FEATURE ARTICLE

How to Build a Super Predator:
From Genotype to Phenotype

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ABSTRACT
We present a drawing discovery lab that crosscuts multiple disciplines in biology and links concepts in genetics and evolutionary thinking to enhance understanding of the genotype-to-phenotype transformation. These combined concepts are also linked to ecological frameworks in nature through the model of biological plasticity. Students and teachers explore drawing skills to flesh out the future of a predator while engaging with the computational software MEGA, which introduces students and teachers to nucleotide changes, mutations, variation, phylogenetics, and molecular evolution.

Key Words: genotype; phenotype; MEGA; phylogeny; drawing discovery; plasticity; predators; ecology.

Introduction
The concepts of genotypes (heritable, nonobservable sets of genes) and phenotypes (expression of genes) comprise the terminology of population genetics. Students are often guided to understand Mendelian genetics by recognizing that the genotype of an organism becomes, in some way, the phenotype or appearance of that organism. The importance of understanding these two terms extends into overarching evolution-based models, which dictate that genotypes in their expressed phenotypic states become compatible or adapt to an ecological condition in time through the process of natural selection. The genomically active state of a cell carries its molecular script into actualized physiology through networks of genes described as “upregulated” or “expressed.” The complexity of these networks is not well understood and is the result of many interfaces, environmental prompts, and influences, both local and distant. An arts-based teaching approach to this complexity offers students a glimpse into how biological systems interact with each other, giving teachers a way to deliver crosscutting concepts that reflects actual biological systems. After modeling these complex processes by computational methods, students can benefit from the solid visual foundation of art, which students can use as a synthesis for complex biological ideas, turning the crosscutting experience of biological disciplines into a tangible and scientifically creative process.

By the end of eighth grade, students are expected to have a basic understanding of fundamental genetic inheritance (MS-LS3 Heredity: Inheritance and Variation of Traits). This expectation opens opportunities to expand the concept of inheritance into the context of evolution and ecology as well as systems thinking. The important crosscutting concepts of evolution and ecology can be introduced through engaging, discovery-based drawing activities before students reach high school. Middle school L.S.4 states that “students should understand change of life forms through history,” with “emphasis on finding patterns of changes in the level of complexity of anatomical structures in organisms.” The changing forms and patterns in nature are conceptually challenging for anyone and pose difficulties for both teachers and students who have little experience of combining abstract models into new a paradigm of biological systems thinking. To address the growing complexities and these patterns in biology, we have developed an exercise that combines content in genetics, natural selection, morphology, molecular evolution, computational software, and drawing discovery experiences (Figure 1).

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We present a variation of the drawing discovery process in the form of a drawing heuristic that enables discovery through activity. This process is a variation of one often used by paleontologists; it can assist students in exploring possible phenotypes of the future through deliberate focus on structure and the replication of that structure through classical drawing techniques. Drawing is a focus and skill-based activity that enhances observational and noticing practices (Ainsworth et al., 2011). The images in this work were created by a biology teacher and tested in a lab setting. This activity combines imagination with data to reconstruct a hypothetical organism of the future. We contrast the drawing discovery of...
computational software can analyze DNA and infer evolutionary relationships, offering students insight into molecular (DNA) biology, evolution, and computational biology. This combines phylogenetic trees with physical traits, thus weaving the disciplines of evolution and genetics. By hypothetically evolving those traits to ecological scenarios, one can create yet another association with the discipline of ecology or co-evolutionary concepts. By using the drawing discovery skill, which is a connected component of this exploration, we can realize combined influences and solidify the associations between the word, the concept, the data, and the system as we conclude the activity. Students need to see the connection between changing environments and their outcomes on populations. We ask students to look at phylogenetic trees, to consider relatedness and diversity in a group of mammals, and to draw on the diversity of that group to evolve their own mammal in a changing environment.

We tested this exercise in our computational lab, which was made up of undergraduates and graduates. We focused on the interpretation of data from genotype to phenotype realization through the drawing discovery process. Many of our lab members found the drawing experience challenging but immensely beneficial in conceptualizing the genotype–phenotype problem, offering us insight into this work.

The tools for this project include MEGA software (Molecular Evolutionary Genetics Analysis), paper, and colored pencils. We describe the activities in detail below; for an outline of the steps, see the Appendix.

○ Exploring the Evolutionary Tree of Felines as a Big Picture: MEGA Software

Some biology teachers may not have had experience with MEGA or other computational software available to explore DNA sequences. By introducing the concept of molecular changes in DNA sequences, teachers can naturally crosscut the concept of evolution, mutation, and relatedness in the tree of life. MEGA software can act as a model or teaching tool for enhancing a student’s understanding of molecular evolution. Students and faculty can have easy access to an actual piece of software used by scientists to create evolutionary trees and track evolution (Kumar et al., 2018). This free software performs a comparative analysis of homologous gene sequences, typically from different species, to build phylogenetic trees (MEGA can be downloaded from https://www.megasoftware.net). MEGA relies on four basic steps to create a phylogenetic tree. Discussing the acronym can even serve as an introduction to the activity.

Phylogenetic tree building from DNA is a unique approach for understanding relatedness in the tree of life. This process contrasts with the past method of studying and illustrating morphological characters (the physical traits of an organism) to infer relatedness. DNA phylogenetic trees set the stage for both a molecular or miniature point of view (DNA/nucleotide) and a broad perspective on a group of related organisms. Phylogenetic trees allow students to see the diversity within that group of species and within their DNA. They also offer an opportunity to see changes happening on the small scale (DNA) and to consider those changes at a scaled-up level to reflect the whole organism, represented by the term phenotype. The phenotype is coded by DNA, but DNA does not make phenotypes by itself. DNA must first function within the cell, and there must be multiple interactions and feedback systems between the DNA, the environment, and the organism over time. That phenotype must also be variable and flexible. Phylogenies can show the phenotypes with software that analyzes DNA sequences, which are involved in visualizing phylogenetic trees that represent the species phenotype we see. The drawing discovery requires a skill set that is not taught to scientists or science educators but one that is often the underpinning of visual-spatial skills and STEAM activities. As one of its aims, this article hopes to present the drawing discovery process as critical to conceptualizing biological knowledge and as a tool in the modern biology classroom that educators may want to consider developing themselves. We suggest that students and teachers use this exercise to explore their own personal artistic-discovery skill set while exploring the genotype–phenotype problem computationally. The intended audience for this lab exercise is broad, depending on what pieces teachers decide to use. This activity is suitable for middle school (omitting use of software but discussing computational methods), high school (watching tutorials on software but not necessarily using it), and college students (storyboarding concepts and using software). This lab can be altered to accommodate particular time frames and backgrounds, offering insight into a wide range of overlapping biologically related topics.

○ A Little Background on a Complex Problem

Crosscutting disciplines are another way of offering the student an interdisciplinary window into how scientists must now think when considering molecular biology together with globally complex biological issues. To effectively combine several disciplines, students should experience these disciplines as a practice of applied knowledge.
Teaching Plasticity in Living Systems through Drawing

When students are looking at a set of sequences, they are also looking at variation, which leads to an appreciation of plasticity. Plasticity in phenotypes relies on the ability of a genotype to change over time and settle into a niche that can express those changes into the phenotype and the species we observe in the present. In this exercise, students can consider the underlying genotypic variation with stabilizing or disruptive ecologies and consider what the word plasticity means. Plasticity in phenotypes relies on the ability of a genotype to produce distinct phenotypes when exposed to different environments throughout life (Staron & Johnson, 1993), which supplies another dimension of variability. For the drawing, students must linger, deliberate, and challenge themselves to recreate and modify the phenotypes they are rendering. In this way, the tangible (what we observe) and intangible (processes of change and complexity of the system) become internalized. This will challenge both teacher and student to imagine intermediate forms and the pliability of morphing systems. Observing living relatives of the cat family, as a familiar example, through sketching helps strengthen the appreciation of adaptations evolved for effective predation, and these give rise to students imagining future phenotypic possibilities of their predator based on ecologically variable conditions. For this, we have students compare the variety of species and consider the skeletal anatomy of saber-toothed cats as distant ancestors. Looking at a range of species of felines helps students identify the features that make that species adaptable to its environment. We have also provided a page of predator skulls (Figure 2) to consider the various types of jaws and skulls that make up large terrestrial predators, not just felines. Students can either draw the skulls themselves or trace them into their notebooks. Extinct predators such as bears and marsupial lions were amalgamations of diverse predator characteristics.

Teachers can consider bridging the MEGA experience into the construction of a hypothetical phenotypical predator with questions

● Steps in Using MEGA for Phylogenetic Trees

Students need not use MEGA software with an intended outcome or assessment. Exposure to the program in this activity is meant for students and teachers to gain an appreciation of its functions. MEGA can be used to teach about the process of building a phylogenetic tree. First, teachers can briefly introduce “what molecular evolution is” by discussing the acronym MEGA and showing a grid of DNA sequences from multiple species in a tutorial – along with an introductory discussion of similarity in the gene sequences of several species within that grid, a single PowerPoint slide could be shown noting different DNA bases (A, T, C, and G). Second, students can “play” with the software, after watching a tutorial, and problem-solve together as a group to learn the steps of searching from homologous sequences; they need not be expected to create a phylogenetic tree. Third, it is highly recommended that students storyboard the steps in using MEGA and define terms like phylogeny and homologous sequences. A link to storyboarding has been provided at the end of this article. These experiences serve to prepare students and make the experience of using MEGA teachable and flexible.

Writing out an experiment is not unlike keeping a scientific notebook. Students can be asked to chronicle their struggles as well as their successes with the program. In general, the first step in creating a tree is to identify and acquire a set of homologous DNA or protein sequences, which can be performed using MEGA. This involves a pairwise comparison through a search for a sequence of a particular protein in BLAST (Basic Local Alignment Search Tool), software that compares sequence information for amino acid sequences, DNA, and RNA. BLAST generates the “best hit,” or the closest matches to the query. The student needs to select the best matches.

In step 2, an alignment of these sequences from many species is used to build a phylogenetic tree. Finally, MEGA creates the tree using different methods or algorithms like maximum likelihood and then graphically renders the tree to visualize it for presentation. All these steps are teachable moments and can be emphasized through the storyboarding process, which provides a sequential visualization for the dynamics of any process or procedure. For more information, materials, and links, please refer to the Appendix.

Figure 2. A variety of predator skulls to trace or draw from, to give students a view into variation in skull shape among terrestrial predators extinct and living. Enlarge this image and provide tracing paper for students in lab.
like “How does an organism develop and interact with its environment and with other life-forms?” Students can list all the ways they know that an organism interacts with its environment, creating a web of interactions. Teachers could ask, “Now that you have seen DNA sequence data from genes, can you imagine webs and networks of genes guiding the production of a protein?” While this may seem like an abstract question, teachers might discuss environmental temperature, hibernation, or seasonal gene expression and simply encourage students to visualize a scenario with multiple processes. This contrasts with the linear pathway teachers use to explain the flow of genetic material – that is,DNA to RNA to protein. The teacher could ask, “Can you see the connection between the small, molecular evolutionary process and the larger process of natural selection over time?” Just as a reminder that these systems are connected and changing.

○ With Multiple Concepts, Where to Start? An Introduction to Apex Predators

Regardless of how many topics a teacher weaves together, there must be a beginning. For multiple topics, the use of narrative is essential. Much of the material about felines, speciation, natural selection, and molecular evidence can be introduced in lecture through the narrative of the natural history of the cat family, leading up to the computational and drawing exercise in this lab. In the lecture, simply discussing predators can captivate student attention. Rather than starting with evolution, genetics, MEGA, or ecology, the evolutionary story of the apex predator, the apex predator as a keystone animal, and the decline of predators all entice students into concepts like evolution and ecology with little effort. This also creates a time line that unfolds independently of our own species but then shines a spotlight on our interactions with these creatures and on human-induced global changes to ecosystem dynamics.

Beautiful and powerful animals also generate interest and allow students to connect emotionally to a subject. For this exercise we called apex predators “super predators,” to emphasize specialized adaptations. In lab or lecture, a teacher may discuss physical appearance, coat patterns, predatory adaptations, or even the human fear of predators, the collapse of ecosystems, and human-induced extinction of apex predators. Geological time frames put phylogenetic trees in perspective. By taking students back to two very charismatic apex predators of the Pleistocene, *Canis dirus* (dire wolf) and *Smilodon fatalis* (saber-toothed cat), a teacher can add to the evolutionary drama of the activity. The megafauna ecosystems of the Pleistocene also provide a window into the dramatic changes in climate, gene flow, and migrations that sway and guide the processes of natural selection. Discussing the climate of the Pleistocene provides ecological/environmental background and a time line for understanding the diminishing ecological niches of today and the fictitious ones in this lab.

For this lab, we focused on one animal of the Pleistocene, S. *fatalis*. In either lecture or lab, the discussion of predators and their general features (phenotypes) would include feline behavior and anatomy. To stimulate and start the narrative of a “super predator,” we suggest the National Geographic series *Strange Days on Planet Earth: Predators* (https://www.pbs.org/strangedays/episodes/predators/experts/).

○ Connecting Concepts: Proteins, Predator Strategies & Functional Integration

Students will observe distinctly different-looking cats in different clades of felines, and now we can guide them to another level of knowledge. Before students construct their super predator, teachers discuss how scientists might explore the molecular evolutionary component of apex predator behavior by considering the bite force of feline predators. The concept of bite force focuses students on thinking about mode of nutrition and the molecules that evolved to inform that adaptation. Saber-toothed cats have been found, through computer modeling, to have a weaker bite force than their modern cat counterpart (Wroe et al., 2005). Ask students, “Why might this be?” and “What strategies or phenotype does the saber-toothed cat have that might be different from the modern cat?” This leads us to a brief discussion on the protein myosin, which students should be encouraged to draw (see Figure 3). Drawing this protein solidifies it as an actual form and not just a term or abbreviation. Students may have encountered myosin in their study of the cell’s cytoskeleton or in the study of skeletal muscle contraction. The presence of myosin can be traced in evolutionary time; it is considered a highly conserved protein. There are also many alternative forms of myosin (isoforms) that impart variation in contraction of muscle. This topic can also be introduced in lecture, as it offers a window into how a protein isoform is also a result of selective pressures. We use a cartoon chart of myosin, three feline species, and myosin protein sequences to connect these concepts (see Figure 4).

**Figure 3.** This illustration was created intentionally to connect the idea of the Tpm gene for myosin to the jaw muscles of feline predators. Students can explore the conformational changes in isoforms of myosin and their sequence data to bridge the concepts of genotype and phenotype. We suggest that students use this page as a coloring activity.
The wide range of myosin’s forms may be the result of polymorphisms (Gros & Buckingham, 1987). Polymorphisms can lead into discussions on “types” and varieties within a group and, if time permits, on balancing selection, epigenetics, and phenotypic variants. To date it has been shown that mammals have up to 40 different myosin genes (Foth et al., 2006) and that these proteins can be expressed in different tissues and at different timed events in development, resulting in alterations in the phenotypic expression of myosin. These different forms have arisen over time by the process of gene duplication and mutation. This information offers students another layer of complexity while visualizing a dynamic network in the manifestations of genotype to phenotype. This is also a good place to distinguish between micromutations (simple changes in DNA) and macromutations (gene duplications). This “raw” material of evolution places mutations in a framework that links our super predator activity to using evolutionary software to compare DNA sequences for seeing and analyzing micromutations.

**Using the Sequence Data of Myosin to Explore Evolutionary Change in Genes**

If students are prepared to use MEGA in upper-level classes or even experimentally in general classes, they can start with a search in BLAST of the protein myosin. Using sequence data from the myosin protein family demonstrates that evolutionary history can be traced back to the earliest eukaryotic cells – but what does that have to do...
with apex predators? The behavioral adaptation of a precision bite force in modern cats is something our charismatic saber-toothed cat may not have had. The myosin isoform called “super-fast masticatory myosin” is found throughout Vertebrata and is an expressed phenotype that has undergone evolution to shape the apex predators of today. “Masticatory myosin is widely expressed among several vertebrate classes. Generally, the expression of masticatory myosin has been associated with high bite force for a carnivorous feeding style (including capturing/restraining live prey)” (Wroe et al., 2005). Teachers refer to the skull chart and to the concepts of mutation and molecular evolutionary change, reflected ultimately in anatomy and behavior. We can reconstruct phylogenetic relationships of feline species from the known myosin isoforms; this can assist students in an introduction to phylogenetic tree building through the gene. Teachers may also want to mention the powerful bite force of primates, including chimps and gorillas. Evidence suggests that this fast-masticating myosin was inactivated by frameshift mutations in hominids, a loss that correlates with a change in the size of hominid jaws (Oh et al., 2015). If teachers include the human lineage example in lecture, students can consider similar mutational events for saber-toothed cats in lab, and modern bite-force-based predatory behaviors, alluding to MEGA as a tool to resolve these questions. The multifaceted interactions of mutation, environment, and evolutionary pressures paint an interesting and dynamic impression of eco-evolutionary processes for students to experience, one that is perhaps more reflective of biological systems.

○ Further Discussion: The Tropomyosin Gene & Tracking Evolution

If teachers wish to explore the topic further in lecture or lab, they can consider looking at the gene for tropomyosin (Tpm). Our discussion on molecular evolution involves the Tpm 4 gene, a masticatory myosin. There is an interesting link between the extant species, the clouded leopard (Neofelis nebulosa), saber-toothed cats (S. fata-tlis), and large and small felines. The literature suggests that one living member, the clouded leopard, Neofelis nebulosa, has a skull considered to be characteristic of or like that of saber-toothed felids (Christiansen, 2006). While it would be nice to use the Tpm gene in MEGA, our key cat, N. nebulosa, has not been sequenced for this gene – and, since this species is considered the closest living relative to saber-toothed cats, the tree cannot be built using this gene (see Figure 5), because it would not include the clouded leopard. We had to deviate from the Tpm gene and use a mitochondrial gene (ATP8) to build a feline tree. Ideally, we would want to show the relationship of predators through the myosin gene, but the mitochondrial gene is sequenced for all cat species, which allows a general tree to be made. This is an excellent teaching moment, as it can lead into a discussion on the global biorepository GenBank, its preservation of genetic material, and the fact that not all genes have been sequenced – and that sequencing thus continues, keeping this a very fast-moving, frequently revised database.

○ Creating Your Super Predator

After the background material has been discussed in lecture, have students select about five or six species of the cat family to create their own hand-drawn phylogenetic tree. They can hypothetically “search” MEGA by creating a storyboard of the procedure. If teachers want to create a feline tree, they can rely on the ATPase gene or another conserved gene.

After introduction to MEGA (perhaps one lab period before the drawing), teachers can ask students to make a list of some of the felines’ adaptations. Some adaptations to consider include flexible spines, padded feet, and retractable claws for falling long distances, cushioning those falls; arboreal behavior; and an antenna system (whiskers) that covers the most important parts of their bodies (feet, face, and ears) to triangulate subtle and numerous incoming and outgoing mechanical and sensory signals. The ability to prepare for events by calculating movements before they have happened and by stalking behavior, which requires a great expenditure of energy (remaining tense but ready), can be observed in cats every day. Asking students to simply observe this in their own pet or others makes this lab activity more relatable and fun.

Cats can be solitary and highly social. They possess night vision and sophisticated olfactory senses. They are also obligate carnivores, unlike foxes or even dogs. Given this information, students can consider how these traits may have evolved in a larger macroevolutionary setting. We provide a selection of ecological scenarios (see Figure 6) that can be distributed to students in groups or individually, a “prototype” body of a predator for students to “morph” into their specific ecological scenario (see Figure 7), and a “spec” sheet of physical traits to copy or modify (see Figure 8).

Figure 5. 1 (A) A BLAST display of homologous sequences of felines (using the mitochondrial gene ATP8, or MT-ATP8). Notice that the clouded leopard is at the top and has a 100% similarity (because this is the organism you searched with), while its closest relatives have a 10–15% difference. (B) A phylogenetic tree created in MEGA from 13 homologous sequences of MT-ATP8 (this gene was used because the sequence data for the clouded leopard are not available).
These scenarios will shape the adaptations discussed in modern felines. Again, storyboard is a useful tool to help students transition to these physical and behavioral traits, but students can also just make sketches of what adaptation will be selected for in their hypothetical ecology. Having lots of images around of felines, feline species, and predators in general will stimulate students to think about the diversity of this group when they consider making their own predator.

**Conclusion**

Our apex predator or predation character is not limited to felines and can be applied to all levels of predation, aquatic ecosystems, and protagonists from the microscopic realm, as well as extinct characters. Paleontologists and fossil hunters have relied heavily on both imaginative and accurate portrayals of their discoveries by skilled artists. Similarly, modern software in genomics, metagenomics, and proteomics has offered students an opportunity to examine relationships and patterns through electronic data. Bridging the two art processes and data analysis – helps ground students’ experience in the synthesis of a comprehensive evolution lab with biology concepts that are usually addressed separately but that are necessary to understand the evolutionary process. Through the activities of storyboarding and breaking down the steps in both using computational software and drawing discovery, students can develop focus and skills that assist them in connecting concepts through evolution and to all biological systems.

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Appendix: General Sequence of Activities

Lecture:

- Introduce predators through a video (Strange Days on Planet Earth)
- Discuss the origins of predators back to the Pleistocene
- Show a cat family tree, pointing out specific adaptations and geographic locations
- Introduce the concepts of genotype and phenotype
- Discuss the concept of plasticity and why it exists in nature
- Discuss DNA, mutations, gene duplication, and natural selection
- Give the example of superfast masticatory myosin in feline jaws for a molecular perspective
- Discuss how scientists construct evolutionary trees with molecular data using felines as an example and the Tpm gene
- Show a tutorial from MEGA as an example: https://www.megasoftware.net/videos
Lab:

• Have students storyboard MEGA’s main steps, defining each term
• Depending on the grade level, allow students to “play” with the software without assessment and/or use the software and obtain student feedback about the experience (a paragraph or a survey)
• Print up prototype predator and ecological scenarios for student groups (no more than two or three)
• Have students discuss their scenario
• Have students sketch out and develop their predator’s features
• Trace/draw the skulls and other supplemental drawings
• Have students make a final drawing and list the variations or modifications of the prototype with an explanation of their adaptability
• Link to storyboarding basics: https://www.studiobinder.com/blog/what-is-a-storyboard/
• Link to National Geographic article on cat domestication and ancient DNA: https://www.nationalgeographic.com/news/2017/06/domesticated-cats-dna-genetics-pets-science/
• Link to an evolutionary history of felines from PBS: https://www.pbs.org/wnet/nature/blog/the-making-of-a-cat/ (includes phylogenetic tree)
• Link to National Geographic, Strange Days on Planet Earth: Predators https://www.pbs.org/strangedays/episodes/predators/

Appendix Figure 9. A flow chart for teachers of the activities for “How to Build a Super Predator.” While this is a simplified version of the events, the entire lesson plan/lab experience creates a crosscutting experience that can be arranged to accommodate class schedules and grade levels. Teachers can refer to this graph for ideas (clouds represent concepts).