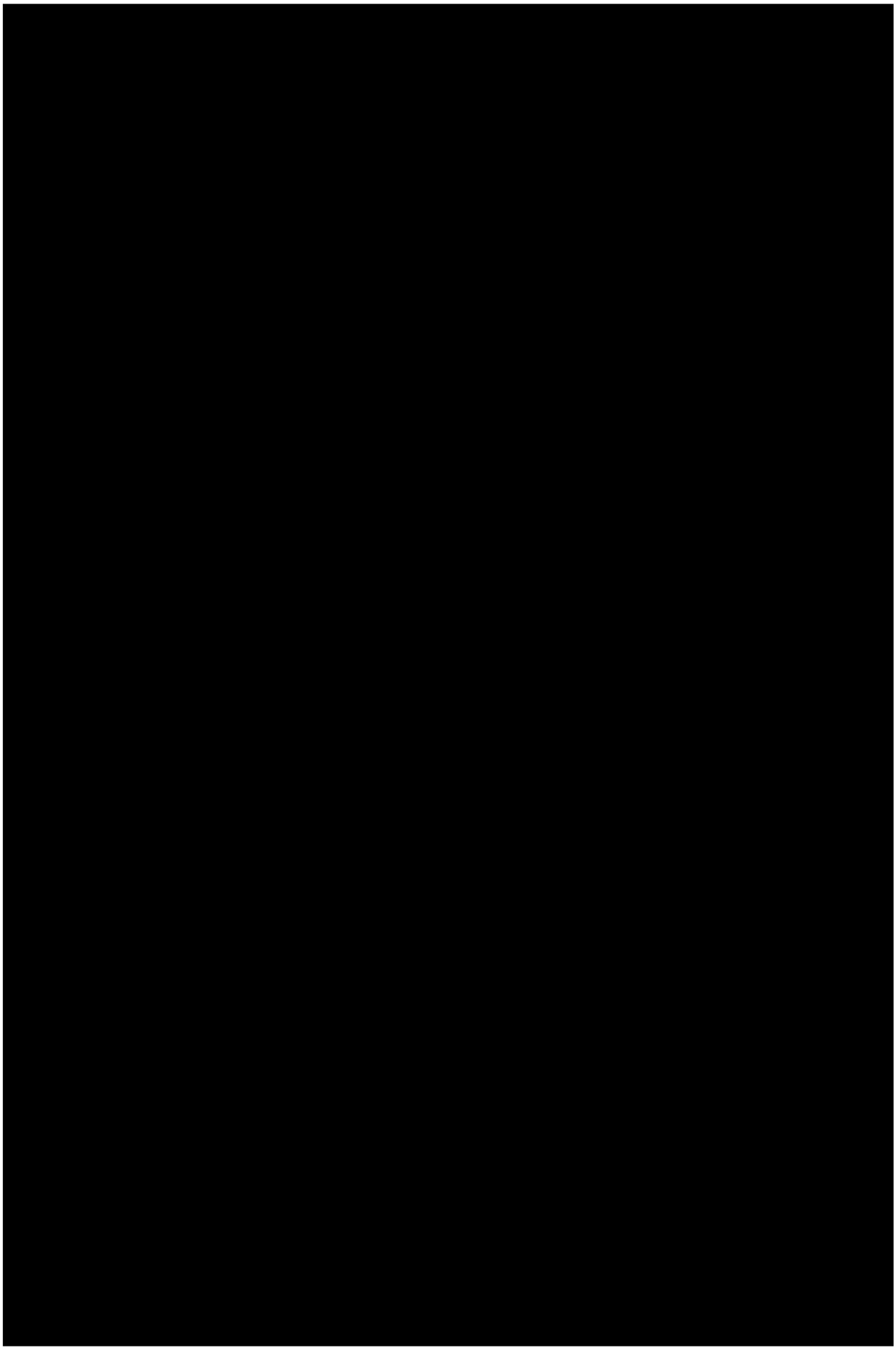

5. GLOBAL CONCLUSIONS

Systems biologists face many challenges when modeling biological processes from available data. It is still not possible to directly use big data, such as transcriptomic datasets, for developing the precise multi-cellular models. One of the open opportunities is to use hybrid models, which vastly reduce the computational costs and able to describe the key components of the processes. The hybrid models approach in biology offers a powerful and promising method to study complex biological systems. By combining different modeling techniques and experimental data, hybrid models enable a more comprehensive understanding of biological processes. The importance of hybrid models lies in their ability to capture the multifaceted nature of biological systems, where interactions between various components can be nonlinear and exhibit emergent properties. Hybrid models provide a means to integrate different levels of organization, such as molecular, cellular, and organismal scales, and bridge the gap between theoretical predictions and experimental observations. Such models allow scientists to generate testable hypotheses, make predictions, and gain deeper insights into the underlying mechanisms driving biological phenomena.

Despite its potential benefits, the development and application of hybrid models in biology pose significant challenges. One major hurdle is the integration of disparate data types and model frameworks. Different types of data, such as genetic, physiological, and ecological measurements, often come with their own complexities and limitations. Harmonizing and reconciling these data sources to create a cohesive and accurate hybrid model can be a technically demanding task.Furthermore, hybrid models require robust computational infrastructure and advanced analytical methods to handle the large volumes of data and complex mathematical algorithms involved. Model parameterization, validation, and calibration become intricate processes due to the increased complexity and heterogeneity of hybrid models. Additionally, model interpretation and analysis can be challenging, as hybrid models introduce new layers of complexity that necessitate scrutiny to differentiate noise from meaningful patterns.

Despite these challenges, the development and application of hybrid models in biology hold immense potential for advancing our understanding of complex biological systems. As computational power and data availability continue to improve, the hybrid models approach offers an increasingly valuable tool for addressing fundamental questions, making predictions, and guiding experimental design in biology. By overcoming the technical difficulties and refining

modeling techniques, hybrid models have the capacity to unlock new insights and contribute to breakthroughs in the field of biology.

Based on this work, the author can confidently conclude that the general-purpose Python language is suitable for the development of new hybrid models in biology. Standard libraries such as pandas, numpy, math, scipy, odeint can become the basis for the development of object-oriented models in biology as well as for discrete-continuous calculations, and matplotlib and other packages are able to visualize the results in both one-dimensional and two-dimensional scales.

However, to move to three-dimensional hybrid modeling of biological processes, standard libraries alone are not enough. There is an urgent need to develop new open libraries for non-commercial use by scientists for popular general-purpose languages which will provide three main components:

1. Standardization of laws and objects, relationships between them. In three-dimensional space, such objects may include cell lattices, transport channels between them, flows of water and metabolites, as well as the mechanistic properties of cells.

2. Standardized visualization of results allowing comparison between experimental and in silico data

3. Possibility of flexible calibration of parameters according to available data.

In addition, it is necessary to provide users with detailed documentation and the ability to add/edit functions. It is difficult to overestimate the technical component for the construction and development of models for non-professional biological programmers; for example, the author spent about a year of PhD work just to correctly implement the two ecological models presented in this work.