Probing visual literacy skills reveals unexpected student conceptions of chromosomes

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Abstract

Molecular biology can be challenging for undergraduate students because it requires visual literacy skills to interpret abstract representations of submicroscopic concepts, structures and processes. The Conceptual-Reasoning-Mode framework suggests that visual literacy relies on applying conceptual knowledge to appropriately reason with the different ways of representing concepts in molecular biology. We used this framework to specifically explore visual literacy related to chromosomes. We conducted 35 semi-structured interviews with students who had taken at least a year of college-level biology courses, and we asked them to sketch chromosomes, interpret an abstract representation of chromosomes, and use the abstract representation to answer a multiple-choice question about meiosis. While many participants used the correct vocabulary to describe chromosome structure and function, probing their visual literacy skills revealed gaps in their understanding. Notably, 97% of participants (34 of 35) held conceptual errors related to chromosome structure and function, which were often only revealed in their sketches or explanations of their sketches. Our findings highlight the importance of scaffolding visual literacy skills into instruction by teaching with a variety of visual models and engaging students in using and interpreting the conventions of abstract representations of chromosomes.

INTRODUCTION

Molecular biology is often a difficult subject for students because the concepts in the curricula cannot be directly visualized (Johnstone, 1991; Newman et al., 2023). To make the invisible visible, biologists often create abstract models to represent molecular biology concepts. Abstract models are composed of shapes and symbols that highlight the salient structures and features that convey contextually important information (Fan et al., 2023). As such, abstract models are useful teaching tools in molecular biology because they allow instructors and students to focus on the most relevant information rather than extraneous or superfluous details. Biology instructors can use abstract models in their teaching to create meaningful opportunities for model-based reasoning that encourage students to deeply think about the purpose and efficacy of particular representations (Quillin & Thomas, 2015).

Abstract models are an important and necessary part of molecular biology education, but they can be a source of challenge or confusion for students, especially when considering how biologists often use different abstract shapes and symbols to emphasize varying aspects of the same concept (Kindfield, 1994b; Offerdahl et al., 2017; Schönborn & Anderson, 2006; Wright et al., 2022). For example, a biologist may choose to represent a gene as a sequence of letters to discuss a mutation, as boxes to point out the positions of exons, or as a double helix to explain the shape of the molecular structure. Given that there are a range of ways that biologists use abstraction in molecular biology, students need to develop skills to understand how, why, and when to use abstract models. The importance of these skills is underscored by their inclusion in the “Modeling” and “Communication and Collaboration” core competencies for undergraduate biology outlined in the *Vision and Change* report (American Association for the Advancement of Science, 2011; Clemmons et al., 2020a, 2020b).

The skills that biologists use to create and interpret abstract representations are also aligned with the broader set of skills associated with visual literacy. Visual literacy describes a viewer's awareness of how conventions in visual representations convey meaning (Messaris & Moriarty, 2004), and definitions of visual literacy are typically refined to reflect the conventions and perspectives in a specific disciplinary context (Avgerinou & Pettersson, 2011; Trumbo, 1999). In molecular biology, visual literacy is often defined as the ability to "read" and "write with" the conventions and symbols that encode meaning in biochemical representations (Offerdahl et al., 2017; Schönborn & Anderson, 2006; Towns et al., 2012).

We can characterize the processes involved in visual literacy using the Conceptual-Reasoning-Mode (C-R-M) framework (Schönborn & Anderson, 2009, 2010). This framework proposes that accurately interpreting the many types or modes (M) of biochemical representations requires an understanding of the content knowledge relevant to the concepts being represented (C) and appropriately applying cognitive processes or reasoning (R) to perceive and decode visual conventions in the representation. These three factors (C, R, and M) are interactive, and the integration of all three factors is necessary for successful interpretation of a representation (Schönborn & Anderson, 2009). We provide an example of how the C-R-M framework can be applied to interpret an abstract representation of chromosomes in Figure 1.

Expert-level visual literacy skills encompassed within the Reasoning factor of the C-R-M framework include the abilities to evaluate the power and limitations of representations, use representations to solve problems, and translate across multiple representations of a concept (Schönborn & Anderson, 2010). While these skills are necessary for reasoning and communication in molecular biology, they take time, practice, and scaffolded support to develop (Offerdahl et al., 2017). Experts develop visual literacy skills through their experiences

interpreting, creating, and communicating with a breadth of visual representations (Arneson & Offerdahl, 2018; Kozma & Russell, 1997), so helping students develop expert-like visual literacy requires intentional allocation of class time for students to practice evaluating abstract representations, complete formative assessments using models to solve a problems, and draw a variety of representations of the same concept.

Instructors who create opportunities for students to develop visual literacy skills ought to be attentive to places where students may benefit from additional support. Previous research found that students may encounter challenges in maneuvering between different abstract representations of the same concept (Cho et al., 1985; Newman et al., 2023). Students may focus on the particular shapes, colors, and symbols that are used in a representation (i.e., the "surface features") without necessarily decoding the meaning of the features in the representation (Newman et al., 2023). In some cases, such as in situations where students are using familiar scenarios and representations, finding patterns in the surface features may be a productive strategy, but solely relying on surface features may steer students away from understanding why a particular representation of a concept was chosen (Arneson & Offerdahl, 2018).

Instructors should also consider that students may need more structured support when they are using visual representations to reason across levels of organization or biological scale (Schönborn & Anderson, 2009). DNA is often a uniquely challenging topic for students to learn because the structure is both incredibly small and incredibly big in terms of the physical size and the number of base pairs, respectively. As such, biologists may choose to represent DNA at the scale of a nucleotide, a gene, or a chromosome, depending on what information they intend to communicate. Because there are multiple representations of nucleotides, genes, and

100 chromosomes, the challenge of understanding visual conventions of scale can frequently be
101 compounded with the challenges of abstraction.

102 We can better understand how students' visual literacy in molecular biology is related to
103 their conceptions of scale and abstraction in visual representations by applying a conceptual
104 framework called the DNA Landscape (Wright et al., 2022). The DNA Landscape categorizes
105 the different representations of DNA in a 3-by-3 matrix to show variation in representations
106 across scale (nucleotide, gene, and chromosome) and abstraction (very abstract, elements of
107 shape and abstraction, and literal shape). Students who are fluent in molecular biology visual
108 literacy should be able to understand the relationships between images that are presented at
109 different levels of abstraction and between representations at different scales, but to develop this
110 fluency students must have previously productively engaged and appropriately reasoned with
111 multiple representations at each scale (Schönborn & Anderson, 2006).

112 We focus here on how students conceptualize abstraction in representations of
113 chromosomes. Conceptual knowledge of chromosomes is necessary for students to understand
114 the *Vision and Change* core concept of Information Flow, Exchange, and Storage (American
115 Association for the Advancement of Science, 2011; Brownell et al., 2014), but historically
116 students often struggle with course content related to chromosomes (Etobro & Banjoko, 2017;
117 Kindfield, 1991; Newman et al., 2012; Saka et al., 2006). Chromosome structure can be a
118 particularly difficult topic for students (Kindfield, 1991, 1994a, 1994b; Newman et al., 2012;
119 Wright et al., 2021), and misunderstandings related to the function and behavior of chromosomes
120 in mitosis and meiosis are so common among students that these concepts are targeted on
121 concept assessments such as the Introductory Molecular and Cell Biology Concept Assessment
122 (Shi et al., 2010), the Genetics Concept Assessment (Smith et al., 2008), and the Meiosis

Concept Inventory (Kalas et al., 2013). Although these misunderstandings are commonly identified in the literature, little work has been done to examine the relationship between how students' conceptualization of chromosomes may be associated with their visual literacy skills in molecular biology (Newman et al., 2023). Thus, we set out to answer the following research question: How do students conceptualize chromosomes and apply visual literacy skills to interpret abstract images of chromosomes?

METHODS

Participants: We conducted semi-structured interviews with 35 students from two different institutions—a primarily undergraduate institution and an R2 research institution (Table 1). All research participants had completed at least a year of biology coursework. We used several recruitment methods to achieve a broad sample of participants, including emailing biology majors at each institution, posting flyers in science buildings, and soliciting volunteers in person during sessions of upper-division biology courses. We also used snowball sampling by asking participants to refer their peers to register for the study after they had concluded the interview protocol. Participants consented to this research and were compensated with a $20 Amazon gift card for their participation. Pseudonyms were selected by the participant or assigned by the researcher. This research was approved and classified as exempt from human-subjects review by Rochester Institute of Technology (protocol 01090823).

Interview Protocol: We developed the prompts in our interview protocol to align with two frameworks to target specific visual literacy skills (Table 2). First, we designed interview prompts to target the factors from the Conceptual-Reasoning-Mode (C-R-M) framework that are

associated with the ability to interpret and visualize biological representations (Schönborn & Anderson, 2009, 2010; Figure 1). We wrote our interview prompts in ways that could elicit evidence of participants' conceptual understanding of chromosomes (Conceptual) as well as the cognitive skills participants engaged (Reasoning) to make sense of visual representations (Mode) of chromosomes. The C-R-M framework indicates that there is a minimum amount of prior conceptual knowledge that is necessary for the interpretation of visual representations (Schönborn & Anderson, 2010), so we scaffolded our interview questions to target conceptual understanding before asking participants to reason about the visual representation we presented in the interview. Second, we refined our use of the Reasoning component of the C-R-M model by classifying cognitive skills according to Bloom's Taxonomy (Anderson et al., 2001; Bloom et al., 1956). We used the Visualization Blooming Tool (Arneson & Offerdahl, 2018) to design interview prompts that target visual literacy skills across a range of Bloom's Taxonomy cognitive skills. As with any prompts or questions designed to target specific cognitive skills, the mental processes that a participant engages may not always align with the targeted cognitive skill level (Monrad et al., 2021).

We incorporated an item from the validated and widely-used Genetics Concept Assessment (GCA; Smith et al., 2008) into our interview protocol. Items that compose concept assessments like the GCA are typically evaluated for statistical measures that indicate item difficulty (i.e., the number of correct responses divided by the total number of responses) and item discrimination (i.e., the ability of the item to distinguish between high- and low-scoring students). We used item #2 from the GCA, which had posttest item difficulty of approximately 0.7 and item discrimination index of approximately 0.4 (Smith et al., 2008). These values

168 indicate that this item is sufficiently difficult and can effectively discriminate between students
169 with high and low test scores.

170 Our interview protocol (Table 2) used a scaffolded structure to prompt research
171 participants to engage in higher-order cognitive skills. We first asked participants to provide a
172 verbal definition of chromosomes and then to draw a chromosome and explain their drawing.
173 Some participants drew additional sketches to supplement their explanations. Next we prompted
174 participants to interpret the illustration that accompanied item #2 from the GCA. The GCA item
175 includes an abstract representation of two pairs of unreplicated homologous chromosomes within
176 a cell (see reproduction of this representation in Figure 1). In this representation, the
177 chromosomes are depicted as single vertical lines with large black dots for centromeres, and
178 alleles on the chromosomes are represented using letters (F,f, Q, q). We asked participants to
179 evaluate the effectiveness of the representation of chromosomes and to describe any confusing
180 elements within the representation. Then we asked them to compare and contrast their drawing to
181 the GCA representation. We next provided participants with the full item #2 from the GCA. The
182 item includes a written description of the image that explains that it contains two pairs of
183 chromosomes on which are two different genes, and it asks them to select the possible sperm
184 genotypes if the cell divides normally. After participants selected their answer from a multiple-
185 choice list, we asked them to draw a picture that represents the option they selected.

186 We conducted and recorded interviews using the video conferencing software Zoom. Of
187 the 35 interviews, 11 participants (31%) created drawings using the Zoom whiteboard and 24
188 participants (69%) drew on their own paper and sent us photos or scans of their sketches via
189 email. The quality of drawings and sketches on the Zoom whiteboards may have varied

depending on the familiarity and comfort with the virtual medium. Audio recordings were transcribed by Otter.ai and then cleaned manually.

This interview protocol was part of a larger interview probing visual literacy across a range of molecular biology concepts. Participants completed the chromosome protocol in approximately ten minutes, while the entire interview was approximately 45 minutes in length.

After 35 interviews, the emergence of new insights or concepts from interviews had slowed, so we judged that we had reached a theoretical saturation of conceptions and reasoning about chromosome structure.

Codebook development: We used initial coding (Saldaña, 2013) to develop our codebook from the interview data. We shared our initial codebook with a group of biology education researchers consisting of two biology professors, two postdoctoral researchers, and four undergraduate students. The group of researchers used the codebook to code one interview transcript that was selected based on a random-number generator. We compared interpretations of the codebook across the members of the research group, discussed areas of disagreement, and subsequently refined our codebook to its present version.

Our codebook consists of sets of codes to categorize participants' verbal definitions of chromosomes, sketches of chromosomes, the accuracy of their description of their sketch, the accuracy of their interpretation of the concept assessment image, the accuracy of their answer to the concept assessment item, and the sketches that represent their response to the concept assessment item.

Verbal definitions of chromosomes: We coded verbal descriptions of chromosomes based on whether they described the shape (e.g. "X-shaped"), structure (e.g. "made of DNA"), and/or function (e.g. "involved in meiosis") of chromosomes.

Sketches of chromosomes: We categorized sketches of chromosomes based on both shape and the style representing the curvature or complexity of the shape. Participants drew chromosomes as either an X-shape (consisting of two structures that cross or intersect at a specific point that may or may not have a visible centromere indicated), an I-shape (a single vertical structure that may or may not have a centromere indicated), or as a side-by-side shape (two structures that do not intersect). For style, participants drew chromosomes consisting of loops (a rounded, continuous curve that creates an enclosed shape), lines (a single continuous pen stroke without additional details), or coils (tightly-would spirals or twists). We also coded sketches for their level of abstraction based on their location on the DNA Landscape matrix (Wright et al., 2022

Accuracy of description of sketch: We classified participants' descriptions of their sketches as either correct (participants used the correct vocabulary to describe the elements in their sketch, like correctly stating that their X-shaped chromosome was a replicated chromosome) or incorrect (participants used incorrect vocabulary or concepts to describe their sketch, like identifying an X-shaped chromosome sketch as a pair of homologous chromosomes).

Concept assessment image interpretation: We classified participants' interpretation of the image from the GCA item #2 as either correct (stated the image consisted of four chromosomes or two pairs of homologous chromosomes and that the letters in the image represented genes or alleles) or incorrect (stated the number of chromosomes was something other than four, mistook the homologous chromosomes for sister chromatids, or said the homologous chromosomes should be connected at the centromere).

Answer to concept assessment: We coded three types of answers to the GCA item #2: the correct answer with the correct reasoning, the correct answer with incorrect reasoning, or an incorrect answer.

Interrater reliability: To establish interrater reliability between coders, two members of the research team (CU and LKW) used the codebook to independently code eight randomly selected interview transcripts (23% of the sample). There was 93% agreement between the two raters across 160 coding decisions. The Cohen's kappa coefficient was 0.83, which indicates strong agreement between raters (McHugh, 2012). The two raters discussed any disagreements until consensus.

Positionality: Author CU conducted the interviews and acknowledges the limitations in this research because of the power differential inherent in their position as an education researcher who is older and has more formal education training relative to the interviewees. This power differential may inadvertently reinforce traditional power structures and hierarchies, thereby shaping the ways in which participants perceived and responded to the interview.

All members of the research team are trained in biology education research, have studied visual representations in biology, and have taught courses where we observed students making errors about chromosome structure and/or function. As such, we had preexisting ideas about how research participants might respond which consciously and unconsciously shaped our analysis and interpretation of participant responses.

RESULTS

We conducted 35 semi-structured interviews to understand how students conceptualize chromosomes. The participants in our study brought with them a wealth of experiences in biology that allowed them to thoughtfully engage in interpreting, evaluating, and creating abstract representations of chromosomes. While it was not directly part of our structured interview protocol to ask students where they previously encountered representations of chromosomes, many participants volunteered information about their experiences learning about chromosomes in high school (n = 2), in their undergraduate courses in introductory biology (n = 3), molecular biology (n = 1), genetics (n = 5), as well as in biology classes or lectures in a broader sense (n = 9). In addition to their courses as sources of information, participants discussed seeing chromosomes represented in textbooks (n = 5), microscopy (n = 5), and karyotypes (n = 2). Although many participants openly discussed their experiences learning about chromosomes, our results as a whole indicate that most participants likely left these experiences with an incomplete understanding of the representations they had encountered.

When we look across the entirety of participant's interview, we found that 97% of participants (34 of 35) demonstrated at least one error in their conceptual knowledge of chromosomes. Using the Conceptual-Reasoning-Mode (C-R-M) framework as a lens for our research, we frequently found that most participants knew the correct vocabulary to describe chromosomes (C) and were familiar with the shapes and symbols used in common abstract models of chromosomes (M). However, we observed mismatches between participants' verbal statements and their drawings that indicated an incomplete conceptual understanding of why and how expert biologists typically use abstract shapes and symbols to convey meaning (C-M). We further probed participants' understanding of chromosomes using an item from the Genetics Concept Assessment and found participants often had difficulties reasoning with the unfamiliar

280 chromosome representation in the item (R-M). Despite misinterpreting the GCA image, the
281 majority of participants correctly answered the associated concept assessment item; however,
282 when asked to explain their thinking, most participants provided an incorrect line of reasoning to
283 justify their answer.
284
285 ***How do undergraduate students define chromosomes?***
286 To investigate what conceptual knowledge of chromosomes participants were bringing
287 into the interview (the C in C-R-M), we first asked them "What is a chromosome?". The
288 majority of participants (86%, n = 30) provided definitions related to chromosome structure
289 (Figure 2). Most participants correctly noted structural elements of chromosomes, such as the
290 fact that chromosomes consist of DNA and contain histones. Participants less frequently
291 mentioned chromosome functions (e.g., the role of chromosomes in gene expression or meiosis)
292 or chromosome shape (e.g., an "X" shape), but when they did mention function or shape, it was
293 typically paired with content related to chromosome structure.
294 While participants frequently provided factually correct definitions of chromosomes, we
295 noted several unrefined ideas, often relating to the size and scale of chromosomes. For example,
296 Jimmy (sophomore biotechnology major) stated, "A chromosome is I guess like a collection of
297 DNA and genes that are—they're like the encapsulation of all your DNA." Jimmy inaccurately
298 implies that a single chromosome contains all the DNA within what is presumed to be a
299 eukaryotic cell. In other cases, participants confused the scale of chromosomes and genes. For
300 example, Evelyn (junior biology major) said, "A chromosome is part of the DNA that can lead to
301 a gene," and Mia (sophomore biology major) said of chromosomes, "basically I think it's like
302 part of the genes."

303 Additionally, we uncovered inaccurate ideas related to chromosome function during cell
304 division. For example, Norma (senior biotechnology major) said, "A chromosome is basically
305 like an X. It can be single, it can also be like the X shape, and it contains genetic information and
306 just I guess, a shaped form. And the DNA can be double stranded—is double stranded—and then
307 if it goes through like the other processes, it could become single stranded and whatnot." While
308 Norma understood that genetic information is divided in "other processes," such as mitosis and
309 meiosis, division does not make DNA into a single-stranded molecule.
310
311 ***How do undergraduate students draw chromosomes?***
312 We asked participants to draw a chromosome to explore how they used shapes and
313 symbols to represent features of chromosomes that convey biological meaning (the M in C-R-
314 M). When asked to sketch a chromosome, 100% of participants drew images that aligned to the
315 "very abstract" representation of chromosomes on the DNA Landscape and drew symbolic
316 loops, lines, or coils to represent X-shaped, I-shaped, or side-by-side chromosomes (Figure 3).
317 We found that participants most commonly drew X-shaped chromosomes (74%) and
318 most frequently represented these X-shaped chromosomes using loops. Participants less
319 commonly drew I-shaped (23%) and side-by-side (17%) chromosome structures. We did not
320 observe any participants drawing I-shaped chromosomes with lines in ways that resembled the
321 representational style used in the Genetics Concept Assessment image.
322
323 ***Why do students draw chromosomes that way?***
324 To understand how participants were representing their conceptual knowledge of
325 chromosomes in their sketches, we asked them to explain the reasons why they drew

326 chromosomes in the way that they did. We group these explanations based on the shape and style
327 of the chromosome sketch.

328 *X-shaped loops*: We found that X-shaped loops were the most frequently drawn
329 representation. In some cases, participants focused the explanation of their sketches on their prior
330 experiences with similar representations. "Well, that's just how I've always seen it in any of my
331 classes—it's always two sister chromatids held together by a centromere," Maddy (junior
332 biology major). For participants like Maddy, their description aligns more closely with how an
333 expert might describe and use this representational form.

334 For other participants, their description of the meaning of the X-shaped loops was more
335 ambiguous or did not entirely align with how experts conventionally use this mode of
336 representing chromosomes (Figure 4). For example, Nadine (senior environmental science
337 major) describes her X-shaped loops as consisting of "two halves that kind of fit together," but it
338 is unclear how or by what process she thought that this "fit" occurs. In another example, Annie
339 (sophomore biochemistry and molecular biology major) described that her sketch contained
340 "four parts" across two sections of genetic code (Figure 4). When the interviewer asked for
341 clarification about this statement and whether the "two sections" (i.e., the two p-arms in a
342 replicated chromosome) that Annie referenced were identical, she responded "Um, no. So like,
343 for example, if you have like, if you are homozygous it'd be like your big E and then like the
344 little e like that." Here, Annie is not only uncertain about the correct vocabulary (i.e.,
345 homozygous compared to heterozygous), but conflated sister chromatids (which must contain the
346 same allele) in her drawing with homologous chromosomes (which could have two different
347 alleles).

We found that some participants used the X-shaped loops to productively reason about chromosome structure. Evelyn (junior biology major), whose sketch is the exemplar of the dimensional X-shape in Figure 3, described it as a "circular thing [so] that you could probably see that there is more like their stuff inside it." Here, the loops are meaningful symbols that reflect her understanding that chromosomes are structures that are composed of "stuff," which we interpret as DNA and proteins.

X-shaped lines: Participants less commonly represented chromosomes using two crossed lines in an X-shape (Figure 4). Even though participants like Pluto (sophomore general science major) asserted that "everyone knows" the X-shaped line style of representing chromosomes, only 14% of participants chose to use this representation in their drawing.

X-shaped coils: We identified two instances of X-shaped coils which seemed to contain both elements of both the "very abstract" and "very literal" portions of the DNA Landscape (Figure 4). These sketches were both X-shaped but used coils as a way of representing the more realistic literal structure of chromosomes at the microscopic level. Jack (senior mechanical engineering major) rationalized that his coiled chromosome could be "filled," which was similar to how participants used X-shaped loops to represent how chromosomes could have "stuff inside."

I-shaped loops: Nine participants drew sketches of I-shaped loops. Two participants referred back to the interview prompt, "Can you sketch a chromosome?," in their explanations. Josie (junior biochemistry and molecular biology major) asked the interviewer "Should I do like — I'll just do one?" and proceeded to draw what we interpreted as a single unreplicated chromosome using I-shaped loops. Similarly, Lacie (junior biotechnology and molecular bioscience major) referred back to the interview question, "'cause I was like 'just one

371 chromosome.'" Both Josie and Lacie mentioned their sketches were not reflecting what they
372 "normally" thought about for chromosomes. Josie remarked that "The way I normally picture
373 them is double. Because we talked about them with like, the replication, but that's—I'm pretty
374 sure they just exist as single." Lacie said that "I was thinking, you know, normally in human
375 beings, chromosomes are drawn in pairs." Neither of these participants used the terms
376 "replicated" or "unreplicated" or discussed the total number of DNA molecules when deciding
377 which type of chromosome structure to draw.

378 *I-shaped lines*: We did not observe any participants using I-shaped lines in their sketches
379 of chromosomes. The lack of this representational style in the initial drawings tracks closely with
380 the first-impressions that participants had to the I-shaped line chromosomes in the GCA image.
381 For example, Donna (sophomore biology and environmental science major) viewed the GCA
382 image and immediately stated "If I saw this, I would not recognize it as a chromosome."

383 *I-shaped coils*: Ristan (senior biochemistry and molecular biology major) was the only
384 participant to draw a chromosome using I-shaped coils (see Ristan's sketch as the exemplar for I-
385 shaped coils in Figure 3). He initially said "I think that's to sort of show that it's not like one
386 strand." Ristan provided a second sketch of a single line for comparison to better illustrate that
387 his first sketch contained "many, many strands of DNA all compressed on top of each other,"
388 suggesting that numerous individual molecules of DNA make up an unreplicated chromosome.

389 Participants sometimes drew coiled chromosomes during follow-up questioning which
390 provided unique insights into their understanding of chromosome structure (Figure 5). These
391 sketches are not included in the initial counts in Figure 3, but they provide contextual
392 information about how students are illustrating meaning with I-shape coils. In Figure 5, we see
393 that Norma's initial sketches include X- and I-shaped loops, stating that a chromosome "becomes

394 an X-shape throughout the process of meiosis." When probed further, Norma produced the
395 follow-up sketch and said "DNA is just coiled up, so it's like this," suggesting a robust
396 understanding of chromosome structure. Conversely, Teresa (junior individualized study major)
397 created follow-up sketches that indicate a *misunderstanding* of chromosome structure. Teresa
398 modified her sketch by filling in the p-arm of the chromosome with the narration that "This is
399 like the clusters of the DNA, big clusters." She stopped at the centromere then continued in a
400 separate pen stroke to fill in the q arm of the chromosome, saying "Yeah, and this is also another
401 cluster of like DNA." Her description shows that she thought of chromosomes as consisting of
402 two separate units of DNA partitioned by the centromere.

403 *Side-by-side loops*: There was variability in the participants' rationale for their side-by-
404 side chromosomes (Figure 6). Alex (senior biology major) drew the side-by-side chromosome
405 with a separate box to represent the centromere, inappropriately indicating that the centromere is
406 a structure separate from the structure of the chromatids. Mia (sophomore biology major) said
407 that she drew side-by-side chromosomes to resemble X and Y chromosomes. Participants Socks
408 (sophomore biomedical science major) and Leo (junior biology pre-med major) both used side-
409 by-side representations, but offered very different explanations as to why. Socks said they drew
410 side-by-side loops "because that's usually how we see it on a karyotype—they're usually paired
411 together like that for mitosis," which we interpreted as a description of unreplicated homologous
412 chromosomes. While homologous chromosomes are typically shown paired in a karyotype, this
413 participant is mistaken because homologous chromosomes do not pair for mitosis. This quote is
414 also interesting because chromosomes in karyotypes are replicated (but sister chromatids are not
415 visible as separate entities), so Socks is likely interpreting the chromosomes in karyotypes as
416 single DNA molecules even though they are not. Leo, on the other hand, said "that would be a

set of sister chromatids." We highlight Socks and Leo as two examples of how the same sketch of a chromosome can be ambiguous if a verbal or written description is not provided.

Side-by-side lines: Christin (sophomore environmental science major) and Regina (post-baccalaureate continuing education) drew chromosomes with side-by-side lines, which they used to indicate different numbers of chromosomes. Christin referenced a centromere in her explanation of her drawing, even though her sketch did not include a centromere, suggesting that she intended the two lines to represent chromatids in a single replicated chromosome (Figure 6). Regina said "When I think of chromosomes, I think of mitosis and meiosis, and they're always paired up with their buddies." To clarify this explanation, Regina drew an arrow pointing to only one of the lines in the sketch and indicated that there were two chromosomes but "they're like a pair." While Christin used the lines to represent one chromosome, Regina used the same style of lines to represent two.

While we focus on what participants did draw in their sketches of chromosomes, it is also important to note what they did not draw. None of the participants in our sample drew chromosomes in a circular shape that might resemble bacterial chromosomes. We did not observe any participants drawing chromosomes with banding patterns like a karyotype or drawing a map with positions of genes.

We necessarily focus our results on the errors related to chromosomes, as these errors provide novel insights into student thinking, but we also want to emphasize many participants (63%, n = 22) used appropriate terms and vocabulary to describe the chromosome structure that they drew.

440 ***How do undergraduate students interpret an abstract model of chromosomes?***

441 To determine how participants use their conceptual knowledge to reason about an
442 abstract model of chromosomes, we asked them to interpret an image from the Genetics Concept
443 Assessment. To correctly interpret the image, participants needed to appropriately engage all
444 three factors in C-R-M and apply their conceptual knowledge (Figure 1). While many students
445 correctly decoded certain symbols, such as the capital and lowercase letters representing
446 dominant and recessive alleles, students may have had incomplete content knowledge and/or an
447 unfamiliarity with the abstract image that prevented them from accurately interpreting all of the
448 key information encoded in the image. Overall, we found that 71% of participants (n = 25)
449 incorrectly interpreted the image from the concept assessment (Table 3).

450 For some participants, accurate interpretation of the GCA image was most directly
451 limited by an unfamiliarity with the mode of representation. G (sophomore biotechnology major)
452 and Donna (sophomore biology and environmental science major) compared the unfamiliar
453 representation in the GCA to the X-shapes they were more familiar with. For example, Donna
454 said "I'm used to seeing chromosomes where they're crossed over and in an X formation, so
455 that's kind of the only reason I wouldn't recognize this because it doesn't have the X formation."

456 Donna and G also held conceptual misunderstandings about the difference between
457 homologous chromosomes and sister chromatids. Upon seeing the GCA image, G immediately
458 asked "The chromatids aren't paired together?". G subsequently described the image as having
459 "all the information needed—it has two different chromatids." These types of conceptual
460 misunderstandings—thinking that sister chromatids can exist independently or that parental
461 origins are mixed in a replicated chromosome—are misunderstandings that underpinned the

462 majority of the misinterpretations of the GCA image, occurring in 19 of the 25 incorrect
463 interpretations.

464 This confusion of homologous chromosomes and sister chromatids persisted even with
465 the visual scaffolding provided by the heterozygous alleles labeled for each chromosome pair
466 (F/f and Q/q). Maddy (junior biology major) incorrectly used the alleles to justify her
467 interpretation of the image as chromatids, stating that the labeled alleles are "just like to specify
468 that they're the same chromosome but different sister chromatids." Heather (sophomore
469 biotechnology and molecular bioscience major) similarly struggled to differentiate chromosomes
470 and chromatids, stating that "in terms of chromosomes that have already been replicated, there's
471 two. So that would be a total of four." In other words, Heather arrived at the correct number of
472 chromosomes in the image by applying the wrong reasoning.

473 In many cases, this misunderstanding of homologous chromosomes stemmed from the
474 idea that the unreplicated homologous chromosomes in the image were "separated" sister
475 chromatids. Participants tended to view the centromere as the place where the chromatids
476 "should have been paired together" or connected. Numerous quotes illustrate this point:

477 "The two halves of the chromosome just pulled apart—Instead of being in that X,
478 they're just portrayed separately" – Eileen (sophomore environmental science major)

479 "I usually imagine [chromosomes] like connected like I did [in my sketch of a
480 replicated chromosome]. Because they're like separate—might be something that I
481 thought was a little confusing at first." – Quentin (junior biology/pre-med major)

482 "[The image is] like showing that there's two separate chromatids, and because like on
483 the initial drawing that I drew [this participant initially drew a replicated chromosome],

a lot of people would just see it as like one X structure and not like two structures that are together." – Jimmy (sophomore biotechnology major)

"In both sketches [participant sketch and the GCA image], it's made out of two parts and you can kind of see that they're supposed to be attached together with the dots on that sketch and on my sketch." – Sylvia (sophomore biochemistry and molecular biology major)

"Typically, I think they're [the chromatids] kind of fused together, so maybe that's one thing that might be confusing to people [who] just started learning." – Jay (Master's student in bioinformatics)

Some participants misapplied conceptual knowledge in how they reasoned about the length of the chromosomes in the image. For example, Annie conflated the length of the chromosomes with dominance in alleles: "Could the longer strands mean it's dominant?" Brittany (senior biology major), interpreted the different lengths as being related to a mutation. "My first thought was that there was like a weird mutation at first on the top part, but then it could have just been like a smaller chromosome or like chromatid or something and they just combined the small." Donna said: "I'm more confused what the small one and what the tall one is? Like why—like, is this one chromosome I'm looking at? I don't know. I guess I'm confused of is this how many chromosomes am I looking at? Is this one? Is this two? Is this the same chromosome?"

Participants also produced unexpected interpretations of the labeled alleles. Jimmy interpreted the two lines for each homologous pair as the "two strands, like the double stranded DNA for each chromatid," and related the capital and lowercase letters from the heterozygous pair of chromosomes as "the uppercase is for the leading strand and the lowercase is for the

lagging strand." The allele "q" was confusing for participants like Donna, who said: "I know there's a q arm. So I'm going to assume that's what the two Qs are. And then I thought it was q and p, but I guess not."

While we focus our analysis here on areas of student misunderstanding to highlight concepts that may need additional instructional scaffolding, we briefly want to emphasize that many participants had productive and insightful ideas about the effectiveness and limitations of the abstract model of chromosomes. Claire (sophomore biotechnology and molecular bioscience major) noted that abstract models are often made when you need to quickly express an idea, such as when making sketches during class: "Sometimes people, will you know, like if they're sketching on the whiteboard, they're not going to draw out the full thing, right? They might just draw lines. And drawing the dots helps you kind of see, oh, like 'top half, bottom half,' especially if you're talking about the location of where a certain gene might be on the chromosome—it kind of helps you see it better." Piper (senior biology secondary education major) emphasized that in addition to the allele labels, the abstract sketch could benefit from additional symbols or notations to emphasize the difference between homologous chromosomes: "I think, like, if I was representing it differently, I'd probably do like maybe a different squiggly line or like a different color—something just because they might get mixed up, especially when people are redrawing them." By evaluating the abstract model, these participants demonstrated their understanding of the conventions used to represent chromosomes and genes as well as their metacognitive abilities for reflecting on how they or their peers might be thinking about visual representations of chromosomes.

How do undergraduate students use visual literacy skills to reason about the division of chromosomes during meiosis?

Three-quarters of participants (26 of 35) selected the correct answer to the GCA multiple choice question, indicating an item difficulty on par with what was previously reported (Smith et al., 2008); however, we found that most participants selected the correct answer for the wrong reasons (Table 3). Only 23% of participants (8 of 35) answered the concept assessment question by correctly representing the division of chromosomes during meiosis in their sketches and associated verbal descriptions.

We were surprised to see that 37% of participants (13 of 35) justified their answer using a Punnett square and that 11 of the 13 participants who drew a Punnett square selected the correct answer to the GCA item. Although Punnett squares were the most common way for participants to approach this concept assessment question, there was a wide range in how participants misunderstood the symbols and misapplied the logic of Punnett squares (Figure 7). Participants like Peggy (junior biomedical science major), Leland (junior biology major), and Piper (senior biology secondary education major) recognized that a two-by-two matrix could generate the four different allele combinations present in the correct answer but did not realize that Punnett squares are not appropriate for identifying combinations of alleles from a single parent. Piper initially had inklings that Punnett squares were associated with a single allele, reflected in her drawings of two separate matrixes for the F and Q genes, but rationalized her final answer with a take on a Punnett square that combined both the F and Q genes. Participants who misapplied Punnett squares tended to demonstrate other novice-like ideas, such as Peggy's conflation of genotype and phenotype, Leland's inconsistent ordering of alleles inside the matrix, and Piper's

use of the term “monohybrid” to describe a Punnett square for a meiotic event rather than a cross.

We also saw participants who drew Punnett squares arrive at the incorrect answer to the concept assessment (Figure 7). The participant G (sophomore biotechnology major) drew the structure for two Punnett squares, one which shows F and Q alleles on separate axes (pictured on the left) and one which has a combination of F and Q alleles on each axis (pictured on the right). G then selected the answer which indicated the possible sperm genotypes included Ff, Qq, FQ, fq, Fq, and fQ, and provided the rationale that “I think that’s the closest to what I got” based on their completed Punnett square. The rationale of the “closest to what I got” is interesting here, as the Punnett square only provides four combinations of alleles while the answer G selected contains six possible combinations.

In addition to puzzling uses of Punnett squares, we saw participants arriving at the correct answer for other unexpected reasons (Figure 8). Many times, participants answered thequestion based on their initial (often incorrect) conceptions of chromosomes even though the text from the item states “two pairs of chromosomes.” Heather (sophomore biotechnology and molecular bioscience major) drew the dividing cell with X-shapes consisting of both homologous chromosomes. Each X-shaped structure in Heather’s drawing contains both the dominant and recessive alleles, and she completed meiosis without replicating the chromosomes and with only one cell division. This may be related to Heather’s initial interpretation of the GCA image where she stated “in terms of chromosomes that have already been replicated, there’s two.” Ristan (senior biochemistry and molecular biology major) produced a similar drawing of the crossed homologous chromosomes. During the initial interpretation of the concept assessment image, Ristan said “I'm assuming that later, we're going to get to the point where the chromosomes start

connecting, and we get into sort of these X shapes. So this figure kind of lends itself to that as well, because you can imagine those two sort of coming together, forming that X." Ristan's drawing here reflects his initial idea of the "connecting" chromosomes.

We saw more unique interpretations of connecting chromosomes in Annie and Christin's drawings (Figure 8). Annie only included two chromosomes in her sketch, and the Q and F genes (initially on separate chromosomes) were now depicted on the same chromosome. Annie also used the telling phrase "when these replicate," indicating that she believed crossing over of alleles occurs during the replication process. We see a similar misunderstanding of crossing over in Christin's sketch in which she stated that two non-homologous chromosomes are connected at the centromere to represent the process by which chromosomes "cross over." Notably, both students chose the correct answer choice.

When we asked participants to create sketches to represent their selected answer to the GCA item, we found that many (n = 12) adopted the visual conventions of the GCA item. Even though none of the participants initially represented chromosomes with the I-shaped lines (Figure 3), we saw many students translating their understanding of chromosomes into this representational format in their follow-up sketches. Four of the participants used this shape and style of chromosomes to correctly answer the GCA item for the correct reasons. The remaining eight participants sometimes arrived at the correct answer but their sketches indicated an incomplete or incorrect reasoning, such as failing to replicate the I-shaped chromosomes, resulting in the products of meiosis as only two cells (rather than four) or as containing only a single chromosome (as a result of dividing non-homologous chromosomes in meiosis I and dividing homologous chromosomes during meiosis II).

DISCUSSION

Chromosome structure and function is foundational knowledge in biology and is necessary to understand the *Vision and Change* core concept of "Information Flow, Exchange, and Storage" (AAAS, 2011). As chromosomes are microscopic and their structural elements (genes, nucleotides) are submicroscopic, biologists often emphasize their relevant features using abstract models. The ability to interpret and use such abstract models are key to *Vision and Change* competencies related to modeling and scientific communication (AAAS, 2011). Despite the importance of these topics in undergraduate education, our findings suggest that chromosomes are parts of introductory biology curricula where college students may need additional instructional support. By probing visual literacy skills, we found a mismatch between declarative knowledge about chromosomes and mental models of chromosomes. Similar to previous research (Newman et al., 2012), our findings here suggest that students' mental models of chromosomes often need additional refinement to align with how experts typically use abstract models of chromosomes. Our work also mirrors findings in chemistry in which undergraduate students presented with multiple representations of the same concept had difficulties interpreting the main features of abstract models and offered conflicting interpretations across representations (Allred & Bretz, 2019). In this discussion, we highlight particular concepts participants misunderstood and how biology instructors might choose to address these concepts in their teaching.

Confronting conceptual misunderstandings of chromosomes

While many students were familiar with the mode of representing chromosomes (M in C-R-M), they differed from experts in their conceptual understanding (C) and the ways in which

620 they used their conceptual understanding to reason about the abstract representations (C-R).
621 Here, we focus on where students' chromosome visual literacy skills were primarily hampered
622 by errors in their conceptual knowledge. For a full list of the conceptual misunderstandings we
623 encountered in this study, see the Supplemental Material.

624 The most common conceptual misunderstanding was the idea that sister chromatids can
625 be separated and exist as independent structures within the same cell prior to cell division—
626 conflating a sister chromatid with an unreplicated homologous chromosome. This conflation was
627 evident in participants who made the statement that sister chromatids are supposed to be "paired
628 together," which at face value is a correct statement, but is incorrect when applied as a
629 description of the pair of unreplicated homologous chromosomes in the GCA image. We have
630 previously documented this confusion of homologous chromosomes for sister chromatids
631 (Wright & Newman, 2011), and our finding here bolsters existing evidence that the distinction
632 between chromatids and chromosomes remains a persistent point of conceptual difficulty for
633 students (Clark & Mathis, 2000; Dikmenli, 2010; Kindfield, 1994c).

634 Similar to how we observed participants identifying homologous chromosomes as
635 "separate" sister chromatids, we also saw the tendency for participants to want to combine two
636 homologous chromosomes into single structure. Previous research has shown that students
637 produced similar representations of "two-DNA-molecule chromosomes" to represent the process
638 of fertilization (Kindfield, 1991). In the previous study, students joined two haploid gametes,
639 each containing a chromosome with the allele "A" or "a," into a single X-shaped chromosome
640 with both "A" and "a" alleles upon fertilization, but here we saw participants using this "two-
641 DNA-molecule chromosome" representation *at the start of meiosis*.

642 One way to address some of this confusion about chromatid and chromosome structure is
643 to incorporate active learning opportunities in which students use physical, hand-held models as
644 reasoning tools for visually distinguishing between sister chromatids and homologous
645 chromosomes (Newman & Wright, 2017; Smith & Kindfield, 1999; Wright et al., 2021; Wright
646 & Newman, 2011). Instructors may also consider asking students to reason about the purpose of
647 centromeres in abstract models as a way to clarify the difference between chromatids and
648 chromosomes. Instructors can present multiple models of replicated and unreplicated
649 chromosomes and ask students to determine the total number of chromosomes, emphasizing that
650 biologists often include centromeres in abstract models because counting the number of
651 centromeres is a way to quickly determine the number of chromosomes in a representation.
652 We were surprised by students who merged non-homologous chromosomes into a single
653 chromosome structure. For example, participants took the two non-homologous chromosomes
654 (denoted with alleles F/f and Q/q), and merged them into a single chromosome containing both F
655 and Q alleles (Figure 8). Interestingly, we provided participants with a representation of
656 chromosomes in a single straight line, but participants that combined homologous chromosomes
657 adopted representations with bowling-pin shapes or curved lines that were not present in the
658 GCA image. It is possible that the students who did not adopt the conventions of the I-shaped
659 line model of chromosomes from the GCA image did not fully understand what the straight lines
660 represented, highlighting the importance of explicitly asking students to evaluate the conventions
661 of chromosome representations when using them as a visual aid when teaching.
662 Curiously, many participants solved the GCA question by creating Punnett squares to
663 describe the outcomes of one cell undergoing meiosis. These participants were relying on their
664 understanding that Punnett squares are associated with alleles, but Punnett squares are used to

665 predict allele combinations of offspring from a cross between two individuals, not one individual
666 cell undergoing specialized division. While a two-by-two matrix can be a productive strategy for
667 visually sorting combinations when one item in each of two pairs will be part of a combination,
668 referring to the model as a "Punnett square" is problematic. Our finding provides a chain of
669 evidence that students are misapplying Punnett squares in many different genetics contexts
670 (Newman et al., 2021; Shaw et al., 2008; Stewart, 1983; Strand & Boes, 2019). To reinforce the
671 intended purpose of Punnett squares, we suggest that instructors encourage students to make a
672 drawing that indicates each allele on the "outside" of the Punnett square is representative of a
673 sperm or an egg.

674 We anticipate that many of these conceptual errors result from the phenomenon that
675 chromosome-related concepts are often presented in "twos." The "twos" occur in the two
676 chromatids within a replicated chromosome, two chromosomes in a pair of homologous
677 chromosomes, and two different pairs of chromosomes (i.e., 2n = 4) that frequently appear in
678 common diagrams of chromosomes (including the one in the GCA). This series of "twos" may
679 in-part explain why participants often conflated two homologous chromosomes in the GCA
680 image for two sister chromatids. To remove one "two" from this challenge, we encourage
681 instructors to use examples with at least 3 pairs of chromosomes (i.e., 2n = 6). Using examples
682 with three pairs of chromosomes may create dissonance with the intuitive ideas that students
683 have about using monohybrid Punnett squares, and may lead to more productive methods of
684 reasoning about the behavior of chromosomes during meiotic events.

685

686 ***Reckoning with unexpected reasoning about chromosomes***

We were surprised at apparent mismatches between participants' conceptual knowledge about the common modes of representing chromosomes (C-M) and how they reasoned about the meaning of the shapes and symbols in those abstract representations (R-M). We describe several examples here of unexpected ways that participants used shapes and symbols, and provide a full list of the unexpected reasoning we encountered in this study in the Supplemental Material.

One of the most common instances of unexpected reasoning was how several of the participants used loop symbols to indicate that chromosomes were like vessels that could be "filled" with DNA or that each loop was a separate "chunk" of DNA. We speculate that participants' reasoning about the meaning of the loops may be related their prior experiences and their intuitive sense for symbolism. Multiple participants (Jimmy, Ristan, Ronette, Norma, Andi) described chromosomes as structures that "contain information" or "contain alleles," and it is possible the colloquial use of the word "contain" may evoke intuitive ideas of chromosomes as "containers" that can be filled. We also saw that some participants thought of separate loops as representing distinct molecules of DNA. Participants described replicated chromosomes as consisting of "four parts" or represented unreplicated chromosomes as two separate "clusters" of DNA separated by the centromere. The misconception that chromosomes contain multiple molecules of DNA has been previously documented (Kindfield, 1991) and was even the subject of an item on the Introductory Cell and Molecular Biology Concept Assessment (Shi et al., 2010), but exactly how we saw participants conceptualizing loops as "chunks" of DNA is a new finding. Our work here provides further support that students may be confused by chromosome structure in ways that hinder their understanding of the nature of the relationship between DNA and chromosomes (Saka et al., 2006). To clarify how loops are used to represent chromosome structure, we recommend that instructors verbally and pictorially explain that each unreplicated

710 chromosome is a single continuous strand of DNA. Instructors may also ask students to work in
711 small groups to compare and contrast models where chromosomes are represented as loops and
712 as lines, particularly using this comparison to probe how students are thinking about what the
713 loops exactly mean.

714 We highlight another example of unexpected reasoning in the side-by-side chromosomes
715 that several participants drew. Some participants used side-by-side structures to represent a
716 replicated chromosome, while to others these structures symbolized a pair of unreplicated
717 homologous chromosomes. Because the drawings lacked centromeres, it was impossible to know
718 whether the participant was using their drawing to represent one chromosome or two without a
719 verbal explanation. This finding echoes previous work which emphasized the importance of
720 including centromeres in chromosome models as a way to clearly signal the intended number of
721 chromosomes (Smith & Kindfield, 1999). While the purpose of abstraction is to make
722 simplifications, we suggest that abstract chromosome representations should not be simplified to
723 the point of erasing centromeres as a visible feature. As biology students are likely to encounter a
724 variety of chromosome models, we recommend that instructors ask students to evaluate the
725 benefits and limitations of representing chromosomes with and without centromeres. In these
726 instructional exercises, we encourage instructors to be intentional about describing the
727 centromere as a specific segment of DNA within the chromosome to avoid reinforcing the
728 misconception that the centromere is a separate structure or introducing confusion between
729 centromeres and kinetochores.

730

731 ***Incorporating visual literacy into instruction and assessment***

732 During our interviews, many participants justified their drawings or interpretations of
733 chromosomes by referring to what they had seen in their biology classes or in textbooks. Given
734 the number of conceptual misunderstandings that we encountered, we suggest that there is room
735 to improve how students engage with representations of chromosomes in their classes and course
736 materials. Traditionally, students are likely to have seen prepared graphics in their textbooks and
737 in PowerPoint slides, and this way of presenting graphics and images reinforces an intrinsically
738 passive method of learning via images (Higley et al., 2024; Rotbain et al., 2005; Wright et al.,
739 2022). Teaching using visual representations should go beyond putting an image up on a slide or
740 labeling visual structures. While labeling visual representations may help students memorize
741 structures such as components of a nucleotide in a chemical structure (Rotbain et al., 2005),
742 using visual representations to memorize facts about science is not an end goal. Rather, the
743 *Vision and Change* core competency of "Ability to Communicate and Collaborate with Other
744 Disciplines," suggests an end goal of science education is for students to be able to *engage with*
745 visual representations and build conceptual models that help them participate in the *processes of*
746 *science* (American Association for the Advancement of Science, 2011; Clemmons et al., 2020b).
747 We encourage biology instructors to adopt a more active approach to using visual
748 representations in their courses. We have provided a few sample active learning tasks in this
749 discussion (e.g., asking students to work in small groups to evaluate models). We recommend
750 instructors consult the Visualization Blooming Tool (Arneson & Offerdahl, 2018) for example
751 active learning tasks that can help foster the development of visual literacy skills.
752 Incorporating visual literacy into instruction does not need to take much class time.
753 Asking students to consider strengths and limitations of a chromosome model requires less than
754 five minutes. In addition to using active learning to teach visual literacy, we encourage

755 instructors to take a few seconds to verbally explain the diagrams, figures, and visuals they use in
756 class. When drawing an X-shaped chromosome, instructors can quickly tell students that the "X"
757 represents a replicated chromosome and circle the two sister chromatids represented within that
758 shape. When drawing a centromere using a circle, instructors can mention that even though they
759 made a separate drawing stroke to create the centromere, it is still part of the same single DNA
760 molecule in that condensed chromosome structure.

761 While interpreting pre-fabricated visual representations is an important part of biology
762 education, we also encourage both instructors and students to be engaged in creating visual
763 representations during class time. Historically, students are rarely asked to sketch in their science
764 classes (Ainsworth et al., 2011; Van Meter & Garner, 2005), but there is tremendous educational
765 value in drawing (Quillin & Thomas, 2015). Drawing is an important process for biological
766 literacy that is associated with understanding representations, developing scientific reasoning,
767 and effectively communicating science (Ainsworth et al., 2011). While some instructors and
768 students may believe drawing requires a strong artistic talent, we emphasize that sketches do not
769 need to be of the highest artistic quality to be meaningful representations in biology (Quillin &
770 Thomas, 2015). In fact, quickly-constructed "bad" drawings that require interpretation can
771 increase student interaction with the representation and help to reinforce that sketches are useful
772 and acceptable in biology (Higley et al., 2024). These quick sketches that use abstract
773 representations can be powerful learning tools because they distill complexity to show only what
774 is most essential to understand a concept.

775 In line with principles of Backward Design (Wiggins & McTighe, 2005), instructors who
776 teach visual literacy should also assess visual literacy. Instructors can assess visual literacy with
777 formative assessments, such as evaluating the drawings and explanations that students make on

whiteboards during class time, but instructors should also assess visual literacy on summative assessments (e.g., exams) as well. Because content on summative assessments signals to students the knowledge and skills that instructors value and prioritize (Jensen et al., 2014; Scouller, 1998; Uminski et al., 2024), if visual literacy skills are something an instructor wants students to develop, visual literacy skills need to be incorporated into summative assessments. When asking students to draw on an exam is not feasible, assessments may effectively measure student understanding of abstract models if they integrate pre-fabricated models into multiple choice options rather than relying on options that are only based in text. We suggest instructors consult the Drawing-to-Learn framework (Quillin & Thomas, 2015) for a robust set of recommendations about facilitating assessment of visual literacy skills.

Our work underscores the importance of valid and reliable visual literacy assessments. We found that the majority of participants in our study selected the correct answer to the GCA item for the wrong reasons. By asking them to create a visual representation of their answer to the GCA item, we uncovered that the majority of participants had incomplete or incorrect mental models of chromosomes or meiosis and were getting the item right even though they had an incomplete understanding of the content the item was designed to assess. Had we relied on the multiple-choice answer alone, we would have vastly overestimated our participants' true conceptual understanding of chromosomes and meiosis. Our finding here emphasizes both the limitations of traditional multiple-choice assessments in which students can select the right answer for the wrong reasons (Cerchiara et al., 2019; Fisher et al., 2011; Fulmer et al., 2015; Haudek et al., 2012) and the importance of assessing students' visual literacy skills as a robust method of gauging conceptual understanding (Arneson & Offerdahl, 2023; Offerdahl et al., 2017; Schönborn & Anderson, 2006). We emphasize the need for biology education researchers

801 to develop valid and reliable assessment instruments that specifically measure visual literacy
802 skills in biology.
803
804 ***The DNA Landscape provides a framework for visual literacy in molecular biology***
805 We highlight the DNA Landscape (Wright et al., 2022) as a useful framework for
806 instructors looking to teach visual literacy skills and for researchers investigating visual literacy
807 in the context of molecular biology. For example, we found evidence of student
808 misunderstanding of chromosomes when their drawings mixed abstract representational
809 conventions. We observed that participants who drew representations of chromosomes that
810 mixed the abstract X-shapes with the more realistic string representations (Figures 4 and 5)
811 tended to have incorrect ideas about chromosome structure and function, often confusing how
812 more experienced biologists traditionally use the string representation. Identifying where
813 students are mixing representations within columns of the DNA Landscape can provide insights
814 into where students may be facing conceptual challenges in molecular biology.
815 We suggest that instructors use the DNA Landscape to inform their choices related to
816 *what* and *how* they are visually representing molecular biology concepts. For example,
817 instructors teaching about chromosomes should attend to asking students to interpret multiple
818 representations within the same column, making sure to clarify that chromosomes can be
819 represented using abstract lines, as maps or ideograms, or more realistically as strings. In
820 addition to providing opportunities for students to reason with multiple representations within a
821 column of the DNA Landscape, we encourage instructors to incorporate small group discussion
822 and ask students discuss with each other why biologists might use one representation over
823 another. We also suggest instructors take a moment of class time to ask students to reason across

824 the columns of the DNA Landscape to help illuminate the relationships between nucleotides,
825 genes, and chromosomes. Instructors can ask students to consider why it is inappropriate to use a
826 Punnett square to answer a question about meiosis or to think about the relationship between the
827 double helix and chromosome structures. Instructors might ask students if the helix "fills" the
828 chromosome, as some of our participants here have suggested. By giving students opportunities
829 to grapple with multiple representations across scale and abstraction in the DNA Landscape,
830 instructors can help their students develop the fluency in visual literacy that is necessary for deep
831 understanding of the foundational concepts in molecular biology.

832 Biology education researchers can use the DNA Landscape as a framework for designing
833 research studies and assessment tasks. Researchers may design future work to study how biology
834 students are thinking about the similarities and differences between representations within
835 columns of the DNA Landscape. There are also opportunities for future work to study how
836 students are conceptualizing scalar relationships across the columns of the DNA Landscape.
837 When developing research studies or assessment tasks related to visual literacy in molecular
838 biology, we suggest researchers consider incorporating representations across the DNA
839 Landscape.

840

841 ***Future directions***

842 We found that misunderstandings of chromosome structure and function are "sticky,"
843 often persisting in students who have already entered advanced undergraduate coursework in
844 their third and fourth year of college. Our findings emphasize areas for future research to
845 investigate where students may have derived some of their incorrect ideas and misconceptions
846 about chromosomes. We anticipate that some of these misunderstandings we observed may be

related to the colloquial ways that students see chromosomes discussed and represented in K-12 life science curricular materials (Cho et al., 1985). We highlight the use of the word "half" to describe chromatids as one example of this imprecise language that we suspect created confusion in students' understanding of chromosomes. Multiple students referred to sister chromatids (or, in a few cases, a pair of unreplicated homologous chromosomes) as being two "halves" of a replicated chromosome. While our participants frequently used the word "half," and the term "half" appears in open education resources about genetics geared towards a K-12 audience (National Human Genome Research Institute, 2024), this term was never used to describe chromatids in undergraduate biology textbooks (Brooker, 2011; Freeman et al., 2017; Lodish, 2008; Morris et al., 2022; Solomon et al., 2015; Urry et al., 2016). Learning theory suggests that schemas are established based on initial learning and these schemas can be difficult to change (Piaget, 1952); thus the incomplete ideas about chromosomes that are assimilated during middle and high school can persist even when more correct information is presented in undergraduate courses. As the first learning about chromosomes can shape what is understood (or misunderstood) in future learning, we recommend further investigation into the representations of chromosomes in K-12 contexts.

We also recommend future research efforts to uncover additional insights into the types of representations, learning activities, and assessments that are most beneficial to the development of visual literacy skills across a variety of molecular biology contexts. Our work leaves questions about how different types of models in molecular biology may help or hinder student understanding of particular concepts. While it was not a focus of our research, we found that that many students mentioned seeing representations of chromosomes in their classes and textbooks, but no students mentioned seeing such models on exams or on other class

assignments. This leads to questions as to how students are engaging with the abstract representations in these in-class, out-of-class, and assessment contexts. There are remaining questions about how is learning in molecular biology is affected by the ways students engage with models during class time, impacted by the frequency with which models are used in a course, and influenced by how models are integrated into summative assessments.

Limitations

We necessarily focus our results on the errors in student conceptualizations of chromosomes, as these errors provide novel insights into student thinking that may be useful for informing instruction and further education research on conceptual understanding in molecular biology.

We recruited participants from two institutions. It is possible that biology instructors at other institutions may emphasize different aspects when teaching about chromosomes, and as such, we do not make claims that the misunderstandings we observed in our sample population are representative of what misunderstandings might be present in other student populations.

We informed the participants in our study prior to the interview that they would be asked to draw during the interview protocol. As a student's attitude, self-efficacy, and interest in drawing biological models can affect their use and engagement with models (Quillin & Thomas, 2015), we likely only recruited students who already had a positive attitude, high self-efficacy, and high interest in drawing biological models. Given this self-selection bias in our recruitment and sampling, we highlight the need of additional visual literacy research conducted in ways that can capture a wider range of student affect towards drawing.

CONCLUSION

Understanding chromosomes is necessary for understanding more complex concepts in genetics. Even though the notion of chromosomes begins as early as middle school, it remains a difficult concept for many undergraduate biology students, which may be tied to their incomplete or incorrect mental models of chromosome structure and function. These difficulties may not be evident in students' verbal descriptions of chromosomes and are only revealed when probing students' visual literacy skills. Asking students to create drawings and interpret visual representations provides key insights into students' conceptualizations of chromosomes. We encourage biology instructors to incorporate opportunities to develop students' visual literacy skills in molecular biology by using multiple representations of chromosomes at different levels of abstraction, engaging students in sketching representations during class, and asking students to evaluate conventions used in visual representations.

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Figure Legends:

Figure 1. Application of the Conceptual-Reasoning-Mode framework to explain how a student might interpret an abstract representation of chromosomes. The representation of chromosomes in the top left is reproduced from item #2 in the Genetics Concept Assessment (Smith et al., 2008).

Figure 2. Percent of participants defining chromosomes based on structure, function, or shape. Overlapping regions indicate definitions that met two criteria, with the central overlap indicating a definition met criteria for chromosome structure, function, and shape. This Venn diagram uses blue color to indicate that definitions are located in the Conceptual factor of the C-R-M framework as shown in Figure 1.

Figure 3. Categorization of participants' initial sketches of chromosomes. Sketches of chromosomes are categorized by shape (X-shaped, I-shaped, side-by-side) and symbolic style (loops, lines, coils). Examples of each representation type are reproduced from participant sketches when available. From left to right, example sketches were from interviews with the participants Evelyn, Catherine, Mia, Donna, Christin, Jack and Ristan. There were no sketches for I-shaped lines or side-by-side coils. Some participants produced several different sketches in response to the interview prompt, so the sum of percentages exceeds 100%.

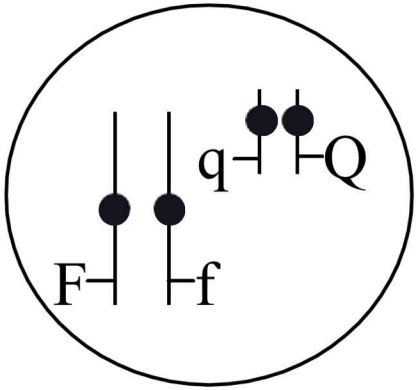
Figure 4. Initial sketches of X-shaped chromosomes and the associated verbal description of the sketch. Nadine, Annie, and Andi drew chromosomes with X-shaped loops. Andi and Pluto drew chromosomes with X-shaped lines. Jack and Heather drew chromosomes with X-shaped coils.

Figure 5. Participant sketches of coiled chromosomes generated during follow-up questioning. Participants' initial sketches are in the first column. The second column shows the follow-up sketches. Note that in Teresa's follow-up sketch, she modified her original drawing.

Figure 6. Initial sketches of side-by-side chromosomes and the associated verbal description of the sketch. Alex and Mia drew chromosomes with side-by-side loops, and Christin drew side-by-side lines.

Figure 7. Participant sketches of Punnett squares and associated reasoning to explain their answer to the GCA item. Participants Peggy, Leland, and Piper selected the correct answer to the GCA item. Participant G selected the incorrect answer to the GCA item.

Figure 8. Participant sketches of their answer to the GCA item and associated reasoning to explain their answer. Participants Heather, Ristan, Annie, and Christin selected the correct answer to the GCA item**.**



Conceptual knowledge to interpret meaning
(e.g., interpreting that there are two sets of unreplicated chromosomes)

Cognitive processes used to interpret and visualize
(e.g., viewing and interpreting that there are four separate structures represented)

Conceptual knowledge relevant to the representation
(e.g., knowing that homologous chromosomes can contain different alleles of the same gene)

CONCEPTUAL
(C)

R-C

REASONING
(R)

C-R-M

C-M

R-M

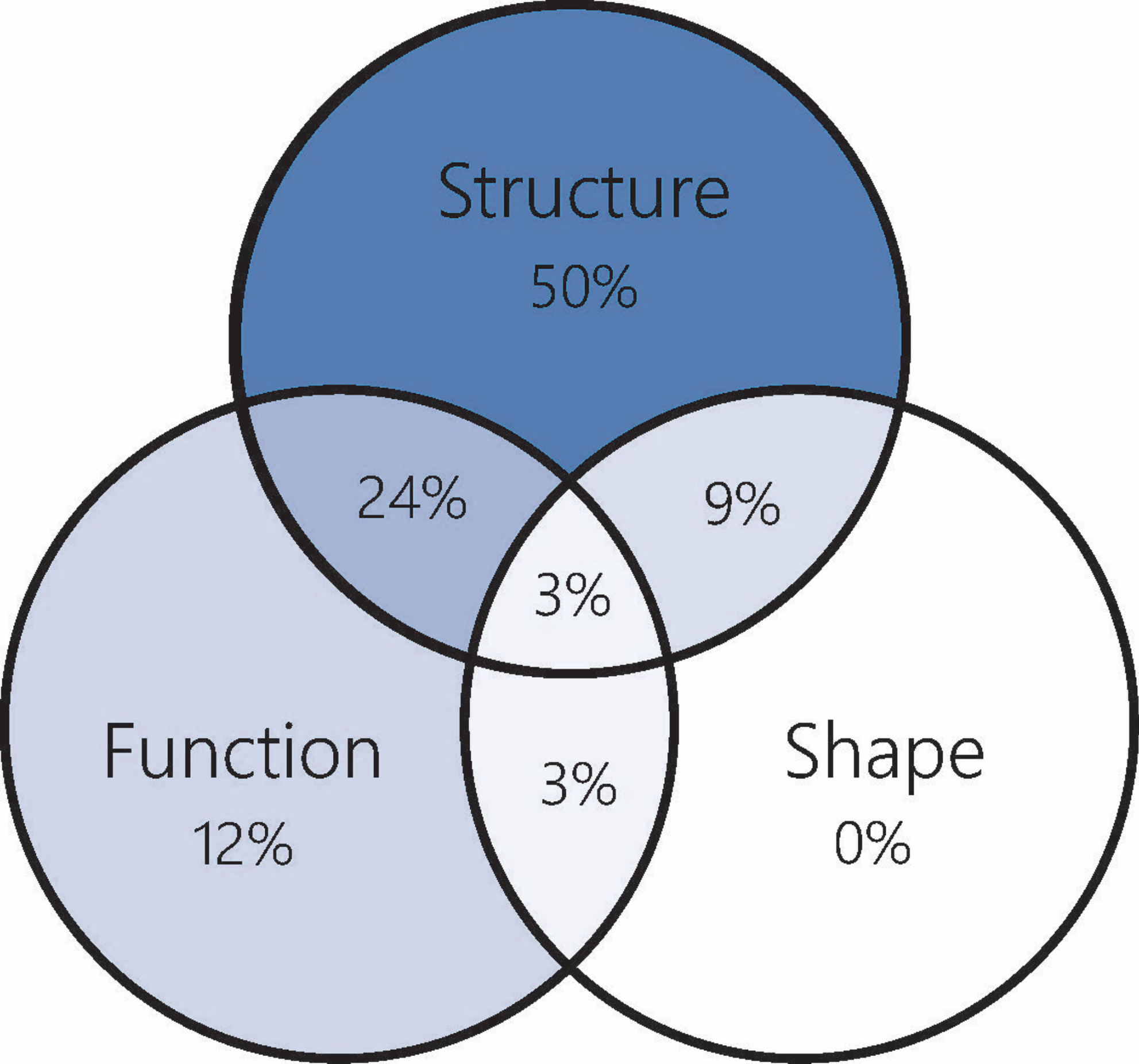
MODE
(M)

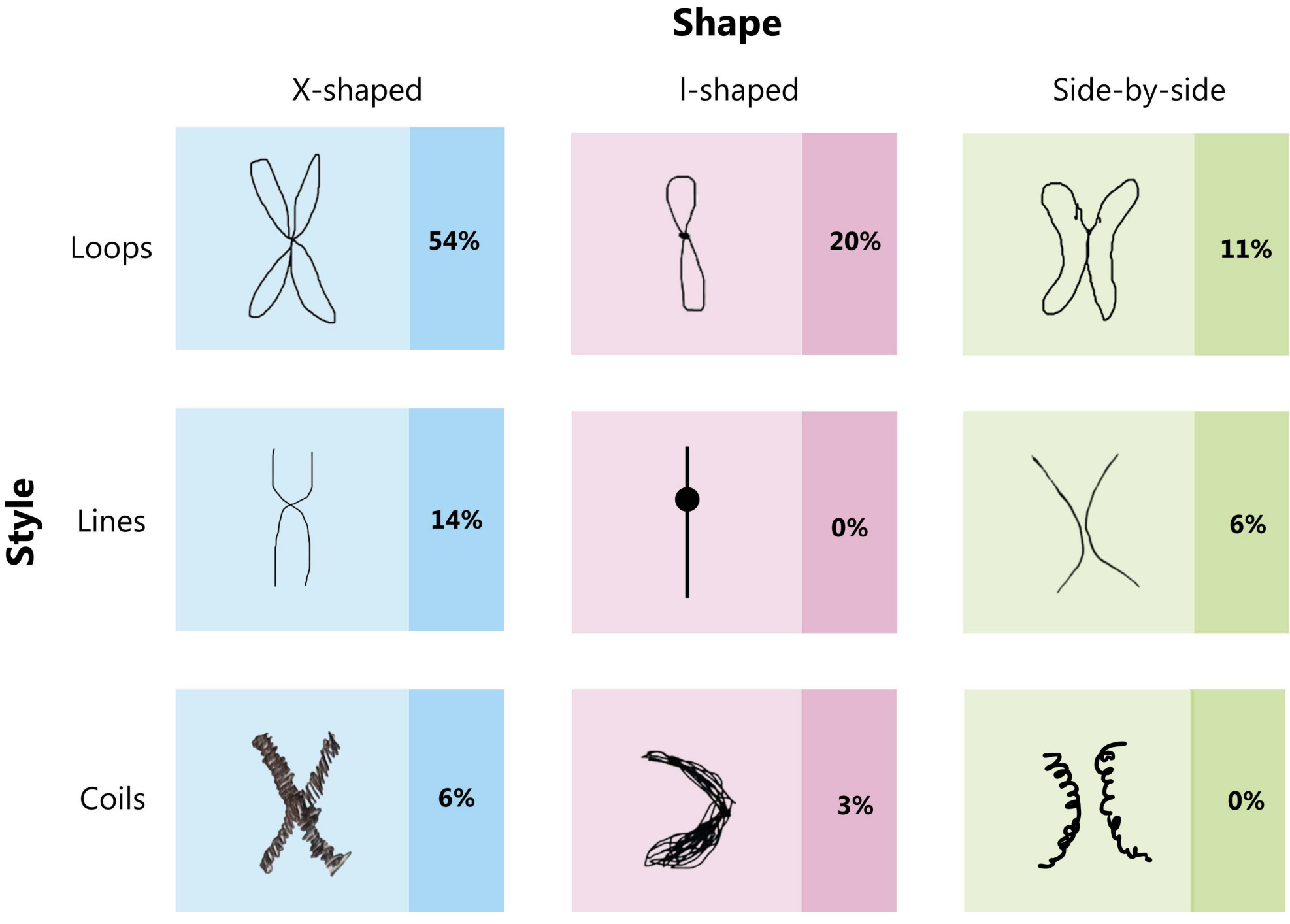
Ability to decode symbolism
(e.g., decoding that the capital and lowercase letters confer different meaning)

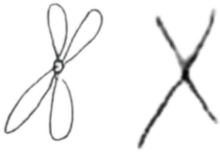
Scientific knowledge represented through symbolism
(e.g., reading the black dots and interpreting them as centromeres)

Symbolic language
(e.g., recognizing that lines, letters, dashes, dots, and letters encode meaning)

Engage all three factors to successfully interpret the representation
(e.g., determining that the representation shows that the cell contains two sets of unreplicated homologous chromosomes and that the individual is heterozygous for genes F and Q)

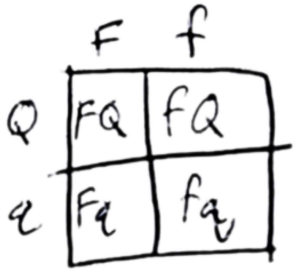
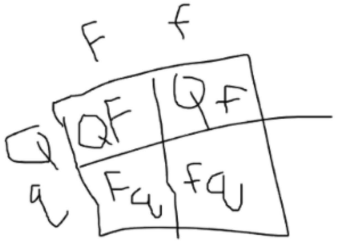
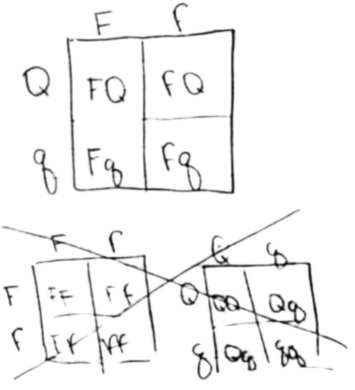
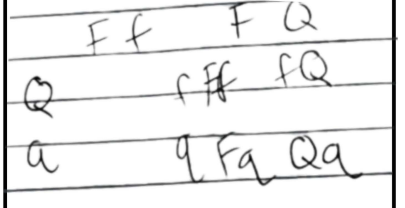

Structure
50%
24%
9%
3%
Function
12%
3%
Shape
0%


Shape
X-shaped
I-shaped
Side-by-side
Style
Loops
54%
20%
11%
Lines
14%
0%
6%
Coils
6%
3%
0%

Sketch of X-shaped chromosome	Participant's description of sketch
	They just are like two halves that kind of fit together, and I just, that's how I see them in all my biology textbooks, they kind of have two halves together. - Nadine
	Because I know that it has like four parts. Well, your, your code, one section of the code will be on this end and one section will still be on that side. It's double. - Annie
	Why did I draw it [the chromosome on the left] that way? Um, it's simple...it's the simplest way. I mean, okay, really the simplest way to draw a chromosome's like the old school way, but it's when chromosomes are condensed. That's kind of what they look like, if we were to, like, image them [referencing the chromosome on the right]. - Andi
	I mostly drew it like that because like, when you see it in class you see all like the different ones, and the most prominent one is usually one that looks like that 'cause that's like the standard one everyone knows. - Pluto
	I thought of filling it in with like, kind of just zigzag lines to represent that it's like not just the solid shape—it's composed of like strings and coils. - Jack
	I drew it kind of like with like the squiggles instead of just straight lines to represent the condensed chromatin. - Heather

Initial sketch of chromosome	Follow-up sketches of chromosomes	Participant's description of sketches
		It's usually single when the process starts, and then it becomes an X-shape throughout the process of meiosis... I guess that's just how we've learned it, and DNA is just coiled up, so it's like this [follow-up sketch]. - Norma
		One's just how they are normally [initial sketch on left]. They kind of just exist and the other one's for when it's being replicated into daughter cells [initial sketch on right]...This is like the clusters of the DNA, big clusters [follow-up sketch on top]. Yeah, and this is also another cluster of like DNA [follow-up sketch on bottom]. - Teresa

Sketch of side-by-side chromosome	Participant's description of sketch
	I drew it with the sister chromatid with the centromere binding the two pieces together. - Alex
	I just drew it like that, because like, that's how I like I saw, like my professors drawing chromosomes and stuff. And like, I think they are explained like, you know, and like in females and males, there's like an X and a Y and or X and an X, you know, and so, something like that, but it could like probably has to do with, I don't know, like, maybe pairs or something. - Mia
	I know they have two I think they're called chromatids and they're connected in the middle, I think it was called a centromere or something like that. - Christin

Sketch of GCA answer	Participant's description of sketch
	And if we're talking about how I got there, I imagined a Punnett Square, because [the item] talks about if [the cell is] undergoing normal cell division, then in theory, there should be equal chance of getting all four types of phenotypes with, there should be two options with a capital F and two options with an uppercase Q and then two with a lowercase of each. And then they should just be matched so that there's no one of the same pair. - Peggy
	I guess the most common way to do [this] is just the Punnett Square. All different. And since it's asking for genotype and not phenotype, this would be all of it. - Leland
	If you're saying that these are just dividing normally, I was going to do a Punnett Square to find all of my variations of the genotypes...So I decided to do them separately, but then I was like, you don't have both. And since it's just to produce sperm, then I was like, 'Oh, well, I can't do that'. And I knew it was monohybrid. So I didn't attempt to do a dihybrid, even though I would never do that by hand. - Piper
	I kind of drew like a [bad] Punnett Square. I put the capital F, capital Q on one, lowercase on the other side, and I got capital F—heterozygous F, heterozygous Q, and then lowercase f capital Q, capital F lowercase q. So I think that's the closest to what I got. - G

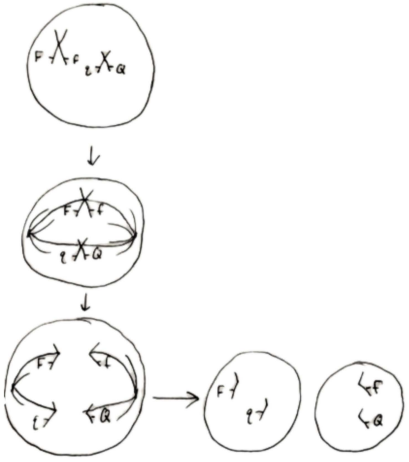
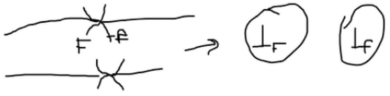
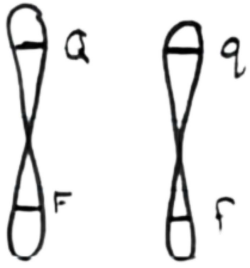
Sketch of GCA answer	Participant's description of sketch
	Yeah, so this is kind of just one example of how it could divide. So I started kind of with how it's represented [in the item]. I kind of mixed the way it's represented on the screen and kind of my representation of more that X. And then the, the, the mitotic spindles-- Well, this, the chromosomes lineup in the center of the cell independently, and then it's the mitotic spindles that come in and pull the pull the sister chromatids apart. And then that splits into two separate cells. - Heather
	There are a host of proteins that will actually pull the combined chromosomes apart, right? So you'll initially look sort of like this. And so if it were to continue, that would be f. And this would be the capital F, which looks very similar, but I promise they're different. That's a little better. So those will be pulled apart. And then in the new daughter cell, it'll only contain the one chromosome with the F. - Ristan
	So it says it's dividing normally to produce the sperm so it would be, I would think it's one allele of one of the half of the chromosome with the other one, or with the other... Okay, so if—when these replicate, this, so this Q [top left] could go with this f [bottom right] and this F [bottom left] can go with that q [top right], but at the same time, yeah, this F [bottom left] could also go with this Q [top left] in this, this f [bottom right] could also go this q [top right], if that makes sense. - Annie
	My first thought would be, I guess that like they cross over. So I guess combining the letters. - Christin

Table 1. Research participant demographics			
	PUI	**R2**	**%**
Gender			
Man	3	3	17
Woman	8	18	74
Non-binary or gender queer	1	2	9
Year in college			
Sophomore	4	9	37
Junior	5	6	31
Senior	3	5	23
Master's or Continuing Education	0	3	9

Table 2. Interview protocol and alignment of interview prompts to visual literacy frameworks

Interview Prompt	**C-R-M[a]**	**Bloom's Level(s)[b]**
"What is a chromosome?"	C	Knowledge
"Can you sketch a chromosome?"	C-M	Knowledge
"Why did you draw the chromosome this way?"	C-R-M	Knowledge/ Comprehension
Participant is presented with the image of chromosomes from Genetics Concept Assessment item #2. "Please explain what you think about this representation of chromosomes."	C-R-M	Comprehension
"What about this image makes this an effective representation of chromosomes?"	C-R-M	Evaluation
"Is there anything surprising or confusing about this representation of chromosomes?"	C-R-M	Evaluation
"How does your sketch of a chromosome compare to this representation? What is similar and what is different between your sketch and this image?"	C-R-M	Analysis
Participant is presented with the text from the GCA item #2. "Please talk through how you answered this question."	C-R-M	Comprehension/ Application
"Can you sketch a representation of the answer you selected?"	C-R-M	Application

Notes

[a]Abbreviations: C = Conceptual; R = Reasoning; M = Mode (Schönborn & Anderson, 2009)

[b]Bloom's levels classified based on the Visualization Blooming Tool (Arneson & Offerdahl, 2018)

Table 3. Participant interpretation and responses to an item from the Genetics Concept Assessment			
	Incorrect interpretation of image	Correct interpretation of image	Total
Correct response to item with correct reasoning	3	5	8
Correct response to item with incorrect reasoning	16	2	18
Incorrect response to item	6	3	9
Total	25	10	35