

EVOLUTIONARY DEVELOPMENTAL BIOLOGY



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Evolutionary Developmental Biology

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This text was compiled on 08/19/2026

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1: INTRODUCTION TO EVOLUTIONARY DEVELOPMENTAL BIOLOGY (EVODEVO)

1.1: What is EvoDevo?

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1.E: Introduction to Evolutionary Developmental Biology (Exercises)

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1.1: What is EvoDevo?

Anomalies

Anomalies refer to anything outside the ordinary, for example conjoined twins or missing limbs. Even though we commonly think of developmental anomalies as inherently "bad", they are not fundamentally so. For example, the average height of an NBA player is 6'7". Although this is anomalous (only 0.045% or one in 2200 American men are this tall or taller), it is not considered at all detrimental to be this tall. If you are a Developmental Biologist, you relish natural variation as a window into developmental processes. For example, you might find out what an NBA player ate growing up, how tall his parents are, if he grew up in a rural area or the city, and compare these data to the data of an average American man. The differences between the two can help you generate hypotheses regarding the developmental processes leading to tall height.



Figure 1: The poster to the left shows an advertisement for a tall man, Franz Winklemeier, who toured Europe in the 1880s. alt Image from [Wellcome Images CC BY 4.0](#)

At a deeper level we also notice something more fundamentally interesting about a very tall (or very short) person. Most people who are quite tall or quite short (say a 4'7" woman), have bodily proportions that are nearly the same as someone of average height. For example, their arms aren't placed too low, their neck isn't particularly long, their legs reach all the way to the ground. In short, their body has been "scaled" to a larger or smaller size. This leads us to think that perhaps development itself is scalable. Surely the blueprints for building a very short person are not so different from the blueprints for building a very tall person. Our current understanding is that signals from growing body parts signal to each other to accomplish this task. The same signals build a short or tall body, but a tall body might undergo more cell division or longer periods of growth than a short body.

*Cell division is an important **developmental process**. What are some other developmental processes?*

Classical Developmental Biology relies on mutations (gene based) and teratogenic (environment based) anomalies to dissect processes like this. Not only can we compare "normal" anomalies like heights that fall two standard deviations from the average, but we can also look at more rare anomalies like conjoined twin tadpoles or a cyclopic (one eyed) mouse. Both of these anomalies have the bonus of being able to be created in the lab. In fact, we can create them using either genetics, or environmental insult (a *teratogen*).

The Evolution of Development or the Development of Evolution?

In a very real way, the fields of Development and Evolution cannot be truly separated. When we study Developmental Biology we are mostly looking at a fine-tuned mechanical and genetic process that has been selected on for eons. Not only can evolution select on the final product - a working, fertile adult - but it also can act at each developmental stage. It is easy to imagine how

evolution acts through natural selection on adults, but how can it act on development itself?

Consider the useful but pesky fruit fly, *Drosophila melanogaster*. Unlike you and me, who have support from our society (family, friends, institutions), *Drosophila* are r-selected. They survive by mating frequently and producing massive amounts of offspring that they leave behind on rotting fruit. Selection on adult *Drosophila* includes things such as ability to fly, to find food, find mates, and evade fly swatters. But other selective pressures affect embryonic *Drosophila*. Since the ability to mate quickly and produce many offspring is such a strong selective pressure on this species, embryos have evolved rapid development in response. In fact, *Drosophila* undergo such unusual developmental patterning in order to accomplish this incredibly fast development (about 24 hours to hatch into a larva), that we can use them as a model to see how evolution can speed up normal developmental processes. Surprisingly, to me at least, speeding up development doesn't just mean that everything moves along more quickly. Instead, *Drosophila* and its relatives undergo a unique type of embryonic development called "long germ-band" development. Instead of developing by slowly elongating and adding new differentiated tissue to the posterior end of the body, as many animals do, long germ-band insects develop all at once - rapidly subdividing an undifferentiated field of cells into the many parts of the larval body.

How else could evolution affect developmental processes?

This example is a classic illustration of evolution acting on developmental processes. There are many other ways evolution acts on development, these are often related to the physical constraints experienced by a growing embryo. On the flip side, we can also think of evolution itself as being constrained by development. Although much of natural selection acts on adults, that adult body must be formed by developmental processes inherited from ancestors and influenced by the environment. This is a limit on the types of bodies that can be produced, even if selective pressures are strong. However, the average member of a species does not show us the extremes of what morphologies are possible given evolutionary and physical constraints. This is where it becomes important to look at anomalies and other variation. When we see that height is scalable, we can look at the evolution of height in the past (what pressures drove the *Homo* genus to become tall with *Homo erectus* and its descendants? for example), pressures on height in the present (for example locomotor issues with very short or very tall heights), as well as the development of height (length of puberty, environmental factors such as food and toxins, for example). By examining our very short and very tall people, we can see the outer limits of these evolutionary and developmental constraints. Likewise by looking at rare anomalies (for example people born without limbs) we can examine the limits of perturbing development and find out what is possible.

One great example of the interconnectedness of evolution and development is the problem of scaling in flies. Briefly, there are closely related fly genera that vary widely in size but have very similar proportions. An outstanding question in EvoDevo is: how do flies change their scale by tweaking or changing developmental processes?

1.2: EvoDevo Case Study

When we look within a species we see tall and short (or large and small) individuals but they are all scaled more or less the same. For example, we don't generally see tall people with abnormally long necks or short people with abnormally short legs. As a general concept, we can say that this kind of scaling must be the result of cross-talk between different cells, tissues, and organs. For example, we might expect a growing bone to stretch muscle fibers and induce them to elongate. We might also expect a bone that grows long to also grow wide in proportion. However, the actual cellular and molecular mechanisms that perform this scaling are often somewhat mysterious. Here we are going to begin to examine a case of scaling in a group of super-manipulable animals - the dipterans.

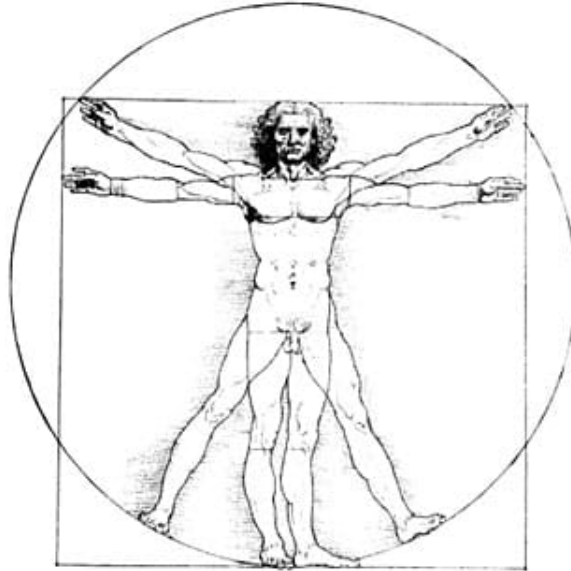


Figure 1: Scaling in humans Leonardo DaVinci noted scaling in humans in this famous anatomical study, Vitruvian Man. This public domain image from [wikimedia commons](#) illustrates the generalized proportions we see across the vast majority of humans, regardless of height.

Bicoid in dipterans: *Drosophila*

Dipterans are a type of fly, and this group includes the flies you are probably most familiar with - houseflies and *Drosophila* fruit flies. As was mentioned in the Introduction, these flies undergo extremely rapid development. This is likely selected for by their transient food sources. Moreover, their embryos and larvae lack protection and are a good source of nutrition for insectivorous predators.

One way to speed up development is to pattern the whole animal at the same time instead of patterning one end first, like we and many other animals do. *Drosophila* and relatives (often called the "higher dipterans") pattern their anterior and posterior ends at the same time. In fact, the oocyte (egg) is patterned before fertilization even occurs, with the "organizer" of anterior patterning (Bicoid) at one end and the "organizer" of posterior patterning (Nanos) at the other^{1,2}. There are many more genes involved in this process, but we will mostly focus here on Bicoid since it has been the subject of intense study.

Both the Nanos and Bicoid organizers in *Drosophila* are proteins that are found in a concentration gradient. Bicoid has its highest concentration at the anterior end, while Nanos is highest at the posterior. Towards the middle of the embryo they are both present at only low concentrations (Figure 2)^{1,2,3,4}. If you deplete Bicoid from a fly egg, you end up with a fly missing anterior (head) structures and an expanded abdomen. If you deplete Nanos, you end up with a fly missing posterior (abdominal) structures and an expanded head. Note that the tip of the head and the tail are patterned by two separate developmental mechanisms, but we are going to ignore this for now.

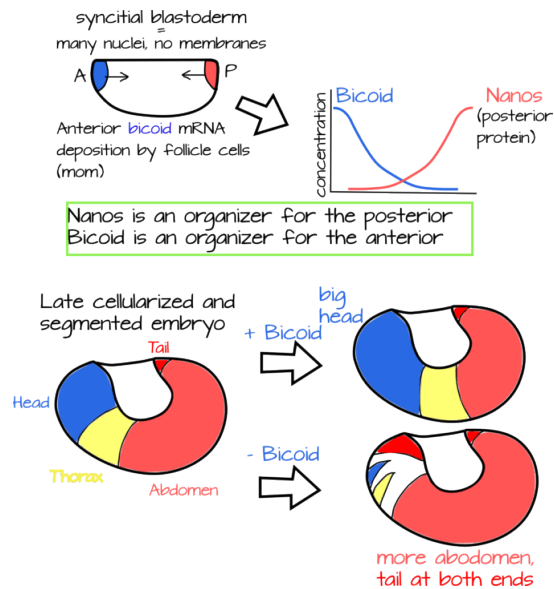
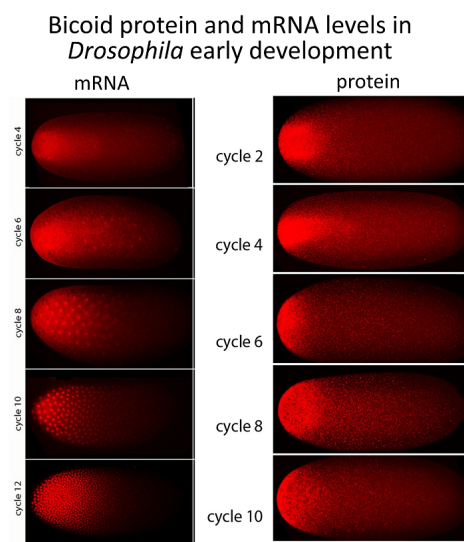


Figure 2: Bicoid and Nanos specify the anteroposterior axis of a developing *Drosophila* embryo. Bicoid is the "head organizer" - it is expressed at the anterior end of the embryo, depleting Bicoid gives a smaller head and increasing the amount of Bicoid gives a larger head. An editable svg file of the figure above can be downloaded at <https://scholarlycommons.pacific.edu/open-images/6/>

At this point, I am going to make our first foray into light molecular genetics. If you get a bit confused, please read [Briefly, Genetics](#) for some clarification.

When Christiane Nusslein-Volhard first made Bicoid mutants, she noticed a couple of interesting things. First, you need to make the moms mutant. This makes sense because it is maternal mRNA that gets pumped into the oocyte, so of course it is the mom's genotype that rules the embryo's morphology in this case. Second, even though she didn't have perfect evidence, she noticed that the Bicoid protein gradient looks like a *diffusion gradient*. A diffusion gradient is the pattern you get when something is diffusing freely from a point source (Figures 2,3,4). Imagine you are baking cookies, a visitor knocks on your door. As you open the door, they notice a faint but terrific smell coming from your house. They make small talk with you and slowly walk towards the source of the smell towards the kitchen. As they get closer to the oven the smell intensifies. You open the oven to take out the cookies, and your kitchen is flooded with the smell of cookie goodness. In this case the baking cookies in your oven are the point source and the smell has formed a diffusion gradient in your house, faintest at the front door and strongest in the kitchen. This mechanism for forming a gradient is called the "SSD model" for synthesis, solute, diffusion. However, in the case of Bicoid, this model has its critics, as we will discover in The Curious Case of Bicoid.



Murthy and Kornberg, 2016 DOI: [10.7554/eLife.13222](https://doi.org/10.7554/eLife.13222)

Figure 3: Bicoid gradient along the A/P axis of a *Drosophila* embryo. This figure is a derivative of Murthy and Kornberg, 2016 published under a CC BY 4.0 license.

The Problem of Scaling in Dipterans

The SSD model looks good on paper, but there is a confounding fact about dipteran embryos that forces us to take a closer look at it. Dipteran embryos vary in size tremendously. *Drosophila* embryos are teensy-tiny, about a third to half a centimeter long. Housefly embryos, on the other hand, are a whopping 1.5 centimeters long. What does this mean for the SSD model? Imagine your point source in our cookie example: a delicious smell coming from your oven. Only now you are cooking in your University's dining hall. Someone walks into the dining hall, what is the likelihood that they are going to smell those cookies? Pretty low, unless they have a great sense of smell. The door is now way too far for detectable odor to reach by diffusion. Likewise, in a large *Musca* (housefly) embryo, the diffusion gradient would only reach 1/5 (or less!) as far down the embryo as the same diffusion gradient in a small *Drosophila* embryo (Figure 4). How do flies solve this problem? There are several possible solutions that we will consider in The Curious Case of Bicoid.

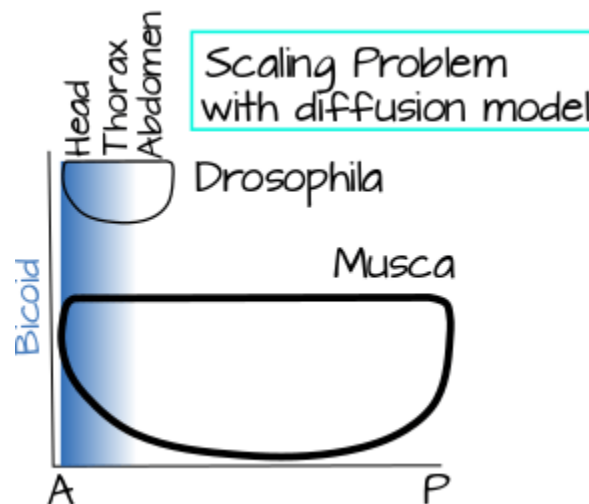


Figure 4: The scaling problem with the diffusion model. If Bicoid protein specifies the A/P body axis by forming a gradient (head at high concentration, thorax at medium/low concentration, abdomen at no Bicoid), then how do much larger flies get a proper A/P axis? If Bicoid is passively diffusing, it should form a much shallower gradient/body length in larger flies. What are some ways that the developing flies could compensate for this?

An editable svg file of the figure above can be downloaded at <https://scholarlycommons.pacific.edu/open-images/5/>

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4. "Bicoid gradient formation and function in the *Drosophila* pre-syncytial blastoderm". 2016, eLife, Zehra Ali-Murthy and Thomas B Kornberg, [10.7554/eLife.13222](https://doi.org/10.7554/eLife.13222)

1.3: Briefly, Genetics

Some Basic Terms

DNA is a double stranded nucleic acid polymer that is the primary hereditary material in most living organisms. Each strand of DNA is made up of covalently bound nucleotides. Each nucleotide is made up of a sugar, a phosphate, and a nitrogenous base. The covalent bond between nucleotides occurs between the sugar of one nucleotide and the phosphate of the next. In DNA, there is a single type of sugar but 4 types of bases. These bases - adenine, thymine, cytosine, and guanine - give each nucleotide its name (abbreviated A, T, C, and G).

The two strands of DNA are bound together by hydrogen bonds between nitrogenous bases. Each nitrogenous base has a "complementary" base. A pairs with T, and C pairs with G. If we think of a DNA strand having an orientation of sugar-phosphate-sugar-phosphate-sugar-phosphate then the second strand of the molecule has an orientation of phosphate-sugar-phosphate-sugar-phosphate-sugar. We call this type of orientation *anti-parallel* and we refer to the two strands as *reverse complements*. Reverse complementarity refers both to their opposite orientation (reverse) and the A=T G=C bonding pattern (complement).

RNA has a similar structure, though it is typically found as a single strand. It is capable of forming double strands (or even more complex structures, for example tRNA) through hydrogen bonding with reverse complement sequences. It can hydrogen bond with itself or with other RNA molecules.

The Central Dogma of Molecular Biology

For the most part when we think about Molecular Genetics we are thinking about making functional proteins from a gene encoded in DNA, though some RNA is functional on its own.

The production of protein from a DNA code happens via two highly-regulated processes: *Transcription* uses RNA polymerase to make a single stranded mRNA molecule from one strand of a double stranded DNA molecule. *Translation* uses a ribosome to make a peptide (part or all of a protein) from the mRNA (Figure 1).

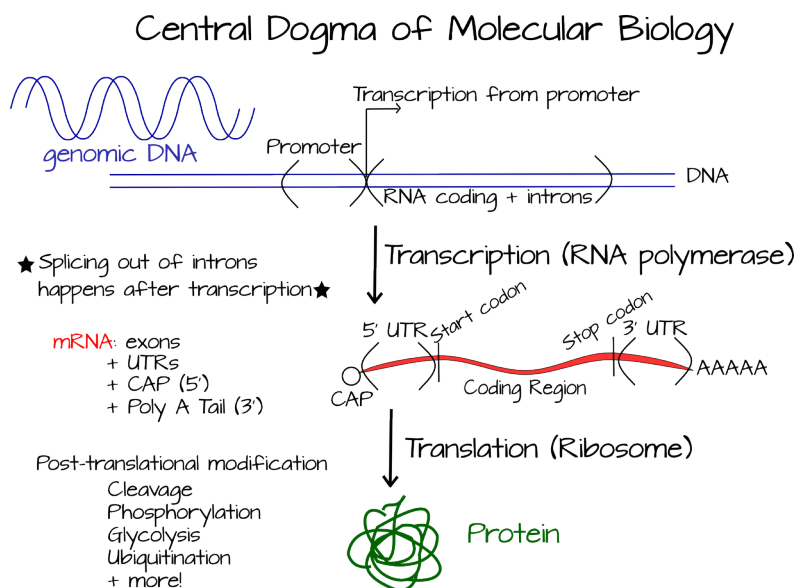


Figure 1: The Central Dogma of Molecular Biology by Kristina Vu and Ajna Rivera. An editable svg file of this figure can be downloaded here: <https://scholarlycommons.pacific.edu/open-images/5/>

Quick side note because many people are confused by some of the terms that we use in genetics, evolution, and development. A *gene* refers to a piece of DNA that codes for RNA. This RNA is often an mRNA but can also be a tRNA, rRNA, snRNA, miRNA, or a different "non-coding" type of RNA. *mRNA* is RNA that codes for protein. Most of the time "gene expression" refers to the production of mRNAs and/or the proteins they code for. *Gene expression* is the process of using DNA to make a functional

product. As you probably know, most of the cells in your body are genetically identical (more or less); they "share a genome." However, your cells have very different functions. These different functions are driven by different environments and different gene expression profiles – that is, the cells express different genes. For example, we might expect to find a lot of Myosin protein in muscle cells but a lot of Aquaporin protein in kidney cells.

The regulation of gene expression (epigenetics)

Gene expression gets regulated at three major levels (that can be subdivided into many many sublevels). Transcription itself can be regulated (we often refer to this as turning a gene "on" or "off"). Regulating transcription is the job of transcription factors, also known as "trans acting" factors. These are diffusible molecules, often proteins, that act in many different ways to turn up or down the rate of transcription of a particular gene. Some transcription factors bind directly to *cis-regulatory sequences*, pieces of DNA usually near the coding region of a gene, and influence the ability of RNA polymerase to bind to the promoter of the gene. They can do this by directly interacting with proteins involved in RNA polymerase recruitment (for example TFIID and Mediator), or by changing the accessibility of the promoter (for example, physically blocking the promoter, adding or removing methyl groups, or interacting with histones and histone binding proteins). Some transcription factors bind to other transcription factors and affect their ability to bind DNA.

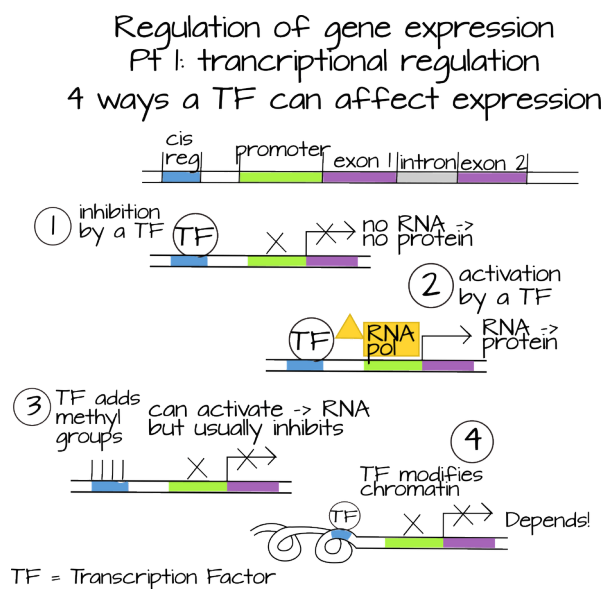


Figure 2: Transcriptional regulation. An editable svg file of this figure can be downloaded here: <https://scholarlycommons.pacific.edu/open-images/7/>

The second major level of gene expression regulation is translational regulation. This includes any kind of regulators that affect the amount of protein/peptide produced by a single mRNA molecule. There are two very well-studied ways that this happens. The first is through RNA interference (RNAi), which you may have heard of. RNAi uses short pieces of non-coding RNA that are exact or very close reverse-complements to part of an mRNA sequence. This targets the mRNA for destruction or blocks translation.¹ The second well-studied way to regulate translation is through RNA binding proteins. These proteins will often bind to specific sequences found outside the protein-coding region in mRNAs. These regions are called the *untranslated regions* or "UTRs". Proteins that bind here can

1. Destabilize the mRNA, lowering translation levels.
2. Stabilize the mRNA, raising translation levels.
3. Localize the RNA to a specific part of the cell.
4. Block or inhibit the binding of translation factors, lowering translation levels.
5. Recruit translation factors, raising translation levels.²

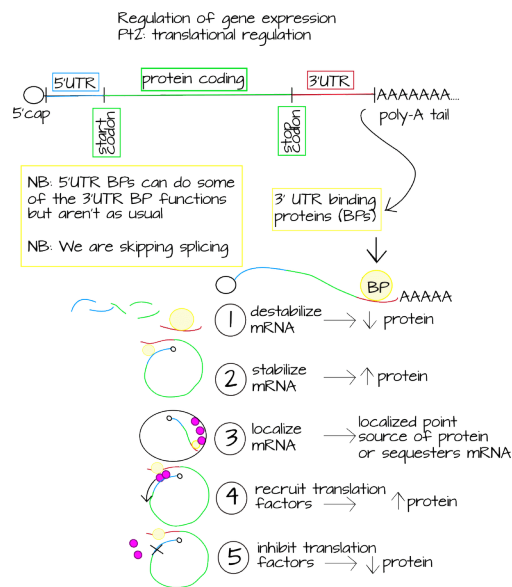


Figure 3: Translational regulation. An editable svg file of this figure can be downloaded here: <https://scholarlycommons.pacific.edu/open-images/8/>

The third and final major level of regulating gene expression is the post-translational level. This level encompasses most of what we think of as *signal transduction pathways*, or the protein-protein and protein-small-molecule interactions that affect protein stability and function. This can include both covalent modification of the protein (for example, phosphorylation, dephosphorylation, glycosylation, or proteolytic cleavage) as well as non-covalent modification (for example allosteric inhibition or activation, homo and heterodimer formation).³ If you aren't sure what these terms mean right now, that's ok - most of them we won't cover and those we do cover are easier to understand in context as you learn about signal transduction pathways.

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1.E: Introduction to Evolutionary Developmental Biology (Exercises)

At-home assignment

Watch the YouTube video at youtu.be/ydqReeTV_vk (Evo-Devo Despacito) and respond to the following two prompts:

- 1) What is one thing mentioned in the video you already know about?
- 2) What is one thing mentioned in the video you would like to learn more about?

Discussion and Reading Guide Questions

The questions below were originally written for students reading *Freaks Of Nature* by Mark Blumberg and *Endless Forms* by Sean Carroll. However, the **questions in bold** are discussion questions that can be answered without these books.

The "Reading Guide" questions are appropriate for short homework answers and the "Discussion Guide" questions are appropriate for open in-class or online discussion.

Discussion Questions

1. **How does studying anomalies help us understand how development and evolution work?**
2. **How does studying development give us insights into evolutionary biology?**
3. **What is the argument for DNA being only a part of the process of development?**
4. **Why is it that tinkering with an embryo can sometimes produce an animal that has anomalies that still can function integratively? Does tinkering always result in an integrated adult animal**
5. **What animals did you draw as a child? Do you still allow yourself to be awed and inspired by nature? What (if anything) has changed since your childhood?**
6. **If you had to go on a nature vacation anywhere, where would you go and why?**
7. **Why do we study evo-devo and what are the central questions in the field?**
8. **What evolutionary adaptations most interest you?**
9. **How do "just so" stories differ from the scientific process?**
10. **What are some examples of "building blocks" and modular design in animals?**
11. Do you think that hopeful monsters are a way that evolution happens? Why or why not?
12. What does it mean that some anomalous characters are "discontinuous" rather than continuous?
13. What are punctuated equilibria? Are they real or an artifact?
14. What is the genetic toolkit and what is the toolkit paradox? How can we resolve this paradox?
15. How does the "gene centric" view of this book differ from *Freaks*?
16. Can you think of any exceptions to "Williston's Law"?

Freaks Reading Guide: Chapter One

1. What are evolutionary constraints?
2. What is teratology?
3. What is Developmental Systems Theory?

Endless Forms Reading Guide: Intro and Chapter One

1. How does studying an organism's development help us understand its evolutionary relationships?
2. What is regulatory DNA?
3. What is a developmental constraint?
4. In what ways can evolutionary changes in the development of an organism affect its morphology?
5. What are *Hox* genes, and what role do they play in evolution?
6. What are serial homologs?

7. What will be covered in the first and second halves of this book? What do you already know about?
8. Does ontogeny recapitulate phylogeny? Why or why not?

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2: Fertilization and Cortical Rotation

Fertilization

For sexually reproducing species, the completion of fertilization marks the start of embryogenesis. You are already aware that species have different reproductive isolation mechanisms that prevent interspecific fertilization. In the case of sea urchins (links below) you will see that there are several steps that can mediate species-specific recognition. These include chemo-attractants (chemicals secreted by the egg that the sperm responds to) and sticky molecules held by the sperm (bindins) that only interact with the same species eggs.

The sperm-entry point is also significant in many species in that it marks the first break in egg symmetry. Imagine a fairly symmetric ball. Now draw a single dot on that ball, this dot is now a landmark. You can be opposite the dot, close to the dot, or right on the dot. The sperm-entry point, like the dot, acts as a landmark to break symmetry. It does so in at least two related ways, by donating a centrosome to the egg, and by triggering cytoskeletal rearrangements. The sperm centrosome, a tiny organelle that acts as a seed for microtubule formation, can act as an organizer of the spindle apparatus. By putting one side of the spindle near the sperm-entry point, it can define the axis of the first cleavage (Figure 1).

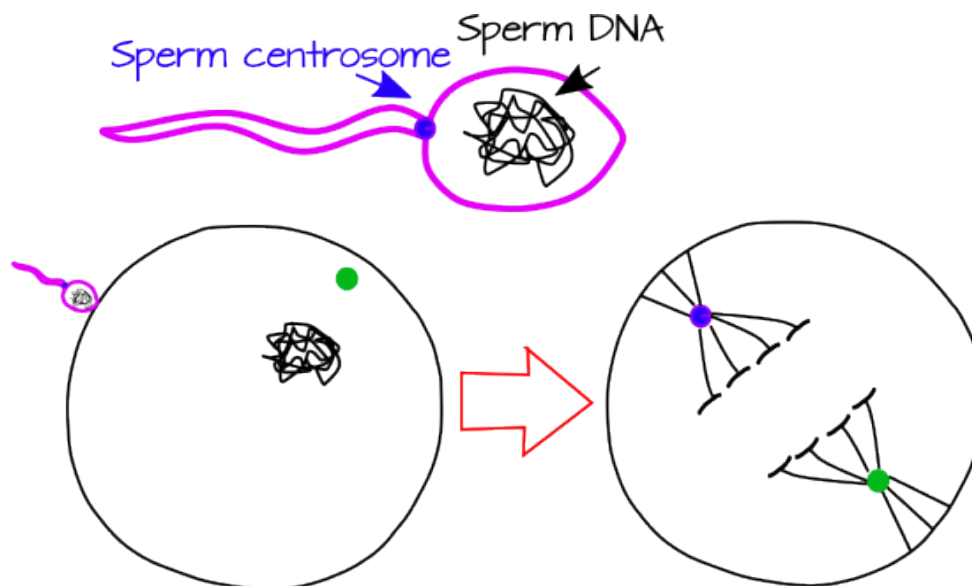


Figure 1: The sperm entry point polarizes the oocyte/zygote. The sperm cell donates DNA plus a centrosome (blue circle) to the egg. The centrosome acts as a microtubule organizing center (MTOC) for the spindle. The sperm and egg MTOCs move to opposite poles of the cell as the nuclear envelopes disintegrate and the cell becomes diploid. In mammals, the sperm entry point is often perpendicular to the zygotic metaphase plate and first cleavage¹. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/9/>

Additionally, the sperm-entry point can be a trigger for cytoskeletal shifting, including a process called *cortical rotation*, wherein the outer layer of the cell (the cortex) rotates relative to the inner cytoplasm. While cortical rotation is limited to a few species (that we know about), studying it has led to breakthroughs in EvoDevo and developmental biology in general.

Cortical rotation's significance as a symmetry breaking event is underlined when we see what happens if we prevent it. Brief treatments of frog (*Xenopus laevis*) fertilized eggs with agents that affect microtubule polymerization prevent cortical rotation and result in a "ventralized" embryo - that is, it has ventral structures but lacks dorsal structures including those that would build a spinal cord. We call an embryo like this a "belly piece." What is it about cortical rotation that turns a frog into a frog, rather than into a mass of belly tissue?

Cortical rotation as an EvoDevo example

Even though cortical rotation is a process found in only a few animal species, it is an example of several fundamental concepts in EvoDevo

1) Establishment of organizers. *Organizers* are parts of the embryo that produce signals that pattern nearby or distant tissues. In *Drosophila*, *Bicoid* mRNA marks the head organizer, for example. Depletion of Bicoid leads to headless flies, too much Bicoid leads to big-headed larvae. Later in the semester we will examine the ZPA organizer in vertebrate limbs.

Frogs have a Spemann-Mangold organizer (named after student Hilde Mangold and her advisor Hans Spemann who discovered and characterized it)². Removal of the organizer tissue leads to a ball of belly tissue. Addition of an organizer to another location on the embryo leads to twinned tadpoles.

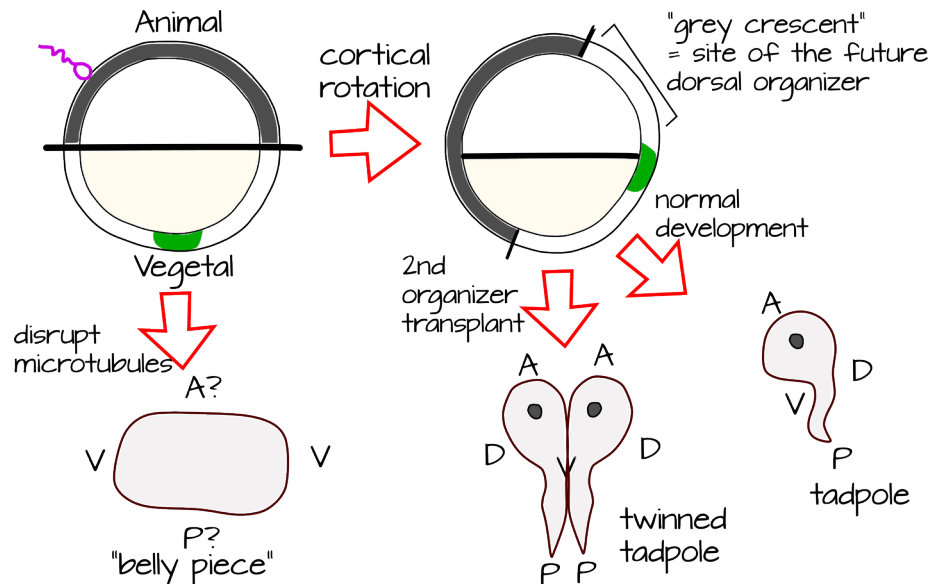


Figure 2: **Cortical rotation and the grey crescent.** Sperm (purple) entry triggers the rotation of the cortex of the cell relative to the inner cell mass. This rotation moves determinants on the vegetal (yolky) side of the egg (green) towards the animal/vegetal border. A grey "crescent" appears where the clear vegetal cortex overlies the grey animal inner cell mass. The animal cortex is darkly pigmented, hiding the inner cell mass underneath it. The grey crescent is the site of the future organizer that runs along the anterioposterior axis. If cortical rotation is disrupted, then this organizer is never formed and the embryo becomes a mass of ventral tissue (belly piece). If a second organizer is added to the embryo, a second axis is formed, leading to twinning. An extreme case of this is a complete twinned tadpole. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/10/>

2) Induction of cellular differentiation. Many cells have a "default" path that they will follow over time if they are not given additional instructions. One of the main roles of an organizer is to make and send off those additional instructions. Organizers do this through cell-signaling molecules and gene regulation. The Bicoid example in *Drosophila* is an example of gene regulation. The frog example demonstrates both gene regulation and cell-signaling, as I will soon describe.

3) Development happens in 4D. Not only do we have to think about complex 3D objects when we are thinking about development, but we also need to consider time. Over the course of time, cells and parts of cells change their positions. This changes the "local environment" - changing the types of interactions that can happen. This will be more important in gastrulation, but we will see the start of it here in cortical rotation. In the case of cortical rotation, the rotation changes the locations of mRNAs and proteins. These will eventually be inherited by different cells and will give these cells starting instructions for differentiation. These cells will later change their position and, as they come into contact with new neighbors, will differentiate further and give out new instructions.

Sperm entry triggers changes in the egg cell

An unfertilized frog oocyte is not perfectly symmetrical. It has *radial symmetry*, that is you can stick a pin from top to bottom and it will be symmetric around the pin. However, the top and bottom sides differ. The bottom (*vegetal pole*) is enriched with dense yolk granules while the top (*animal pole*) is enriched with less dense cytoplasm and pigment. Similar animal/vegetal symmetry is

seen in many species. Frog eggs are also asymmetric in that they are made of two layers. An outer *cortex* and an inner cell mass. The animal and vegetal poles differ in the mRNAs and proteins (see [Briefly, Genetics](#) for a refresher on molecular biology terms)³ and so do the inner and outer parts of the oocyte.

Sperm entry induces a change in the way cytoskeletal microtubules interact with each other. They go from being a loosely organized network across the cell to a parallel array with highest density in the cortex and at the cortex/inner cell mass border. These microtubules rotate the cortex relative to the inner cell mass, taking with them the asymmetrically localized cortical determinants². Because the pigmentation in the egg was unevenly distributed, this creates a "grey crescent" where the vegetal cortex now lies over the animal inner cell mass (Figure 2)

The interaction between vegetal cortex molecular determinants and animal inner-cell mass molecular determinants makes this grey crescent unique compared to either the animal or vegetal pole of the fertilized egg. The grey crescent marks the future dorsal side of the frog. You can see now why elimination of cortical rotation or elimination of the molecular determinants that populate the grey crescent lead to ventralization of the frog. Duplication of the grey crescent, either through transplantation or genetic manipulation, leads to an extra dorsal axis and "twinning" of the frog.

In the next chapter, Cleavage and Gastrulation, we will examine the development and genetic of this organizer and think about the consequences of organizing tissues in evolution.

References

1. "Mitosis in the human embryo: the vital role of the sperm centrosome (centriole)", 1997, Histology and Histopathology, A. Henry Sathananthan [PMID: 9225167](#)
2. "Induction of Embryonic Primordia by Implantation of Organizers from a Different Species," 1924, Archiv für Mikroskopische Anatomie und Entwicklungsmechanik, Hans Spemann and Hilde Mangold., [PMID: 11291841](#)
3. Reviewed in: "Cortical rotation and messenger RNA localization in *Xenopus* axis formation", 2012, WIREs Developmental Biology, Douglas W. Houston doi: 10.1002/wdev.29

Further Reading: Developmental Biology

www.ncbi.nlm.nih.gov/books/NBK9983/

- [Introduction to fertilization](#)
- [1. Sperm and Egg](#)
- [2. How do you do? Sperm and egg recognition](#)
- [3. One sperm per egg: the cortical granule reaction](#)
- [4. OPTIONAL: The activation of egg metabolism](#)
- [5. OPTIONAL: Two become one, fusion of genetic material](#)
- [6. Breaking symmetry: Rearrangement of the egg cytoplasm](#)
- [7. Summary](#)

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2.E: Fertilization and Cortical Rotation (Exercises)

At-home assignment

Free response question:

[A recent paper](#) examines the effect IVF may be playing on human evolution. Look at one or two of the traits being selected on by IVF ([Table 1](#)) and think about the role IVF might be playing in future evolution of that trait. Is the effect of IVF large enough to drive significant human evolution?

Discussion Questions and Reading Guide

The questions below were originally written for students reading *Freaks Of Nature* by Mark Blumberg and *Endless Forms* by Sean Carroll. However, the **questions in bold** are discussion questions that can be answered without these books.

The "Reading Guide" questions are appropriate for short homework answers and the "Discussion Guide" questions are appropriate for open in-class or online discussion.

Reading Guide for "Further Reading"

1. **What are the four major events of fertilization?**
2. **What are the parts of an animal sperm cell?**
3. **Where does the power for sperm propulsion come from?**
4. **How does an animal egg differ from a sperm cell?**
5. **What are the different layers of an egg cell?**
6. **What is polyspermy? How does an urchin egg prevent this?**
7. **What processes produce the first asymmetry in frog embryos?**

Discussion Questions

1. **What are some other mechanisms that reduce the rates of interspecific fertilization?**
2. **What selective pressures drive pre-zygotic barriers between species?**
3. **What is the significance of an egg packed with protein and mRNAs?**
4. **What are different ways eggs and zygotes protect themselves in different environments? Consider, for example, urchin eggs, chicken eggs, and mammalian eggs.**
5. **Why is it important for animal embryos to "break symmetry"?**
6. **What is the connection between eugenics and evolution?**
7. **Why do we consider eugenics to be unethical?**
8. **What does it mean when we say that cells "express" a gene?**
9. **How are evolution and development linked as fields of study? What is the link between both and genetics?**
10. **How are evolution and development both affected by environmental interactions?**
11. How does the Kirschner/Gerhart view of EvoDevo differ from the West-Eberhard view?

Freaks Reading Guide: Chapter Two

This chapter brings in the dimension of "time" into the picture. Two of the examples in this chapter are covered in this online text, Sonic Hedgehog and the Segmentation Cascade.

1. What is cyclopia? Is it genetically or environmentally induced?
2. What is induction and how does it relate to cyclopia?
3. **What is eugenics?**
4. **What is the difference between teratogenesis and mutagenesis? Which of these are used by developmental biologists?**
5. **How can the same mutation produce different phenotypes?**
6. What are the different types of twins?

7. What subjects in this chapter are you interested in following up on later in the semester?

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3: CLEAVAGE AND GASTRULATION

3.1: The Curious Case of Bicoid: Models for Making a Gradient-A Model Emerges

3.2: Cell Division and Movement: Cleavage - Frog Gastrulation and the Dorsal Lip of the Blastopore

3.3: The EvoDevo of the Blastopore: The Spemann-Mangold Organizer - Gastrulation in Evolution

3.E: Cleavage and Gastrulation (Exercises)

3.S: Cleavage and Gastrulation (Summary)

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3.1: The Curious Case of Bicoid: Models for Making a Gradient-A Model Emerges

Models for Making a Gradient

Morphogenic gradients are incredibly important in development for patterning tissues. If a tissue is completely homogenous (every cell doing the same task) it cannot be complex and is often not resistant to environmental fluctuations. In the case of Bicoid in *Drosophila*, we are considering a morphogenic gradient that patterns the anteroposterior axis - as mentioned in the Introduction, too much Bicoid gives us a giant head and too little gives us no head at all. There are somewhere around 60 different genes that respond to various levels of Bicoid protein and one of the earliest to be discovered is Hunchback. Hunchback gives positional information to a fly embryo, it sharpens the boundary between the anterior and posterior of the fly. While the Bicoid protein gradient is very broad and resembles a diffusion gradient, the Hunchback gradient is sharp, with significantly different protein levels within 10 μm , or about 2 nuclei¹.

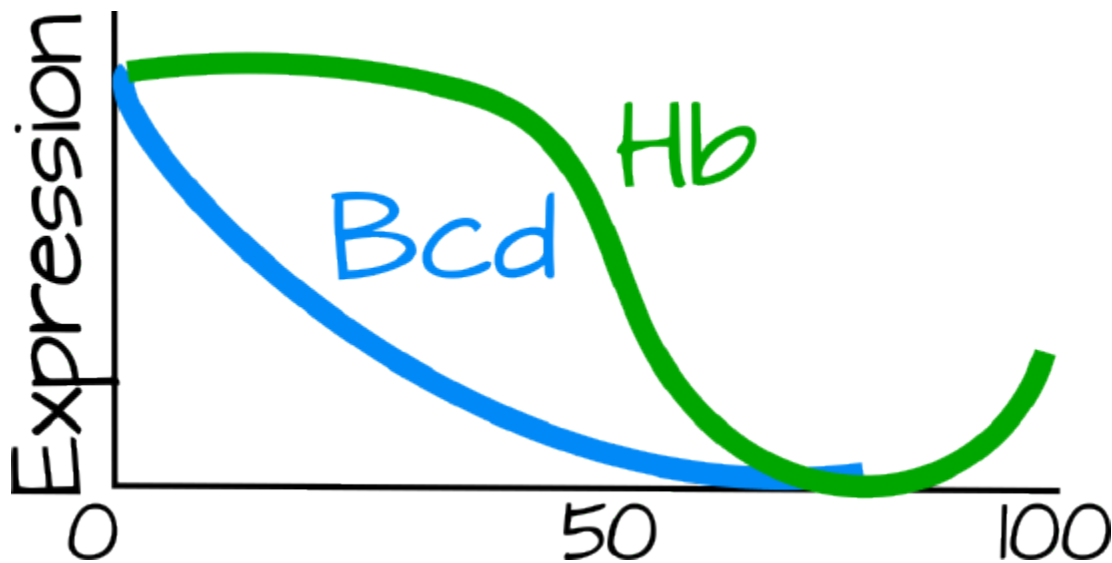


Figure 1: The Bicoid (Bcd) gradient is shallow, Bicoid concentration (y-axis) degrades over about 1/2 of total embryo length (x-axis). However, this shallow gradient provides positional information for Hunchback (Hb), which is translated as long as there are even low levels of Bicoid protein^{1,2}. This sharp Hunchback gradient neatly splits the embryo into anterior and posterior halves. Later in development the presence of high Hb + high Bcd, high Hb, low Bcd, and low Hb + low Bcd will be read by different transcription factors to produce broad stripes in the embryo (see Regionalization and Organizers for more information).

Obviously the levels of Bicoid must matter tremendously since the Bicoid gradient and its readout (for example Hunchback levels) are incredibly precise. An early model for the observed Bicoid gradient in *Drosophila* is the SSD model (Synthesis, Solute, Diffusion), where a point source of Bicoid protein (translated from anterior bicoid mRNA) sets up the concentration gradient. The related SDD model (Synthesis, Diffusion, Degradation) incorporates degradation over time of Bicoid protein, keeping the gradient from reaching the posterior end of the embryo. Several other models have been proposed that would allow for fluctuation in embryo size but still lead to a correct Bicoid readout:

1. More mRNA/protein in larger embryos: This would extend the gradient out further in larger embryos.
2. Nuclear shuttling: The same as the SDD model, but Bicoid protein gets taken up by nuclei as it diffuses by them. Under this model, the more densely packed the nuclei are (smaller embryo) the more quickly the Bicoid protein gets pulled out of the syncytial cytoplasm. In larger embryos, the protein can diffuse farther.
3. ARTS model (Active mRNA, Transport, Synthesis): The cytoskeleton actively transports Bicoid mRNA to form an mRNA gradient that gets readout as a protein gradient as it is translated. In larger embryos, the cytoskeleton can transport the mRNA further.
4. mRNA diffusion and degradation: This is similar to the ARTS model, except that in this model Bicoid mRNA degrades over time to maintain an mRNA gradient. Larger flies could have lower degradation rates of Bicoid mRNA or faster transport.

5. Facilitated diffusion of protein. This is similar to the ARTS model, only it involves transport of Bicoid protein by the cytoskeleton. In this case, larger flies could have faster transport of Bicoid protein.

There is some evidence for and against each of these models, suggesting that the Bicoid protein gradient is likely maintained by a combination of these models. Additionally, Bicoid protein readout might be more complex than we think - multiple proteins could be affecting how Bicoid interacts with its targets.

Evidence for and against the models

Data from both *Drosophila* and other, larger, dipterans have provided evidence supporting each of the models listed above. Additional testing has falsified the predictions of some of the models, and that is the subject of this section. Below I describe each model and a test of that model, I also include a figure that illustrates what the model expects and what was actually observed. I recommend this type of super-reductionist figure to help summarize a large set of data, but we always need to keep in mind that it does not tell the whole story.

1. More protein model. An embryo can get more protein by increasing the amount of mRNA, increasing the rate of translation of that mRNA, or decreasing the rate of protein degradation. Supporting this is the finding that putting in extra copies of Bicoid (more mRNA and more protein) into a fruit fly increases the Bicoid gradient and the size of the head. To see if this is what flies with larger embryos do to make a larger gradient, researchers put Bicoid DNA, complete with cis-regulatory sites and UTRs, from large embryo flies (*Calliphora*) in *Drosophila*. They found that the Bicoid gene from large embryos did not make a longer gradient in *Drosophila*. Therefore, if there are any factors affecting the mRNA stability or protein degradation, they must be specific to the larger flies³.

2. Nuclear shuttling. Early experiments on this suggested that adding a nuclear localization signal to Bicoid protein made a shallower gradient³. Later experiments tested this by making *Drosophila* with a mutant version of Bicoid that did not accumulate in nuclei. If nuclear import made the Bicoid protein gradient steeper, then they would have seen a broader gradient after inhibiting nuclear import. However, they saw a fairly normal looking Bicoid protein gradient, arguing strongly against this model⁴.

- 3 and 4. mRNA gradient models. In both models 3 and 4, mRNA is actively transported around the cell by the cytoskeleton. This is in direct opposition to the SDD model, where Bicoid protein is translated from anteriorly-tethered Bicoid mRNA. In these two mRNA gradient models, the protein gradient is simply a "read-out" of the mRNA gradient. Evidence for these two models comes from careful measurement of Bicoid mRNA in developing embryos (Figure 2). These careful measurements show that Bicoid mRNA itself forms a gradient, and is not simply a point source⁵. Its movement is dependent on the cytoskeleton, if the cytoskeleton is disrupted, the mRNA gradient is also disrupted⁶. Evidence against this model comes from the finding that Bicoid protein at the posterior of the embryo is older than Bicoid protein at the anterior. If Bicoid mRNA diffuses towards the posterior to make a gradient, then Bicoid protein is made from that mRNA, we would expect to see younger Bicoid protein at the posterior and mixed age Bicoid protein at the anterior end. However, [Durrieu et al](#) found the opposite, suggesting that even though Bicoid mRNA may form a gradient, this is not the main determinant of the protein gradient⁷.

5. Facilitated protein diffusion. Disruption of the cytoskeleton by treating a *Drosophila* embryo with anti-actin or anti-microtubule drugs leads to disruption of the protein gradient. Since mRNA tethering at the Anterior pole is also dependent on microtubules, just disrupting the cytoskeleton wouldn't tell you whether it is mRNA transport or protein transport that leads to the protein gradient. One study used a funny trick - if you raise *Drosophila* embryos in low-oxygen (hypoxic) conditions, the Bicoid mRNA will stay at the anterior end even if microtubules are disrupted. This study found that Bicoid protein still forms a gradient even if Bicoid mRNA does not, and that this gradient is dependent on the cytoskeleton⁸.

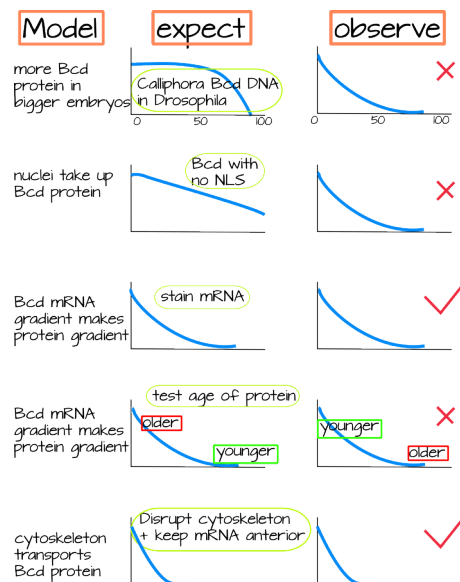


Figure 2: Evidence for and against Bcd gradient models^{3,4,5,6,7,8}. Four major models of Bicoid gradient generation are shown. An experiment that supports or falsifies each is circled in yellow. Expected Bicoid concentration gradients after experimental intervention are on the left, observed concentration gradients are on the right.

A Model Emerges

Despite all of the seemingly contradictory data, a model is beginning to emerge that both explains the robustness of the Bicoid protein gradient and explains how the gradient itself can scale to larger or smaller bodies. First, Bicoid mRNA is mostly sequestered at the anterior end of the embryo, but also travels along the cortex (outer edge) of the embryo via microtubules. Bicoid protein is translated off of the Bicoid mRNA, with a higher amount of translation happening at the anterior end, where the concentration of Bicoid mRNA is higher. Some Bicoid protein is likely also translated off of the lower concentration Bicoid mRNA that has moved posteriorly via microtubules. This leads to a gradient of Bicoid protein that is slightly broader than what we would expect if there was a simple point source of Bicoid protein (i.e. translation from anterior pole Bicoid mRNA).

This protein gradient is also modified as Bicoid protein gets transported around the embryo by the cytoskeleton (via actin filaments and microtubules). In this way, there is control over the gradient by the cytoskeleton and by the proteins that mediate interaction between Bicoid (protein and mRNA) and the cytoskeleton. These mediating proteins may be sensitive to local Bicoid levels and may allow Bicoid to move more quickly or slowly depending on the anteroposterior position and the gradient level. For example, a large fly might have less Bicoid protein by diffusion at 15% of its length (EL) than a small fly does at 15% of its length. However, all of the Bicoid protein at 15% EL in the large fly can get taken up by the mediating proteins and moved posteriorly to broaden the gradient. On the other hand in a small fly, the larger amount of Bicoid protein at 15% EL could oversaturate the mediating protein and be transported less efficiently.

Additionally, Bicoid proteins from different species of fly are known to have slightly different properties, for example Bicoid from larger fly species such as *Calliphora vicina* and *Lucilia sericata* are not able to completely rescue *Drosophila* Bicoid mutants³. These different properties could also play a role in protein-protein interactions involving the cytoskeleton. Finally, the mediating proteins between the species could also differ, some might bind to Bicoid more efficiently and/or move along the cytoskeleton more efficiently.

3.2: Cell Division and Movement: Cleavage - Frog Gastrulation and the Dorsal Lip of the Blastopore

Cleavage

Cleavage stages of embryogenesis are typically very similar across animals. During cleavage a single cell embryo rapidly divides to form a ball of cells, called a blastula. This ball may be hollow inside, have a hollow region, or be fairly solid. Early cleavage stages are different from later cell divisions in that they quickly produce many very similar looking cells. If morphogens are asymmetrically distributed in the egg, then these cells can differentiate via autonomous specification even at early stages. Conditional specification typically begins to occur towards the end of cleavage stages when cell division slows down and cells begin to communicate more extensively with each other. Sometimes this is called the "mid-blastula transition" or MBT. The MBT demarcates the transition between using mostly maternal cell and fate determinants (RNAs and protein) to the embryo producing its own RNAs and proteins. One interesting experiment that shows the extent of this transition is to block transcription using the drugs alpha-amanitin or actinomycin D - two inhibitors of RNA polymerase action. If these drugs are applied to an early cleavage-stage embryo cleavages proceed normally, suggesting that developmental processes and cell division at this early stage are using maternally-derived factors already present in the embryo. If the drugs are applied to later-stage embryos development halts, suggesting that at this point zygotically derived transcripts are necessary for development and cell division. The timing of the MBT and the sorting of maternal proteins and RNAs into different cleavage-stage cells varies widely among different animals.

Read more details about Cleavage [here](#), focus on any section you find interesting and especially *figure 8.5*. www.ncbi.nlm.nih.gov/books/NBK9992/#_A1678_

Gastrulation

Gastrulation refers to a time of great flux in animal embryos. During gastrulation the cells produced during cleavage begin to move relative to each other. As they move, they change their local environment and come into contact with new cells. This change in local environment means that the cells may a) be exposed to different extracellular morphogens and b) make contact with new cell-cell signaling partners. The movement of cells (Figure 3) over (epiboly), under (involution), between (intercalation), internally (invagination) or away from an epithelial sheet (ingression and delamination) creates layers of tissue. As the cells comprising these tissue layers encounter new morphogens and new signaling partners, they become specified as germ-layers.

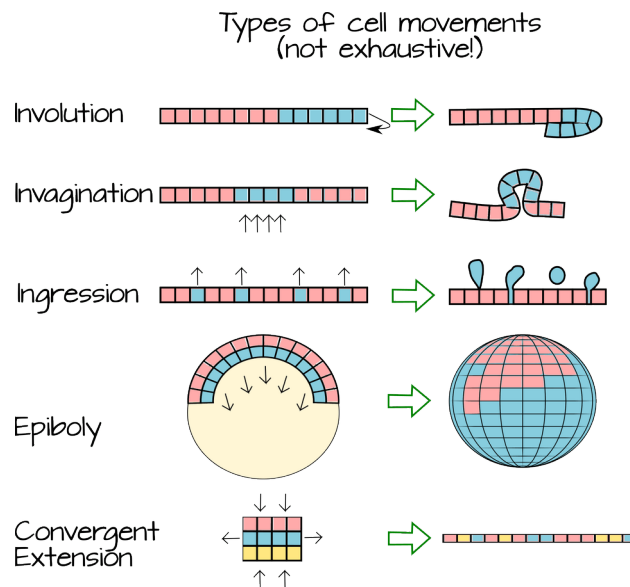


Figure 3: types of cell movements during development. In involution, a single layer of cells curves under itself to form two layers. An invaginating sheet of cells pokes inwards at one point to make a lined cavity. Ingression cells (blue) delaminate from a sheet of cells. Epiboly is a set of complex movements including intercalation (cells moving between each other), cells crawling, and cells changing shape that results in a sheet of cells covering a surface. Convergent extension is cell intercalation and cell elongation to turn a short/wide tissue into a long/narrow tissue. An editable svg file of this figure can be downloaded from <https://scholarlycommons.pacific.edu/open-images/15/>

The following video shows a live-frog embryo undergoing cleavage and gastrulation and also shows an animated internal view of how the cells move inside the embryo. Note the different kinds of movements. <https://www.youtube.com/watch?v=riSA1mo86Kg>

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

[View this YouTube Video online](https://www.youtube.com/watch?v=riSA1mo86Kg)



Changing Partners

As mentioned earlier, gastrulation performs two key functions in a developing animal embryo. First, it creates tissue layers (germ layers) from set of fairly homogenous cells. Second, it allows for new interactions between cells and places cells in new morphogenic positions. As cells change their partners over time, they become fated to a specific path. For example, compare a picture of a fate-map of a blastula-stage frog to the fate map of a gastrulated frog (Figure 4).

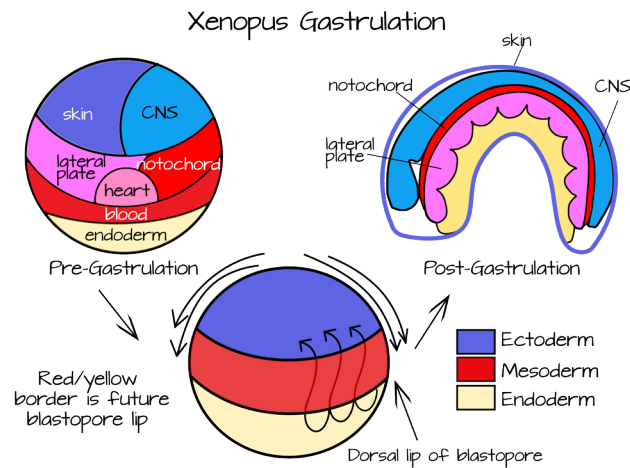


Figure 4: *Xenopus* gastrulation by Kristina Vu and Ajna Rivera. A *Xenopus* blastula (pre-gastrulation embryo) will undergo predictable movements in gastrulation to form its three tissue layers, the ectoderm, mesoderm, and endoderm. A "fate-map" (top left) shows which regions of the blastula will develop into specific parts of the larva after normal development. The blastula germ layers are stacked from animal pole to vegetal pole. The process of gastrulation moves them relative to each other and elongates them. During gastrulation, the ectoderm epibolizes over the other germ layers as the mesoderm turns under itself at the dorsal lip of the blastopore. In this way, the endoderm will end up lining the gut with the mesoderm overlying it and the ectoderm will cover the entire embryo and form the nervous system. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/14/>

While the blastula-stage frog seems almost random in its mapping, the gastrulated frog has all of its tissues/organs in roughly adult positions. The blastula stage has not undergone extensive cell movements yet and the cells are largely unspecified. If cells are removed during the blastula stage, other cells will often be able to take on the fate of the missing cells. For example, identical twins develop from a single embryo that has "split" into two after multiple cell divisions. Rather than each half of the embryo making only half an animal when contact is broken between the two cell masses they each develop into an entire animal. One of my favorite sets of experiments of all time, the Spemann-Mangold organizer experiments, shows the extent to which cell-cell communication can rule development. Before I discuss that I would like to briefly summarize frog gastrulation (no offense to other animals, but they are my favorite gastrulators). A more in-depth look at frog gastrulation can be found here: www.ncbi.nlm.nih.gov/books/NBK10113/ and an interactive fate-map for *Xenopus* is available from <https://www.xenbase.org/anatomy/static/xenbasefate.jsp>

Frog Gastrulation and the Dorsal lip of the Blastopore

After fertilization and cortical rotation, the frog embryo is divided roughly into four quadrants. These are demarcated by the two major axes A/V (animal-vegetal) and D/V (dorsoventral). There is no Anteroposterior axis yet, this axis will be generated during gastrulation. <https://www.youtube.com/watch?v=EPMgHMnwW28>

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

[View this YouTube Video online](https://www.youtube.com/watch?v=EPMgHMnwW28)



Over the course of gastrulation, the red area (future mesoderm) in Figure 5 will involute under the blue area (future ectoderm) and extend to form a layer at the roof of the blastocoel (Figures 3 and 4). This involution occurs most extensively at the dorsal side of the embryo (the side opposite sperm entry), but also occurs along the rest of the "blastopore lip". To visualize the blastopore

lip, open your mouth wide. Your lips represent mesoderm and the rest of your face represents ectoderm (sorry no endoderm in this one!). Use your lips to cover your teeth. This is physically similar to involution. Now imagine that your lips could grow thin and long and cover the inside of your mouth. This is how the mesodermal cells end up as a second layer under the ectoderm. Finally, begin to close your mouth. This is similar to epiboly - when the ectodermal cells move over the surface of the embryo to cover the mesoderm and endoderm.

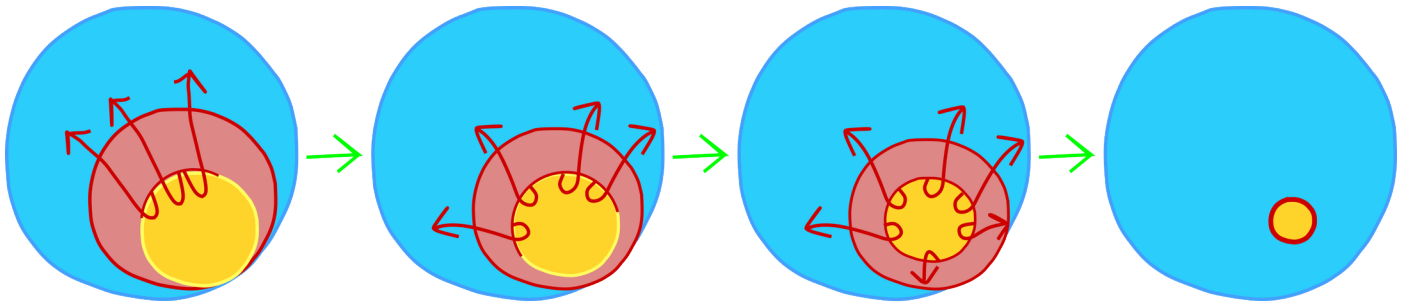


Figure 5: Movement at the lip of the blastopore (dorsal view). In an early gastrula, mesoderm (red) involution occurs at the dorsal lip of the blastopore. Most mesoderm will involute here. Over time, involution spreads around the blastopore lip as the lip gets smaller and smaller due to epiboly by the ectoderm (blue). An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/>
13/

To understand the role of the Spemann-Mangold organizer, you need to keep these movements in mind. In particular, you need to think about the dorsal lip of the blastopore, the place where involution first begins and happens most extensively. If you look at the Figure 4 fate map, you will notice that the dorsal mesoderm (the mesoderm at the dorsal lip of the blastopore) will become the notochord. These cells are the first mesodermal to internalize during epiboly. This block of cells will elongate and thin out to become a rod of mesodermal tissue that spans the length of the embryo. This will run along the anteroposterior axis, just like your spinal column runs along your anteroposterior axis. Additionally, protein signals from the notochord will help to pattern the rest of the embryo, as Hans Spemann and Hilde Mangold famously discovered.

3.3: The EvoDevo of the Blastopore: The Spemann-Mangold Organizer - Gastrulation in Evolution

The Spemann-Mangold Organizer

Hans Spemann and his graduate student Hilde Mangold perfected a technique to do cross species transplantations of the dorsal blastopore lip to new locations in the host's body. Spemann had wondered for years what exactly the dorsal lip of the blastopore did. He knew it was important because if he sliced an embryo in two, the half that got the dorsal blastopore lip developed into a tadpole, while the other half developed into a "belly piece." He also knew that if he carefully divided the embryo down the middle of the dorsal lip of the blastopore, both halves would develop into a tadpole. One possibility was that a morphogenic signal emanated from the dorsal lip that caused cellular differentiation in a time gradient, such that the closest cells to the dorsal lip differentiate first. Without this signal, cells wouldn't differentiate. Spemann thought he had support for this hypothesis when he did transplantation experiments in newts. When he transplanted the dorsal lip from one newt to another, he got conjoined twin newts - suggesting that some signal from the dorsal lip was indeed patterning the main body axis^{12,13}.

Soon Spemann revised his hypothesis, largely based on careful observation of gastrulating embryos and on the cross-species transplantation work by Mangold. Mangold took the blastopore lip from an unpigmented newt species and transplanted under the ventral ectoderm of an early gastrula pigmented newt from the same genus. In this way she was able to see which tadpole tissues came from the donor (the unpigmented newt) and which from the host (the pigmented newt). They found that only a tiny portion of the twinned tadpole tissue was unpigmented - the notochord (plus some additional cells here and there - the technique was not completely perfect). This meant two important things: 1) The dorsal lip of the blastopore develops into the notochord. 2) The notochord patterns the tissue around it to make the primary body axis¹⁵.

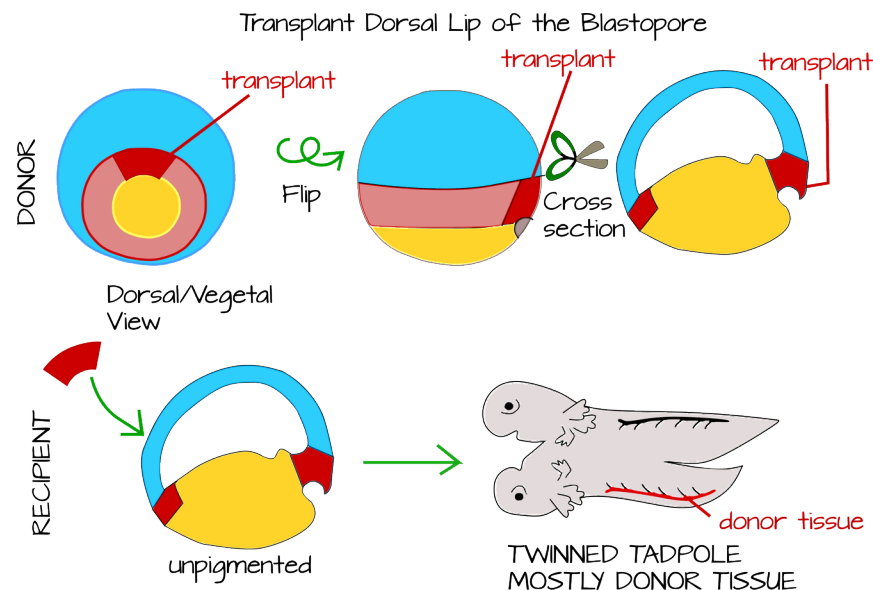


Figure 6: The Spemann-Mangold experiment. Transplantation of the dorsal lip of the blastopore from a donor to a host of a closely related species gave a second body axis. Most of this second body is made of host tissue. The donor tissue primarily forms the notochord. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/16/>

Gastrulation in Evolution

In general, gastrulation involves the involution and invagination of outer-layer cells to become internalized. Other outer layer cells epibolize to cover these internalizing cells. In this way, an embryo goes from a single cell type to multiple distinct cell layers. The place where this invagination or involution (where cells internalize) occur is called the blastopore. The blastopore can be a discrete "hole" in the embryo that elongates (called an archenteron) as in the case of sea urchins (Figure 7), or it can be a broad lip of cells that narrows as epibolizing cells expand as in frogs and snails (Figures 5 and 8).

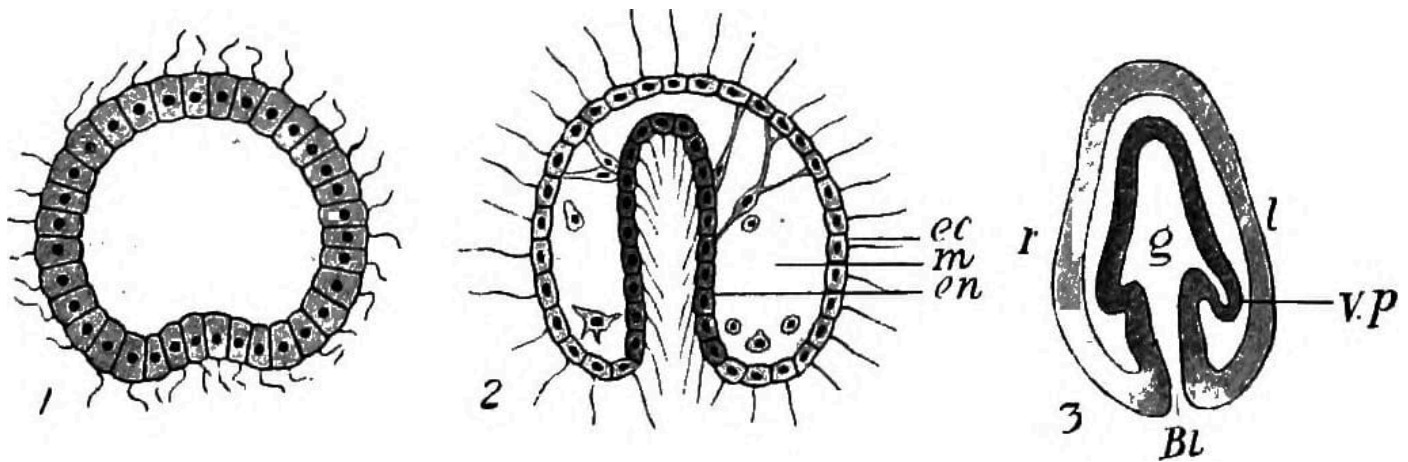


Figure 7: Gastrulation of a Sea Urchin from Outlines of Zoology by J. Arthur Thompson⁹ published under a [CC BY-NC-SA 4.0 license](#).

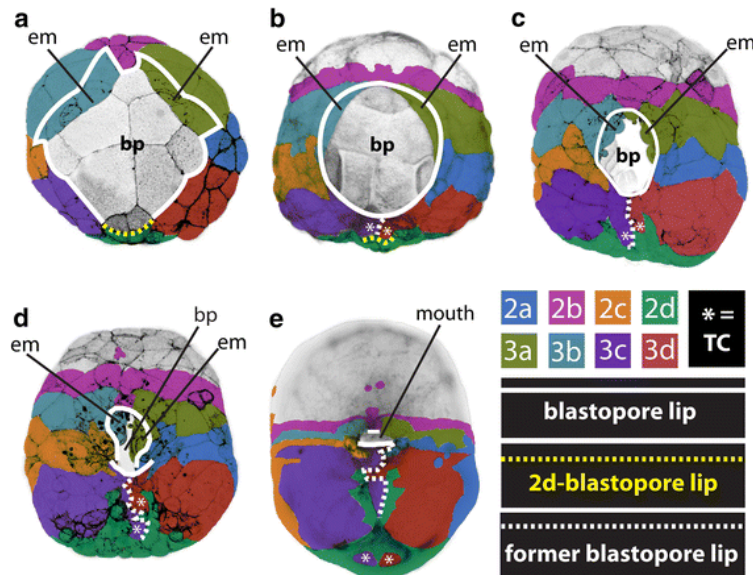


Figure 8: Snail blastopore. A-E are a time series of snail gastrulation viewed from the vegetal surface of the embryo. Colors represent different cell lineages (from cell fate mapping). The blastopore is outlined in white and yellow. The dotted white line shows where cells that originally formed the lip of the blastopore have now fused to close the blastopore as it shrank. Unlike frog and urchin gastrulation which occurs via a combination of internal movement (invagination, ingression, and involution) and external movement (epiboly), snail gastrulation occurs primarily through epiboly. From Spiralian Gastrulation by Dede Lyons et al.¹⁰ published under a [CC BY 4.0 license](#).

How can two such different structures be compared and even homologized? We consider gastrulation and blastopore formation to be homologous across Planulozoa (the group containing most animals, Figure 9) for two main reasons:

1. Other multicellular organisms do not undergo the massive tissue rearrangements we see in animal development. In this way, gastrulation represents an animal (or at least a Planulozoa) synapomorphy.
2. There are commonalities between various modes of gastrulation. The initial site of gastrulation is nearly always opposite the site of polar body extrusion, on the vegetal pole of the animal¹¹. And of course the end result of gastrulation - a tube with ectoderm lining the outside and endoderm lining the inside with mesoderm or mesenchyme in the middle - is clearly homologous among planulozoans.

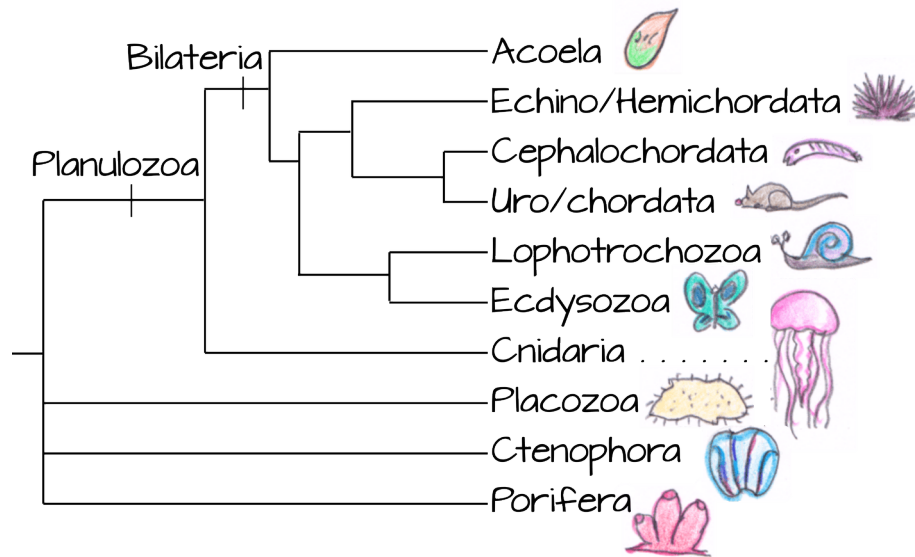


Figure 9: Animal relationships. I conservatively make the base of the tree a polytomy of Planulozoa, Placozoa, Ctenophora, and Porifera since the relationships between these phyla are not completely resolved. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/17/>

3.E: Cleavage and Gastrulation (Exercises)

At-home assignment

Make an illustration (by hand or computer-aided) for one of the topics/concepts we covered this week. Please cite your sources.

Group assignment

Choose a Bicoid model to explain to the class. Your explanation should include:

- 1) The location of mRNA and protein in the embryo
- 2) Any factors that impact the location of mRNA and protein (e.g. degradation)
- 3) How this model could be used to explain scaling in higher Dipterans (i.e. how can you use this model to build a small or a large embryo?)
- 4) How the model was tested and whether it held up to the test
- 5) BONUS: other experiments that researchers could run to test the model and what their results would tell us.

Discussion Questions and Reading Guide

The questions below were originally written for students reading *Freaks Of Nature* by Mark Blumberg and *Endless Forms* by Sean Carroll. However, the **questions in bold** are discussion questions that can be answered without these books.

The "Reading Guide" questions are appropriate for short homework answers and the "Discussion Guide" questions are appropriate for open in-class or online discussion.

Chapter 2 and 3 of Endless Forms

Reading Guide

1. **What is a morphogen? Give examples**
2. **What is a homeotic change?**
3. **What is polydactyly? Is it a homeotic change?**
4. How does the lac-z operon help explain cell-type differentiation?
5. What is the central dogma of molecular biology? At which step is the lac-z operon regulated?
6. What are the two features of gene logic in bacteria?
7. **What is a homeobox and how does it relate to homeotic transformations?**
8. Homeoboxes are incredibly conserved between disparate animal species, what kind of selection is this?
9. What is a conserved function of Pax-6 genes?
10. What does the Distalless gene do in general?
11. What does the tinman gene do in general?

Discussion Guide

1. **What experiments showed that the "dorsal lip of the blastopore" acts as an organizer tissue?**
2. What are the general properties of an organizer?
3. Freaks discussed the importance of environment and plasticity in Chapters 1 and 2, how does *Endless Forms'* discussion of hopeful monsters contrast with this?
4. **In homeotic fly mutants, the antennae/wing look fairly normal - why is it that we recognize them as normal structures in the wrong place?**
5. **How do cells get differentiated and why is it important?**

6. Explain the lac-z operon using the terms "cis" and "trans"
7. What did Monod mean when he said "What is true for *E. coli* is also true for the elephant."?
8. **What are some similarities between building a fruit fly and building a human?**
9. What is similar between Tinman, Pax6, Dll, and Hox genes?
10. What is the "genetic toolkit" and what are its implications for evolution?

3.S: Cleavage and Gastrulation (Summary)

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9. "Outlines of Zoology" (1899) J. Arthur Thompson [digitized version](#).
10. "Spiralian gastrulation: germ layer formation, morphogenesis, and fate of the blastopore in the slipper snail *Crepidula fornicata*" (2015) *BMC: EvoDevo* Deirdre C. Lyons, Kimberly J. Perry and Jonathan Q. Henry <https://doi.org/10.1186/s13227-015-0019-1>.
11. "Evolution of Development: The details are in the entrails" (2013) *Current Biology* Mark Martindale. DOI: <https://doi.org/10.1016/j.cub.2012.11.023>.
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15. "Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren" (1924) *Archiv für Mikroskopische Anatomie und Entwicklungsmechanik*, Hans Spemann and Hilde Mangold

Further Reading

- [Introduction to Cleavage and Gastrulation](http://www.ncbi.nlm.nih.gov/books/NBK9992/). www.ncbi.nlm.nih.gov/books/NBK9992/
- [Spiral cleavage and snail gastrulation](http://www.ncbi.nlm.nih.gov/books/NBK10074/). www.ncbi.nlm.nih.gov/books/NBK10074/
- [Why does gastrulation matter: changing cell-cell interactions](http://www.ncbi.nlm.nih.gov/books/NBK9968/). www.ncbi.nlm.nih.gov/books/NBK9968/
- [PAR proteins](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2964935/pdf/nihms34081.pdf). www.ncbi.nlm.nih.gov/pmc/articles/PMC2964935/pdf/nihms34081.pdf
- http://www.hhmi.ucla.edu/derobertis/teaching/lectures_2-3.pdf.
- Giant, Krüppel, and caudal act as gap genes with extensive roles in patterning the honeybee embryo. <https://www.sciencedirect.com/science/article/pii/S0012160609014055>

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4: GENETIC TOOLKIT

4.1: Introduction: The Big Picture

4.2: Signaling and Fate Restriction: Cell-cell communication - Within the cell

4.3: EvoDevo: How does this all evolve?

4.E: Genetic Toolkit Exercises

4.S: Genetic Toolkit (References)

4.1: Introduction: The Big Picture

We already began examining Developmental Genetics and got a taste of the complexity of genetic interactions. For example, the *Drosophila* gene Bicoid is transcribed into mRNA in maternal cells and pumped into the embryo. There its expression is regulated by localization signals on the mRNA, as well as degradation and localization of the protein. Bicoid protein itself acts as both a regulator of transcription and translation of downstream genes. The end effect is an embryo with different genes expressed along the A/P axis.

Now we will take a step back and look at a broader picture of Developmental Genetics, from the point of view of signaling pathways. A signaling pathway allows cells to communicate with their external environment. In Developmental Biology, this is usually cell-cell interactions. These kinds of interactions are incredibly important because each cell needs to follow its own developmental trajectory in coordination with all the cells around it. For example, imagine a growing mammalian limb bud. Each cell in the limb bud needs to know if it is on the thumb or pinky side of the limb and how close to the body it is. Not only that, it also needs to know how long development has progressed. Imagine if an osteogenic cell started forming bone matrix early in limb development. It would not undergo enough cell divisions to make the correct number of osteoblasts, leading to too little bone and an animal with a malformed limb.

The Genetic Toolkit

(An introductory PDF can be found here: Rediscovering Biology www.learner.org/courses/biology/pdf/7_gendev.pdf)

One of the major findings of Evo-Devo is that organisms share a common set of genes to build their very diverse bodies. In particular, animals share a suite of "body plan" genes that perform similar functions in development. Mixing and matching different gene expression patterns across tissue and cell types seems to be the primary mechanism for generating the unique bodies we see in each animal phylum. Novel evolutionary features (like spines, feathers, chambered hearts, tentacles, etc) appear to rely on the reuse of toolkit genes at new times and places in development (see Endless Forms for more details). Below, we will start to take a look at the genetics behind this genetic toolkit - how do these genes interact with each other?

In Evo/Devo, there are two main ways we can think about genetic interactions, but since they are interconnected so we will consider both simultaneously. One is Signal Transduction Pathways, which have an external signal (in Developmental Biology this is usually a secreted protein) that is usually received by a cell-membrane receptor that only certain cells express. When the ligand (secreted protein) and receptor bind, this triggers a cascade of protein modifications that lead to transcriptional activation of downstream genes (Figure 1).

The second way Evo/Devo thinks about signaling is Gene Regulatory Networks (GRNs). This includes the signal transduction pathway and its downstream effects on gene expression. If we turn up the expression of one gene, what other genes does it affect? GRNs are often represented as wiring diagrams with arrows and bars showing the effect of proteins on the expression of downstream genes (Figure 2).

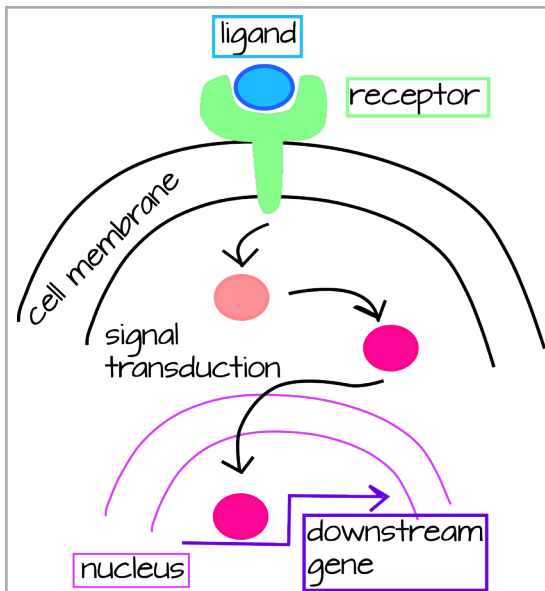


Figure 1: Generic signal transduction pathway. An extracellular ligand binds to a membrane-bound receptor triggering the activation of a signal transduction cascade in the cytoplasm. This cascade may be simple (as in the case of the Notch pathway below) or very complex requiring multiple steps and types of input. The final step of the transduction cascade is translocation of a cytoplasmic transcription factor to the nucleus where it will activate or inhibit the transcription of a downstream gene. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/18/>

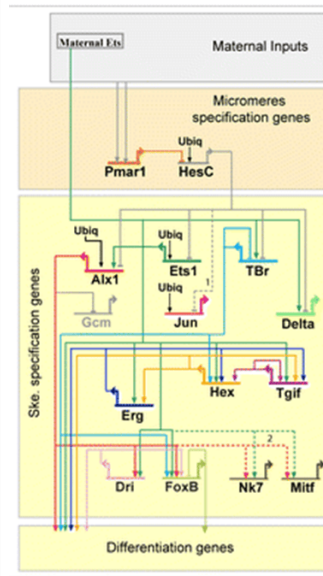


Figure 2: Partial Gene Regulatory Network wiring diagram for the specification of skeleton-forming mesoderm in the sea urchin, *Strongylocentrotus purpuratus*. Each gene is represented by a horizontal line attached to an arrow of the same unique color. The horizontal line indicates the cis-regulatory region of the gene and the arrow indicates transcription of the mRNA coding region. Lines emanating from the mRNA coding region indicate how the protein product of the gene regulates other downstream genes. Inputs onto the cis-regulatory regions by the protein products of other genes may be activating (arrow) or inhibitory (bar). “Ubiqu” is unknown activators expressed in all cells. Figure from Dylus et al¹ Published under a [CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/). Figure has been cropped from original.

The big picture: Cell fates are progressively restricted as development proceeds

Over the course of development all organs, tissues, and cell-types must be specified. In animals this is accomplished by repeated fate restriction². For example, the totipotent embryo undergoes several rounds of division to form a blastula (ball of cells). Some of these cells will take on a mesodermal fate. At this point, they can become any type of mesodermal cell. In some animals experiments have been performed to show that a generic mesodermal cell can be induced by its neighbors to develop into a huge variety of mesodermal cell types³. Over time, however, these mesodermal cells divide, undergo shape changes, migrate, and differentiate. As they receive signals from their environment (mostly the cells around them) they differentiate into more specific cell types. For example, Figure 3 shows a mesodermal cell differentiating into muscle, bone, and blood cells. As time progresses, different muscle progenitor (myogenic) cells will differentiate into different types of muscle, for example smooth and striated. Each of these cell-fate decisions is dependent on the gene expression pattern in the cell, which is dependent on the cell's history and its neighbors.

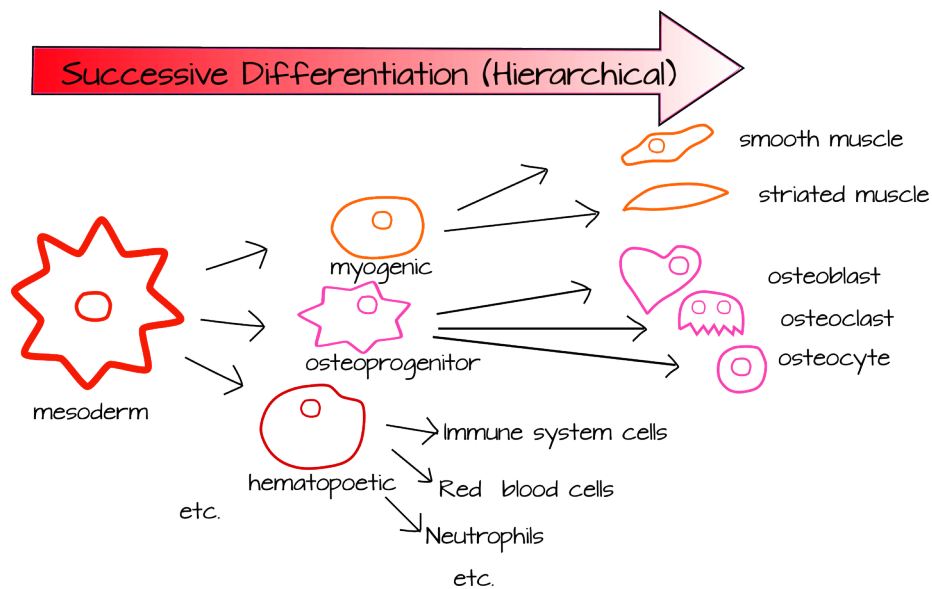


Figure 3: Differentiation of a mesodermal cell. Time flows from the left to right. Cells become more differentiated to the right. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/19/>

We call this type of progressive differentiation "hierarchical" with more generic cell types (like mesoderm, Figure 3) at the top of the hierarchy and more specific cell types (like neutrophils) at the bottom⁴. As development progresses, we move down the hierarchy. Development begins axis specification, these axes are soon read out into regions - for example head, trunk/abdomen, and tail. Within these different regions tissues get specified, first at the level of germ-layers and later at more specific levels. For example, ectoderm splits into neuroectoderm and epidermal ectoderm depending on the location of the tissue. Next, cell-types within the tissues are specified. For example, neuroectoderm can become neurons or glial cells. Both of these cell types later differentiate into the many specific types of neurons and glia. At this point, cells are more or less terminally differentiated - their adult fates are specified and they cannot return to pluripotent forms. However, most cells are still receptive to the environment and can respond to environmental signals even once they are terminally differentiated. We refer to this as cellular plasticity.

The GRN in Figure 2 mirrors this hierarchy, with early acting genes at the top specifying more general tissues and later acting genes at the bottom specifying specific cell types. However, it is important to note that the genes acting at these different hierarchical levels are reused at other levels of the hierarchy and in other developing tissues. In fact, core parts of GRNs and signal transduction pathways are reused throughout development and have different outputs depending on their context.

4.2: Signaling and Fate Restriction: Cell-cell communication - Within the cell

Cell-cell communication: Signal transduction pathways

Signal transduction pathways couple external signals to changes in gene expression within a cell. That is, an external signal triggers a cascade of biochemical changes in the cell resulting in higher or lower transcription of a set of genes. Fewer than a dozen major signal transduction pathways commonly regulate animal development^{5,6}. Here, we will focus on four of these named after their receptor or ligand: Notch, Hedgehog (Hh), TGF- β (also called Dpp or BMP), and Wnt (Figure 4). These signal transduction pathways act as **switches** for Gene Regulatory Networks (discussed below) which they turn on using different mechanisms. Two main factors affect what types of patterning and specification processes a particular Signal Transduction Pathway is good for.

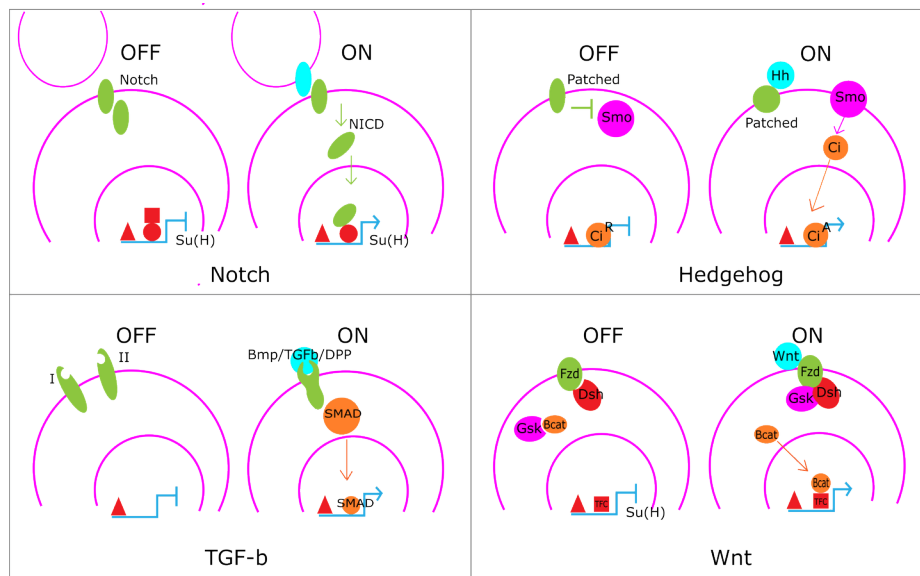


Figure 4: Major developmental signaling pathways by Ajna Rivera and Kristina Vu. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/20/>

First, how is the signal communicated? Does it use a long-range ligand (**paracrine** signaling), does it require cell-to-cell contact (**juxtacrine** signaling), or does the ligand act on the same cell it was secreted from (**autocrine** signaling)? Long-range paracrine pathways, like TGF- β and Wnt are great at forming gradients across a large tissue and are often used as early morphogens patterning body axes or multiple cell types across a tissue. Short-range paracrine pathways, like Hh, often act as morphogens on a smaller scale - fine-tuning regionalization patterns. Other factors modify the signaling range of these ligands, for example the amount of ligand secreted, neutralization by extracellular matrix proteins, the number of responsive cells, and whether responsive cells are expressing inhibitors or coactivators of the target genes⁶. Juxtacrine signals, like the Notch pathway, involve the association of two membrane-bound receptors. In the case of Notch, this is typically a Delta/Serrate/Lag-12 (DSL)-class ligand and a full length glycosylated Notch protein. The Notch pathway is often used in on/off cell-fate decisions, famously in lateral-inhibition where an "on" signal in one cell triggers an "off" signal in all the surrounding cells.

The second important factor governing the utility of a Signal Transduction Pathway is its regulation. Most pathways can be regulated to some extent by their downstream target genes either through negative or positive feedback. In negative feedback the downstream target genes eventually turn the pathway off. For example, a signaling pathway could increase the transcription of a pathway inhibitor. In positive feedback they downstream targets keep the pathway on. For example, a signaling pathway could increase the transcription of its own receptor. Thus, negative feedback is good for promoting a transient one-time signal, while positive feedback converts a transient signal into a permanent cell-fate decision. Pathways can also be regulated by each other and their output can be modified by local transcription factors, which can differ among cell types. This topic is covered more extensively in Three habits of highly effective signaling pathways, by Borolo and Posakony.

Within the cell: Gene regulatory networks

In the early 2000s, Eric Davidson and Isabelle Peter wrote a series of papers proposing a philosophical framework for understanding Gene Regulatory Networks (GRNs). In 2011 they wrote a seminal paper on the evolution of these networks. They defined three basic types of core genetic interaction that are used to specify cell-types:

1. **kernels:** these are evolutionarily inflexible interactions that specify a body part.
2. **batteries:** These are involved in cell or tissue differentiation and are more flexible evolutionarily than kernels.
3. **plug-ins:** these are small sub-circuits that get used in many different developmental contexts⁷.

As a mother to Lego enthusiasts, I use Lego kits as an analogy to understand this. A kernel would be specialized pieces that go together, like a pair of wheels and an axle. They specify a particular function and are inflexible in their interaction - one wheel must be snapped onto each side of the axle to make it useful. A "battery" is more flexible but still usually used for a similar set of purposes. An example of this would be a literal battery pack in a Lego kit - it is used to power movement but is flexible in that it can power many types of constructions. A plug-in is similar to standard bricks, these can be used to build whatever cuboid objects your imagination suggests. In order to make fancy constructions, however, you also require kernels and batteries.

How do we link these types of interactions to a developing embryo? Peter and Davidson conceive of animal development as being alternating steps of **patterning** and **specification**⁷. Patterning is dividing up a body or a set of tissues into smaller parts. Specification is a fate choice for the cell or tissue that was patterned. In this conception, the body axes and germ layers are first defined using body patterning genetic networks. Next, each section of the body is specified: neuroectoderm, gut, pharyngeal arches (in chordates), circulatory system, etc. Then, another round of patterning of these specified units occurs - each body part now gets patterned along its own axes. For example, the tips of the fingers vs an elbow or the anterior vs. the posterior of the heart (Figure 5).⁸

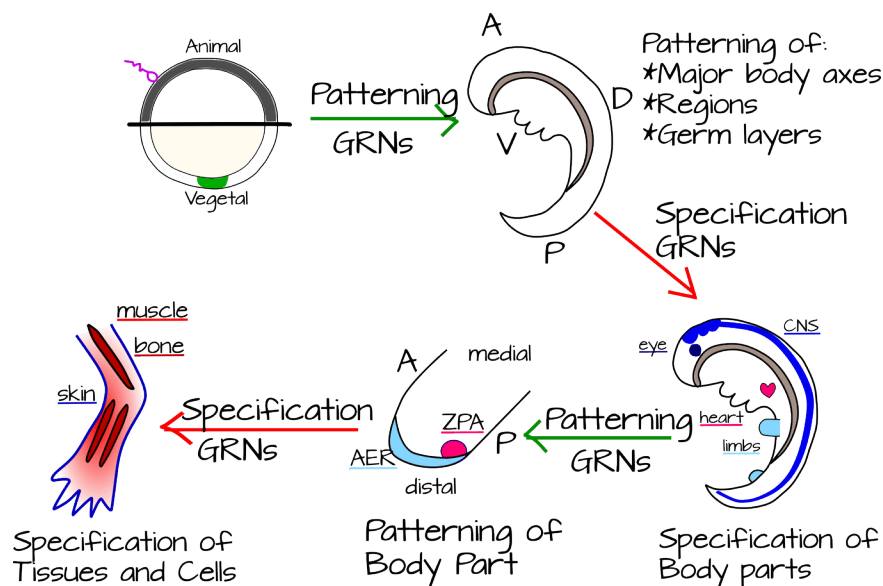


Figure 5: Patterning and Specification. Gene regulatory networks are genes (and their products) that interact with each other. Patterning GRNs specify axes, regions, and broad tissue types. Specification GRNs take the positional information from the pattern and specify cell, tissue, and organ types. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/21/>

4.3: EvoDevo: How does this all evolve?

The system of Signal Transduction and GRNs is incredibly flexible. Even though there are conserved units (like kernels and signal transduction pathways) the way these conserved units are connected to each other can be diverse both within a developing embryo and between different species. As new interactions evolve, this can lead to novelty. For example, the yellow box in Figure 2 contains a GRN battery that is used in skeleton formation across echinoderm classes. In the sea urchin shown this has been reused to make a special larval skeleton derived from a group of cells called the micromeres^{1,8}. Loss of features can also be correlated with changes to connections between the genes in GRNs. For example, the gene *pitx1* is a switch for skeleton patterning (it is a transcription factor that controls a battery that patterns mesodermal tissues)⁹. Loss of a cis regulatory element that turns it on in pelvic regions leads to a reduced pelvis. This mutation is common in freshwater fish populations, where a smaller pelvis may be beneficial, but not in marine fish populations, where a more robust pelvis may be beneficial¹⁰ (Figure 6).

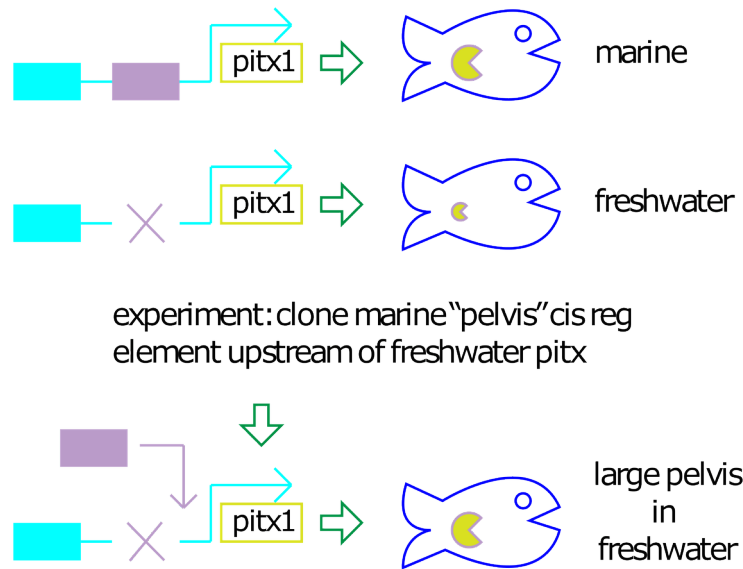


Figure 6: Evolution of a novel enhancer element by Amanda Lo and Ajna Rivera an editable version of this figure can be downloaded at: <https://scholarlycommons.pacific.edu/open-images/22/>

There are many more examples of GRN evolution leading to changes in body plan, and we will cover some of them later. But I would like to mention one final example that you are already familiar with. The Hox genes are an animal synapomorphy, that is they are unique to the animal evolutionary lineage and found in most animals. In animals where Hox genes have been genetically examined, Hox genes are often involved in the same process - early regionalization along the A/P axis. Different sets of Hox genes are turned on at different locations along the A/P axis of the animal body. Interestingly, this appears to be an ancient conserved kernel for body patterning since many diverse species exhibit the same patterning (Figure 7).

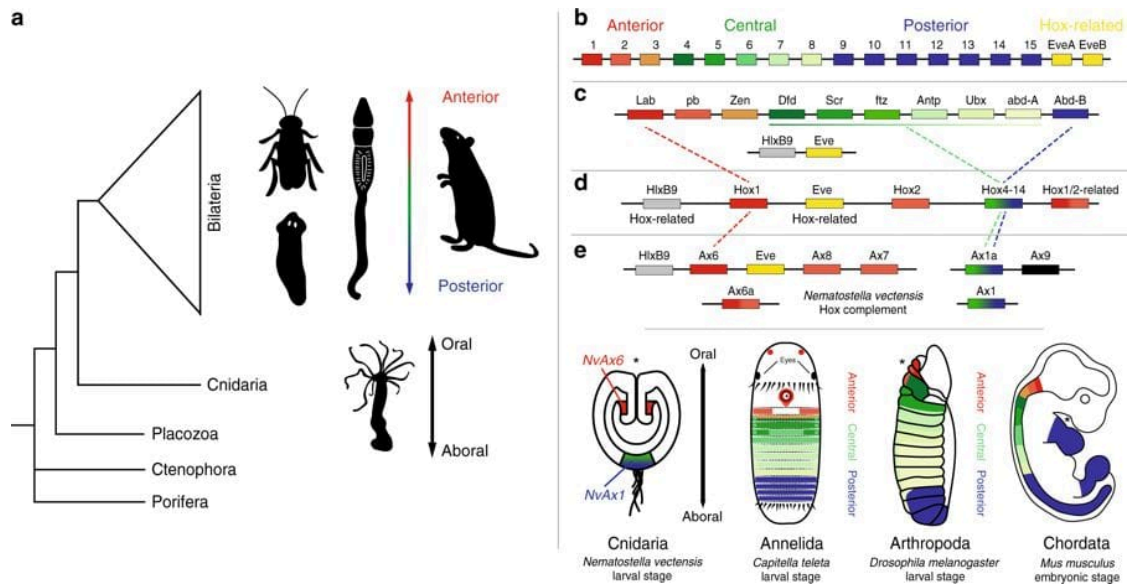


Figure 7: Hox genes and their expression patterns in Cnidaria and Bilateria. From DuBuc et al, 2018 published under a [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/) license

4.E: Genetic Toolkit Exercises

Journal Club

This week's reading is [Three Habits of Highly Effective Signaling Pathways](#)

Reading Guide Questions

Signaling flips a switch

1. What are the three habits of signaling pathways?
2. What is an SPRE and what is its significance in signaling pathways?
3. What might be the purpose of experimentally activating a signaling pathway?

Activator Insufficiency

4. Define activator insufficiency and zones of competence
5. How do the authors explain the finding that SPREs are insufficient in vitro, but sufficient in cultured cells?

Cooperative Activation

6. What is a signal-independent activator?
7. What is the purpose of co-activators?

Default Repression

8. How do developing organisms solve the problem of leaky expression?
9. Compare two types of default repression and how signaling releases the repression

Functional/mechanistic/evolutionary considerations

10. What role does chromatin modification play in developmental signal transduction cascades?

Class Discussion Questions

Signaling flips a switch

1. Chose one habit to illustrate with a drawing.
2. Explain how Notch, Wnt and Hh accomplish transcriptional switching.

Activator Insufficiency

3. What do the authors mean when they say that signaling pathways exhibit "selective transcriptional responsiveness of target genes to pathway activity."?
4. What ways can cells limit the activation of a target gene by an activated signaling pathway?

Cooperative Activation

5. What is a potential drawback to cooperative activation?

Default Repression

6. Illustrate the different types of default repression
7. Illustrate one example of default repression

Functional/mechanistic/evolutionary considerations

8. Explain Figure 5: how is it a summary of the output of signaling pathway habits?
9. Explain how Koide et al tested whether default repression is separate from activator insufficiency.

Group exercise

This HHMI activity explores the evolution of cis-regulatory elements. You can complete the exercise without watching the suggested movie (available on YouTube and the HHMI website).

[Modeling the Regulatory Switches of the Pitx1 gene in Stickleback Fish](#)

4.S: Genetic Toolkit (References)

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5: REGIONALIZATION AND ORGANIZERS

Animal development can be seen as a series of successive fate decisions where cells take internal and external (signals from other cells) information and use it to become progressively more specified. Regionalization refers to subdividing an existing embryo or tissue into smaller parts with unique fates. This can occur at a large scale, for example Bicoid and Nanos broadly regionalizing the embryo into anterior, middle, and posterior, or it can occur at a small scale, for example Shh regionalizing an autopod (hand) into thumb and pinky sides. Regionalization typically occurs in three main steps: First, a morphogen broadly patterns a tissue by forming a gradient. Next, this gradient is read out by a series of transcription and translation factors that turn the gradient into discrete domains of gene expression. Finally (or concurrently) the cells expressing these unique combinations of genes are fated and begin to exhibit different properties. This chapter first broadly examines examples of regionalization and specification and then considers the role of organizers in these processes.

[5.1: Splitting up the A/P axis: Beginning-Hox Genes, Another Level of Regionalization](#)

[5.2: Organizers-Other Organizers](#)

[5.S: Regionalization and Organizers \(References\)](#)

5.1: Splitting up the A/P axis: Beginning-Hox Genes, Another Level of Regionalization

Broad Scale Regionalization: Hox Genes and the Segmentation Cascade

We already took a quick look at Hox genes, both in your readings and in the [Genetic Toolkit](#) section. Now we will put them into a broader Evo-Devo context by looking at a specific example of Hox patterning (following up the [The curious case of Bicoid](#) story) and by seeing evidence that regionalization by Hox genes is conserved across animals. Some cis-regulatory elements driving Hox gene expression patterns are also conserved. However, the earlier developmental events that set up Hox expression domains are not conserved across animals, they differ widely sometimes even within a phylum. For example, even though all arthropods are segmented in *Drosophila* segments are patterned simultaneously, while in some other insects and arthropods they arise one at a time. The conservation of the Hox pattern despite the variability of broader A/P pattern generation suggests that Hox patterning represents a developmental constraint in animal evolution. That is, once the Hox pattern evolved, it was so advantageous that it was mostly retained for millions of years of animal evolution.

We focus on *Drosophila* even though it is an odd case of simultaneous regionalization because it is very well-studied and easier to understand than many other model organisms. Our look at *Drosophila* A/P regionalization and patterning will illustrate three major concepts in Evo-Devo:

1. There are multiple ways to get to constrained developmental stages.
2. Much of development occurs by repeatedly dividing up tissues/regions and specifying them into more restricted fates.
3. Multiple gene expression outputs can overlies each other to give a cell/tissue its specific identity.

In cleavage and gastrulation, we looked at the idea of symmetry breaking in creating axes and germ layers in animals. We also saw that opposing Bicoid and Nanos protein gradients are able to specify the first anteroposterior regionalization in developing fruit flies. But if we think about an adult fly, they have many more anteroposterior regions than simply a head and a tail. For example, only some segments bear wings or legs, other bear eyes or antennae and others have genitalia or excretory organs. How does the developing fly "regionalize" - that is go from having two opposing anteroposterior gradients to having fine-scale anteroposterior patterning? To answer this, we will look at the developmental genetics of the segmentation cascade - the series of steps that divides the embryonic fly into finer and finer subdivisions.

The *Drosophila* Segmentation Cascade

Here I will very briefly summarize the segmentation cascade, for more details please visit [The Origins of Anterior-Posterior Polarity](#)

The segmentation cascade is hierarchical in both developmental time and regulation of gene expression. Maternal effect genes are expressed earliest and they regulate the gap genes. Gap genes are expressed next, acting with the maternal effect genes to regulate the pair-rule genes. Pair-rule genes and gap genes regulate the expression of the segment-polarity genes, which are expressed last (Figure 1).

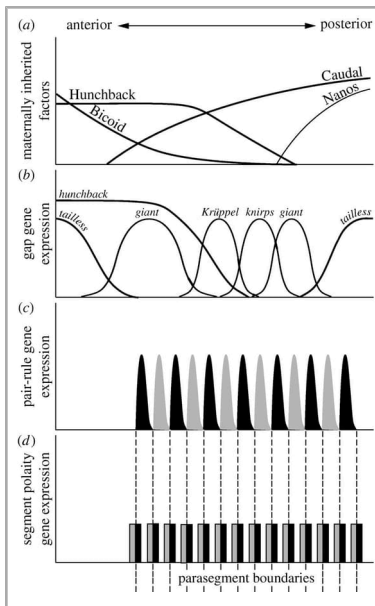


Figure 1: The Drosophila segmentation cascade. Drosophila divides up its segments all at once, but most arthropods (an animals in general) grow from a posterior growth zone where anterior-most regions develop first. Consider what parts of this expression and specification hierarchy might be conserved across different modes of development and which parts could not be.

From "The evolution of developmental gene networks: lessons from comparative studies on holometabolous insects" Andrew Peel, 2008 in Philosophical Transactions of the Royal Society, B. Published under The Royal Society Academic Institution Single Site Licence DOI: 10.1098/rstb.2007.2244

Maternal effect genes: Bicoid, Nanos, Hunchback, and a handful of other Drosophila genes are known as "maternal effect" genes. This means that if you mutate them in a female fly, the mutant phenotype will be seen in her offspring. Since you already know that Bicoid and Nanos mRNAs are made by the mother's cells and pumped into the oocyte before fertilization it should be pretty clear why this is. The maternal effect genes set up broad anterior and posterior regions in the fruit fly as well as dorsal/ventral regions (which we will not cover here, though it's a pretty juicy story).

Gap genes: The maternal effect genes are transcription and translation factors that affect the expression of the gap genes. The gap genes are transcription factors that are expressed in broad strips along the A/P axis (Figure 2). They are called gap genes because if one is mutated it leads to a "gap" in the embryo - the larva will be missing a large portion of its body².

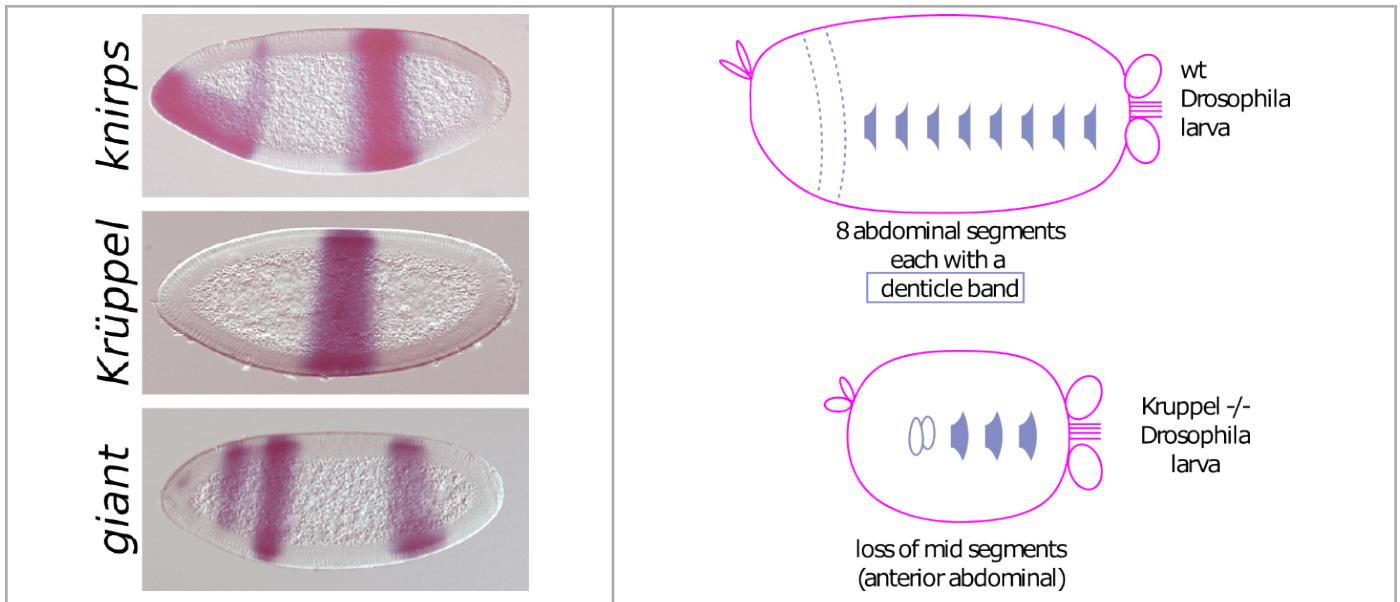


Figure 2: Gap genes. Left panel shows mRNA expression of three Drosophila gap genes, from Wikimedia commons by user Celefin. This is a derivative of an original figure by Haecker A, Qi D, Lilja T, Moussian B, Andrioli LP, Luschnig S, Mannervik, M. DOI [10.1371/journal.pbio.0050145](https://doi.org/10.1371/journal.pbio.0050145) published under a [CC BY 2.5 license](https://creativecommons.org/licenses/by/2.5/) Right panel shows the effect of a homozygous *Krüppel* mutation in an embryo. Grey shapes are denticle bands (bristles) found in each abdominal segment. The *Krüppel* mutant has lost 6 of these bands, corresponding with *Krüppel* expression over the first 6 abdominal segments. Right panel by Amanda Lo and Ajna Rivera. An editable svg version of this figure is available at <https://scholarlycommons.pacific.edu/open-images/24/>

Pair-rule genes: Gap genes and maternal effect genes affect the expression of the pair-rule genes - which are each expressed either in odd or even segments². These are particularly interesting because these genes bridge the gap between regionalization and modularity. They are expressed in a modular pattern, but get their input from a regionalization pattern. Genetically, this means that the expression of each of these genes is activated by multiple combinations of gap and maternal effect proteins. For example, the even-skipped gene is expressed in 7 stripes along the A/P axis. It has 4 major cis-regulatory enhancers that control this. One element controlling stripes 3 and 7 has binding sites for Hunchback and Knirps, both of which repress the expression driven by the 3+7 enhancer (Figure 2). This enhancer is activated by the ubiquitous (expressed everywhere) activator Zelda³. The element controlling stripe 2 is even more complex with binding sites for Bicoid, Hunchback, Caudal, Kruppel, and Knirps (Figure 3)⁴.

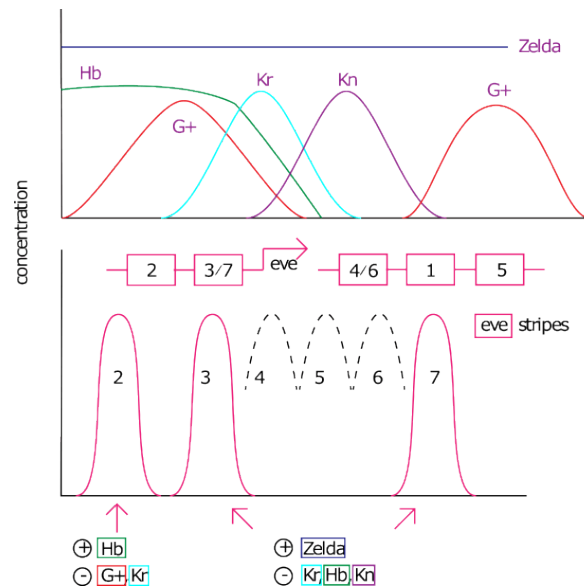


Figure by Amanda Lo and Ajna Rivera. An editable svg file of this figure is available at <https://scholarlycommons.pacific.edu/open-images/26/>

Segment polarity genes: Segment polarity genes are expressed in either the anterior or posterior of each segment and help to define segment boundaries as well as the A/P patterning of each segment (Figure 4). Like the pair-rule genes, these are expressed in stripes along the entire A/P axis, rather than in a regional pattern like Maternal Effect and Gap genes. The expression domains of the segment polarity genes are regulated largely by the pair-rule and gap genes.

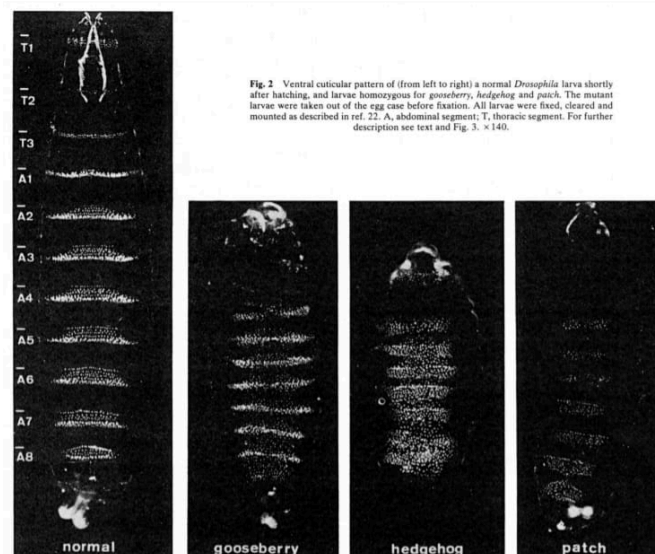


Figure used with permission from publisher.

Hox genes: Another Level of Regionalization

While the maternal effect genes and the gap genes regionalize the *Drosophila* embryo to some extent, the adult animal will have an even more regionalized body. The Hox gene pattern provides segment-level regionalization. It specifies where legs, wings, antennae, mouthparts, genitalia, and other unique structures will go (Figure 5). As you already know, most animals use the Hox genes to do A/P patterning and it's a bit of a mystery how and why so many diverse early developmental strategies coalesce onto the same A/P patterning, and then diverge into diverse body plans later in development.

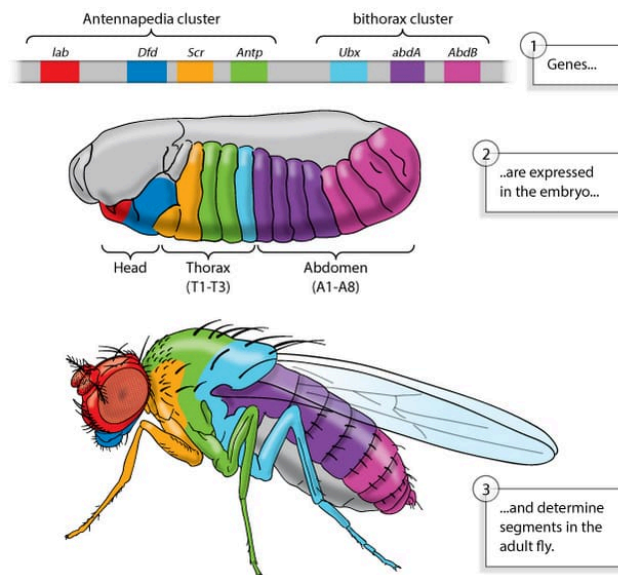


Figure 5: Hox gene patterning in *Drosophila*. From "Genetic Signaling: Transcription Factor Cascades and Segmentation" Nature Education, 2008¹ © 2013 **Nature Education** All rights reserved. You may reproduce this figure, without modifications, in print or electronic form for your personal, non-commercial purposes or for non-commercial use in an educational environment.

How conserved is the Hox patterning? First, nearly all animals have Hox genes and many of them are syntenic - meaning that the order of the Hox genes is conserved along the chromosome. This is related to the regulation of the Hox genes, they have distal regulatory factors (one of which we will see later in [Ectodermal Appendages](#)) but also have proximal regulatory factors. In vertebrates, some genes in the cluster can share regulatory factors⁵. Because of this, gene order matters. An analogy is: if you share a car with your neighbor, you cannot move to a new neighborhood without losing access to the car. This sharing of elements is an evolutionary constraints that has resulted in the conservation of these regulatory factors and the preservation of synteny of the Hox genes. In invertebrate lineages, synteny may be preserved due to tight clustering of the genes with each other and their proximal cis regulatory elements⁵. Some species and lineages however, have lost this conserved synteny, notably the nematodes and the tunicates⁵. Despite these losses, synteny still seems to be the rule with at least some conservation of gene order found in most phyla examined, including the most basally branching Hox-containing phylum, the cnidarians⁶.

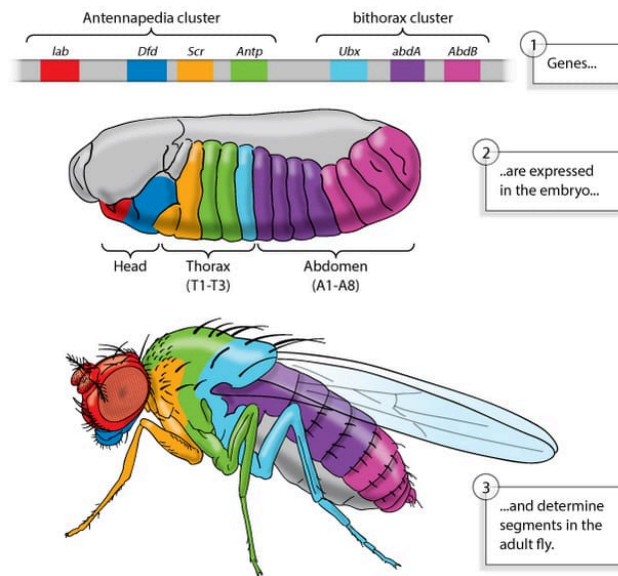


Figure from DuBuq et al, 2018 published under a [CC 4.0 BY license](#)

Cnidarians (such as jellies and sea anemones) are an interesting case in which to look at the Hox genes because while the Hox genes are known to be conserved A/P patterning elements, the cnidarians lack an A/P axis. How can they be missing something so important? Well it's because of how we define anterior and posterior. A standard definition is that anterior is towards the head and posterior is towards the tail. What about animals that don't have an obvious head? We count their mouth and any conglomeration of major sensory organs as the head end and keep moving along their long axis to the nearest tip of the body. Typically, along the long axis of the body, the tip nearest the mouth is the anterior end and the tip nearest the anus is the posterior end. Cnidarians, however, not only lack a head they also lack major sensory organs and a mouth. Instead they have a ring of tentacles (that can point up or down depending on the species) that surrounds a single opening that serves as a mouth and anus.

Many evolutionary scenarios have been proposed to homologize the cnidarian "radial" axis with the A/P and D/V bilaterian axes. Hox genes are one way to look at this. DuBuc et al⁶ found that cnidarian homologs of anterior Hox genes (red in Figures 6 and 7) are expressed at the oral end of an anemone (Cnidaria) embryo and cnidarian homologs of central/posterior Hox genes (blue/green in Figure 6) are expressed at the aboral end (the end opposite the mouth/anus). They also found that Wnt, a signal transduction gene involved in many developmental processes including anteroposterior specification, was expressed between the expression domains of Ax6, an anterior Hox gene, and Ax1, a posterior Hox gene

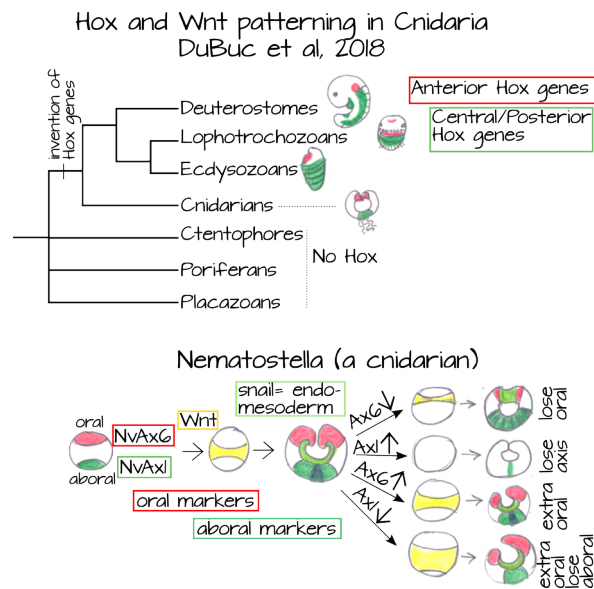


Figure 7: Homologizing the oral/aboral axis in Cnidaria to the anteroposterior axis in Bilateria. The top panel shows anterior (red) and central/posterior (dark green) Hox gene expression patterns in representative Bilateria, including a vertebrate, an annelid, and an insect. Bottom panel shows the results of perturbation experiments in *Nematostella*. DuBuc et al raised (up arrow) or lowered (down arrow) the expression levels of an anterior Hox gene (Ax6) and a posterior Hox gene (Ax1). They found that lowering Ax6 or raising Ax1 caused a loss of anterior structures and a mispatterning of the endomesoderm. Raising Ax6 or lowering Ax1 caused the formation of extra oral tissue and lowering Ax1 additionally caused the loss of aboral structures. Together these data support the homology between the oral/aboral axis of Cnidaria to the anteroposterior axis of Bilateria⁶. An editable svg file of this figure is available at <https://scholarlycommons.pacific.edu/open-images/25/>

5.2: Organizers-Other Organizers

Organizers

One of the most interesting things about building animal bodies is the diversity we see across and within bodies. For example, even though the Hox gene patterning in fruit flies and mice is very similar, the end results (an adult mouse or an adult fly) are extremely different. We see intriguing diversity within bodies as well. For example, a thoracic segment in a fruit fly might express a single Hox gene across a segment, yet parts of this segment take on many different forms. Much of this differentiation is ruled by local organizers and master control genes. These are locally expressed genes that are often expressed in many segments in similar spots. Depending on the Hox gene expressed in that segment, they will activate different body parts. For example, we will look later on at wing master control genes -these genes build wings in thoracic segments and different structures, like gin traps, in other segments. The type of wing - elytra, haltere, flying wing, etc. is also dependent on the position of the segment in the body and the species it is expressed in. For example, dragonflies have two flying wings, while the first wing in a beetle is a protective elytron, and the second wing in a fly is a proprioceptive organ.

This type of programming is known as **serial homology**. We will be coming back to this concept later, but it is a conceptually important idea in Evo-Devo. Serial homologs are a special type of homolog wherein the same type of tissue expressing a core set of genetic regulators (an organizer) is found in multiple spots along a body axis. One easy to understand example of serial homologs are arms and legs. Developmentally, they are nearly identical for the first part of their growth, they only grow substantial differences later. They express the same core limb genes (as we will soon consider) and have the same bones, albeit in slightly different shapes. Serial homologs are incredibly important in our understanding of evolution because they are genetically "cheap," since they use the same core gene regulatory network, but they can provide novel functions -like tool use plus locomotion. The fate of a serial homolog often depends on the Hox genes that are expressed in the region of the serial homolog organizer.

Organizer outputs are not only affected by the Hox code, they can also themselves set up the Hox code (as in the case of Nanos and Bicoid) or can set up a different axis (like the dorsoventral axis of a frog or the anteroposterior axis of a limb). Now that you have a firm grasp of gene regulatory networks, we can take a closer look at a famous organizer we have already considered - the Spemann-Mangold organizer of the frog gastrula.

The genetics of the Spemann-Mangold organizer and the notochord

If you need a quick refresher on the Spemann-Mangold organizer, check out Cleavage and Gastrulation. Briefly, the Spemann-Mangold organizer is mesoderm found at the position of the grey crescent, the dorsal pole of the frog (or newt) embryo. This organizer, as it develops into the notochord, induces the formation of dorsal structures like the central nervous system and spine. Amphibians with an extra Spemann-Mangold organizer grow a second A/P axis (conjoined twin tadpoles) and amphibians missing a Spemann-Mangold organizer develop into a "belly piece."

Using genetics, we can explain two huge questions: 1) What causes the organizer to develop? and 2) How does it induce the formation of dorsal structures? The first step in organizer formation is cortical rotation itself, as shown in Figure 1 of Cortical Rotation. In cortical rotation, microtubules rotate the outer cortex of the fertilized egg relative to the inner mass. The vegetal pole has several localized proteins and mRNAs tethered to its cytoskeleton. In the inner mass this includes VegT and Vg1^{7,8}. The outer cortex has vegetally localized Dishevelled (Dsh) protein, a component of the Wnt pathway, which is transported towards the animal pole during cortical rotation⁹. The displacement of Dsh creates a new zone - a part of the embryo with Dsh, but no Vg1 or VegT (Figure 8)¹⁰. Dsh helps localize and stabilize β -catenin, the transcription factor of the canonical Wnt pathway. Now we have Vg1 and VegT at the vegetal pole and Dsh and β -catenin at the future dorsal end.

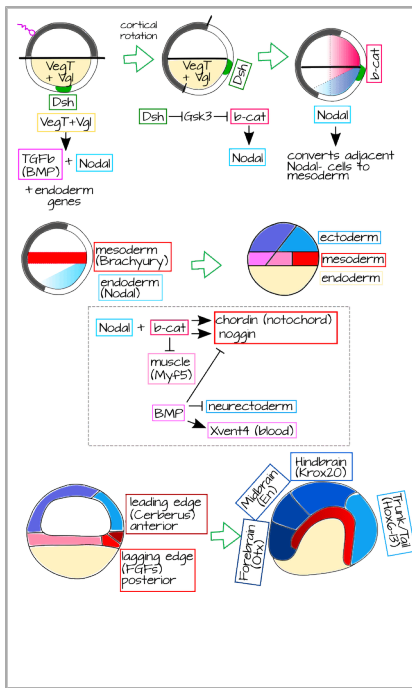


Figure 8: Genetics of the Spemann-Mangold organizer. Maternally derived proteins set up an Animal/Vegetal axis in the unfertilized egg, including VegT and Vg1 in the Vegetal pole inner cortex (orange). After fertilization, cortical rotation brings the Vegetal pole outer cortex protein Dishevelled (Dsh, green) into contact with the Animal inner cortex, driving the accumulation of b-catenin in dorsal cells. Vegetal pole proteins activate the expression of TGFb (BMP) and Nodal. Nodal converts the adjacent Nodal+ cells into mesoderm (red), resulting in a stripe of mesoderm just above the vegetal pole. Meanwhile, b-catenin enhances Nodal expression in the dorsal-most part of the embryo - resulting in high Nodal expression in the dorsal vegetal inner cortex. This, plus b-catenin, drives the expression of organizer genes like Chordin and Noggin, while repressing ventral mesoderm fates like muscle. Chordin and Noggin specify the notochord, which will elongate and induce the dorsal ectoderm (all ectoderm is blue) that lies over it to become neurectoderm. BMP represses organizer activity on the ventral side of the embryo, in particular ventral ectoderm will form skin, not neurectoderm. The notochord itself has a leading (anterior) and lagging (posterior) edge which express different genes and will induce different parts of the central nervous system.

An editable svg file of this figure is available at <https://scholarlycommons.pacific.edu/open-images/27/>

As cleavage proceeds, VegT and Vg1 induce the expression of Nodal (specifically *Xenopus* nodal related protein or Xnr) in the vegetal-most cells. These cells will become the endoderm. Nodal, a homolog of TGFb, signals to nearby cells lacking Nodal (Nodal-cells), these cells will become the mesoderm. Nodal expression is also induced by b-catenin. This makes a gradient of Nodal that is higher at the dorsal side, where it is induced by VegT and Vg1 as well as b-catenin. While b-catenin is stabilized in a wide swath of dorsal tissue, Nodal expression is restricted to the vegetal side of the embryo where it is expressed all over, but highest where b-catenin is present (Figure 8). Where Nodal signaling meets mesodermal cells with high b-catenin, we see the expression of a new suite of genes - the organizer genes Chordin, Noggin, Frizzled, and more (Figure 8)¹¹.

These organizer genes are transcription factors and members of signal transduction pathways. Some of them will specify the organizer mesoderm as "dorsal" (notochord and somites) and some of them will signal to the overlying ectoderm during gastrulation and specify it as "neural" rather than epidermal. The physical mechanics of this process are fascinating, and we can study them later if you are interested, but for now I will focus just on the genetics and briefly summarize the tissue movements. As you saw before, mesodermal organizer cells undergo involution at the dorsal lip of the blastopore and crawl along the overlying ectodermal cells to form a thick layer beneath them. Most of the mesoderm in the gastrulating embryo expresses BMP4, a TGFb family secreted protein. This BMP4 signals to the overlying ectoderm to become epidermis. However, the organizer expresses BMP4 antagonists, like Chordin and Noggin¹². This causes the ectoderm above the organizer mesoderm to take on neural fates.

The organizer itself is not a homogenous structure, it is made up of several cell populations that express different signaling genes and transcription factors. For example, the leading edge of the organizer (the part that will invaginate first and become the anterior-most notochord) expresses a Wnt and BMP4 antagonist called Cerberus¹³. Cerberus and other leading edge genes specify the anteriormost portions of the notochord via gene regulatory networks that will ultimately lead to expression of forebrain gene *Otx2*¹⁴ in the overlying ectoderm. Organizer cells trailing this leading edge express the secreted signaling molecule FGF, which drives the expression of midbrain gene *Krox20* in the ectoderm above it. Both *Otx2* and *Krox20* help make the Hox gene expression pattern, along with other organizer genes expressed in an anteroposterior pattern in the notochord¹⁴. In this way, the notochord is able to direct the anteroposterior patterning of the neural tube (Figure 8).

Other Organizers

There are a multitude of other organizers in developmental biology and their fundamental properties give us insights into the genetics of evolution. They are specified by external factors, for example by the intersection of Nodal-adjacent and b-catenin expressing cells in the Spemann-Mangold organizer. They act on other tissues via secreted signaling molecules, for example chordin inhibiting BMP4 to specify neurectoderm. And their activity on other tissues can vary over time and/or space, for example the ability of the notochord to induce both anterior and posterior nervous system structures.

The notochord has a third organizing activity on the ectoderm (not to mention its organizing activity on the mesoderm), this is patterning the D/V axis of the neural tube. During gastrulation, the ectoderm lying over the notochord responds to signals from it to fold up into a tube and sinks under the epidermis. This tube is polarized into the dorsal side (close to the overlying epidermis) and the ventral side (close to the notochord). A Shh signal from the notochord drives Shh secretion from the ventral side. This Shh gradient opposes a dorsal BMP (TGFb family) gradient initiated by epidermal signaling (Figure 9).

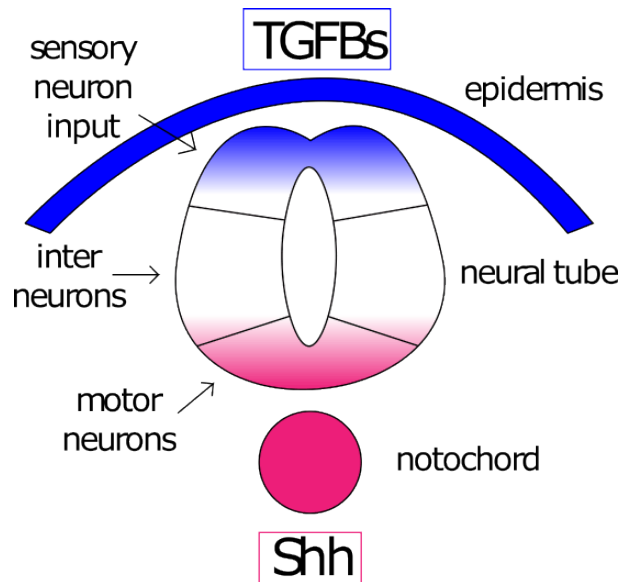


Figure 8: Opposing gradients pattern the neural tube.

TGFβs (blue) from the epidermis and Sonic Hedgehog (Shh, red) from the notochord pattern the dorsal/ventral axis of the neural tube. Neurons exposed to a high concentration of TGFβs will become sensory neurons, those exposed to high levels of Shh will become motor neurons. Neurons exposed to low levels of both will become interneurons. Figure by Amanda Lo and Ajna Rivera

An editable svg file of this figure is available at <https://scholarlycommons.pacific.edu/open-images/28/>

These two opposing gradients lead to gradients of active transcription factors responsive to these signal transduction pathways and the expression of genes with cis-regulatory elements responsive to these transcription factors (Figure 9)¹⁵. Le Dreu and Marti name 11 distinct domains identified by unique transcription factor expression and neuronal subtype¹⁵.

Another famous organizer is one that you encountered in Intro Bio: the tetrapod limb organizer. This is a two-part organizer, with a long thin swath of cells at the distal-most point of the limb expressing and secreting growth-promoting FGFs, and the posterior-most point of the limb expressing Sonic Hedgehog (Shh, Figure 10). The FGF portion of the limb is called the Apical Ectodermal Ridge. It is the signaling center that keeps the limb adding more distal elements onto the end. In this way, the more proximal elements, like the humerus and elbow, develop first. More distal elements, like forearm, wrist, and hands, get laid down later. Interestingly, this system seems to be on a timer, rather than being strictly spatially patterned. If cell-division is inhibited midway through arm development, radius, ulna, and humerus can be small or absent, with a normally proportioned hand and shoulder girdle. This looks somewhat similar to gap mutant in *Drosophila*, though the developmental mechanism is completely different.

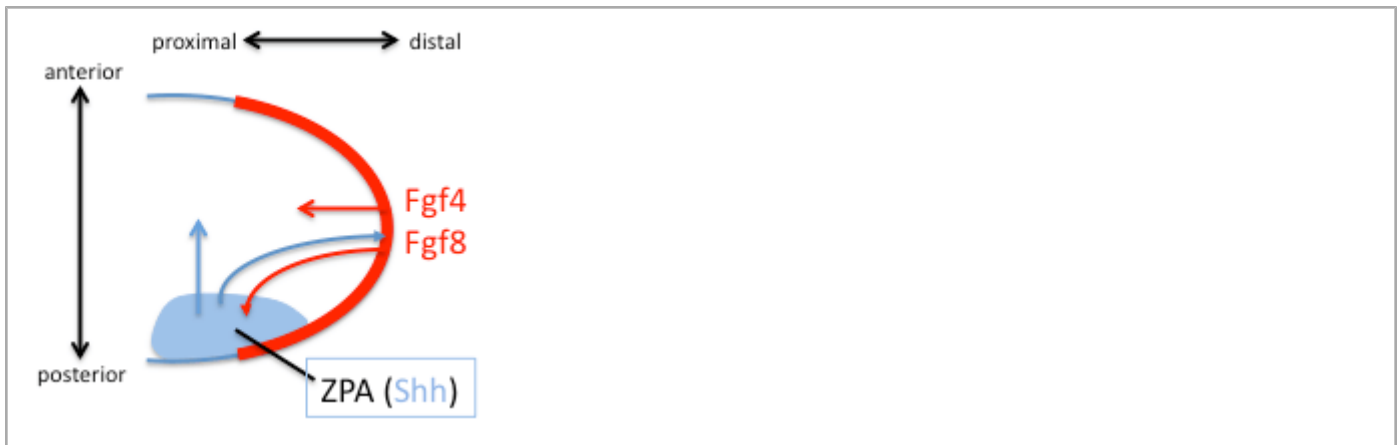


Figure 10: The tetrapod limb organizer. The apical ectodermal ridge (AER, red) secretes FGFs that keep the underlying cells proliferating and maintain Shh signaling from the Zone of Polarizing Activity (ZPA, blue). The Shh protein secreted from ZPA cells forms a gradient along the A/P (thumb/pinky) axis of the limb. Low/no Shh specifies thumb and index finger fates, high Shh specifies pinky and ring finger fates. What happens if we add Shh to the other side of the developing limb? Image from [wikimedia commons user Peteruetz](#) under a [CC BY-SA 3.0 license](#)

The Shh signaling center is a true organizer. It not only secretes a signaling molecule like the AER, but this signaling molecule acts in a graded fashion. High levels of Shh result in the development of posterior elements of the limb, like the radius or pinky, while low levels result in the development of mid-elements, like the middle fingers, and absence of Shh leads to anterior elements, like the thumb.

In general, signal transduction and transcription factor cascades are responsible for much of the patterning of the developing embryo. This gives us a big hint as to how evolution can act on development to change adult morphology. Small changes in the amount or location of a morphogen can have big effects on the adult. The same "toolkit" of genes is conserved in evolution, but is also reused in many different contexts. For example Shh's role in patterning your Central Nervous System as well as your hands. This reuse of toolbox elements in new contexts gives them new functions - depending on the local activator and repressors found there, the signal transduction pathway genes available, as well as epigenetic chromosomal modifications already present. So far we have mainly focused on gradients and organizers in patterning, but in [Patterns](#) we will look at a couple of other ways to pattern an embryo.

5.S: Regionalization and Organizers (References)

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6: GENETIC BASIS OF COMPLEXITY

In Genetic Toolkit, we examined how gene expression (and cell fate) is determined by local activators and on/off signal transduction pathways. We considered "wiring diagrams" for understanding how master regulatory genes can affect a suite of downstream genes in competent cell types. In this module, we will take a brief look at how these switches can evolve taking both organismal complexity and genetic complexity into consideration.

[6.1: Types of Selection: Beginning - Generating Mutants](#)

[6.2: The Evolution of Complexity: Increasing Genomic or Organismal Complexity](#)

[6.E: Genetic Basis of Complexity Exercises](#)

[6.S: Genetic Basis of Complexity \(References\)](#)

6.1: Types of Selection: Beginning - Generating Mutants

Mutations and selection

Evolution at the genetic level begins with mutations generating genetic variants. Variants can be selected for or against if they affect fitness and thus increase or decrease in frequency in a population. If there is no selection on the variant, we say it is "neutral". In this case, it is subject to genetic drift and will increase or decrease in frequency just by chance. Most mutations in animals are in intergenic regions, but some will naturally occur in genes. We usually think of these genic mutations as the ones most likely to be seen by selection. If we are looking over long-ish periods of evolutionary time, we will likely not see many mutations that reduce fitness since these should be weeded out by natural or sexual selection. Instead, we expect to see mutations that are:

1. neutral (no effect on fitness)
2. nearly neutral (very little effect on fitness)
3. buffered (invisible due to epistatic effects) or
4. positive (positive effect on fitness)

When we compare sequences of homologous genes (genes that come from the same common ancestral gene) we can run analyses to tell us whether the sequence is under "positive" or "purifying" selection. Genes under purifying selection have had mutations "purified" out. In this case, there is one "best" sequence and most species in a taxon will have very similar sequences of this gene. When mutations arise in the gene, the individuals carrying the mutations tend to have lowered fitness and will not produce many offspring carrying those mutations. Comparing the sequences of distantly related species we see fewer differences than we would expect to see by chance (or neutral genetic drift).

Genes under positive selection show the opposite - more differences in genetic sequence in a taxon than we would expect to see by chance. A positively selected gene has had a history of mutations that gave individuals higher fitness. These mutations were different in different lineages, giving rise to species with different sets of positively selected mutations. In summary, if most mutations in a gene will make it less functional and result in a negative effect on fitness, we might expect to see purifying selection on that gene (i.e. most mutations will be weeded out by natural selection, Figure 1). But if there are specific mutations that function better in different ecosystems, we might expect to see positive selection on that gene (i.e. different mutations will prevail in different environments).

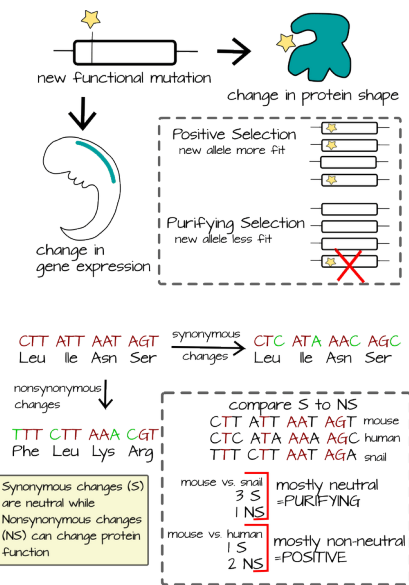


Figure 1: Positive vs. Purifying Selection. A mutation that affects the function of a gene (i.e. not neutral) can change the shape and function of the product (here the product is a protein, but it could also be an RNA) or the expression pattern of the product. If this change in function or expression increases organismal fitness, we expect the frequency of the allele to increase over time (Positive Selection). If the mutation decreases organismal fitness, we expect the frequency of the new allele to decrease over time (Purifying Selection). An editable svg file of this figure is available at <https://scholarlycommons.pacific.edu/open-images/29/>

To find out whether a gene is under positive or purifying selection, we compare the gene sequence across organisms. If one species or lineage has many functional changes in a gene compared to its relatives, we say that the gene is under positive selection. If there are very few functional changes between distantly related species, we say that the gene is under purifying selection. Under purifying selection new gene variants (alleles) tend to decrease fitness. This could be because the gene is already at peak fitness or because the gene affects multiple processes (pleiotropy).

The "background rate" of mutation over evolutionary time can be estimated by the accumulation of neutral (or nearly neutral) mutations. These are mutations that should not change the function of the gene. The easiest place to look for these are synonymous changes to the DNA sequence. Synonymous changes are mutations in protein-coding DNA that do not affect protein sequence. These are often in the third codon. Nonsynonymous changes can affect function by changing the protein sequence. Because synonymous changes don't affect protein function, we can use them to estimate the rate of neutral drift.

In the example shown, mouse, human, and snail sequences in a protein coding region are compared. DNA sequence that is the same for all species is shown in red. Pairwise comparisons counting the number of mutations that result in an amino acid change (NS for nonsynonymous) are compared to the number of mutations that do not result in an amino acid change (S for synonymous). When the number of S changes is higher than the number of NS changes, the gene is likely under purifying selection. That is, the number of functional changes is much lower than the number of neutral changes. When the number of S changes is lower than the number of NS changes, the gene is likely under positive selection.

Mutation location in a gene

In the section above, we categorized mutations by their effect on the phenotype. We can also categorize mutations by their position within a gene. There can be mutations in a coding region or mutations in a cis-regulatory region (there can also be mutations in a "junk" region but we don't need to consider those here).

Cis-regulatory mutations can change the expression pattern of a gene and cause loss of expression, lowered expression, increased expression, or ectopic expression (expression in a new place and/or at a new time). If the gene mutated is a regulatory gene itself, this can also have downstream effects on the expression of its targets. Overall, cis-regulatory mutations can change the "cellular fingerprint," which is the suite of genes expressed in a certain cell that gives that cell its identity and function. This can change the way a cell "behaves", both in terms of its own anatomy and physiology as well as the way it interacts with the cells around it, potentially even changing their fate via cell-cell signaling.

Mutations in coding regions can change the structure and function of a protein or RNA. These changes can be strictly structural in that they affect cellular anatomy, physiology, and/or cell signaling. For example, a mutation in the functional protein Myoglobin

can change how well a muscle cell stores oxygen. On the other hand, a mutation in the Wnt pathway receptor Frizzled can change whether a cell is responsive to Wnt signaling. The Frizzled mutation isn't just structural since it might also affect target gene expression, turning Wnt-pathway targets on or off in a new pattern.

Generating mutants

A third way to categorize mutations is by examining their physical effect on the genome - did the mutational event delete a large portion of the genome? Did it convert one nucleotide into another? The three main types of mutation we will consider are:

1. Point mutations: converting one nucleotide pair into another
2. Indels: Insertions and deletions that add or remove nucleotides from the genome. These can be caused by transposons, slippage during replication, or DNA repair enzymes.
3. Duplications: This is a specific type of insertion mutation that inserts a piece of existing genetic sequence into a new locus without removing it from the original locus.

Of these three, duplications and insertions can add genomic complexity. Here I am defining complexity as the number of unique parts in a system. Genomic complexity would be increased if we add in new genetic information. It seems straightforward to imagine a case where an increase in genomic complexity would result in an increase in organismal complexity. For example, we add in an extra gene for digesting amylose and we increase the amount of grain we can eat - diversifying our diet and making it more complex. However, we can also increase genomic complexity without increasing organismal complexity and we can even increase organismal complexity without increasing genomic complexity.

6.2: The Evolution of Complexity: Increasing Genomic or Organismal Complexity

Increasing genomic or organismal complexity

The case where we increase genomic complexity without a concomitant increase in organismal complexity is fairly elementary. An insertion mutation can add in new "junk" DNA to an intergenic region, or an intron that has no effect on phenotype. Likewise, a duplication mutation (a type of insertion) could duplicate a gene that simply does the exact same job as an existing gene. In both cases, genomic complexity is increased while organismal complexity stays the same.

Increasing organismal complexity without increasing genomic complexity is more interesting. In this case, a point mutation or a deletion affects cis regulatory regions or coding regions in a way that increases the function or expression of a gene product. For example, imagine amylase (an enzyme that digests amylose starches), which is normally expressed in the small intestine. Local activators are present all over the digestive system (including the stomach, mouth, and intestines), but a switch regulated by an signalling pathway is only activated in the small intestine. A series of point mutations in the cis-regulatory regions of amylase now create a novel local activator response element (Figure 2). In a way, this could be seen as an increase in genomic complexity because a new binding site was added, but seen from another point of view, the overall number of nucleotides remains the same. This novel cis regulatory region turns on amylase in the mouth, so now salivary glands produce amylase and starch digestion can begin earlier, diversifying the diet of the animal. In this case, the species has maintained the number of genes and the number of nucleotides in the genome but organismal complexity has increased due to an new cis regulatory site (figure 2).

Exonic (coding) mutations can also increase complexity, without adding genes or nucleotides to the genome. For example, imagine a mammalian protein with two domains - a functional domain and a repressor domain. The repressor domain can be ubiquitinated to inhibit the protein under particular conditions. In this case, our protein is a leg organizer. The repressor domain is bound by Hox genes of the trunk, limiting leg organizer expression to the region anterior to the thoracic and posterior to the lumbar regions of the body. A point mutation that converted a protein-coding codon to a stop codon (a nonsense mutation), at the end of the functional domain, would result in a protein missing the repressor domain. This would cause ectopic activation of leg organizer activity and (potentially) additional legs (Figure 2).

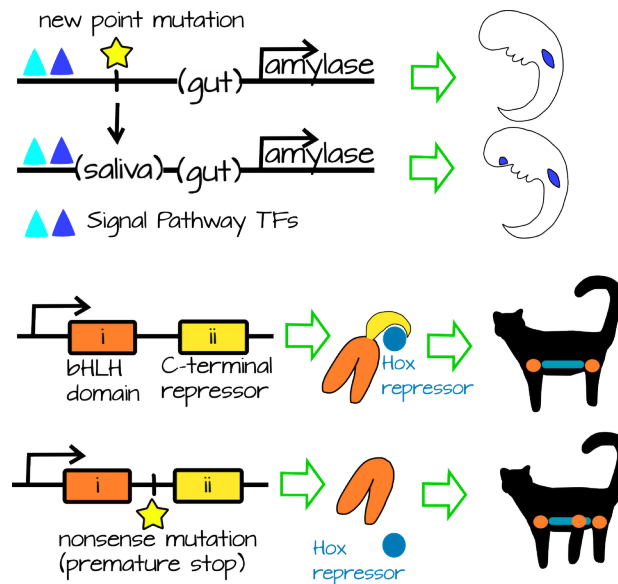


Figure 2: Increasing organismal complexity. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/30/>

Increasing organismal and genomic complexity

Genomic complexity can be added, at the small or large-scale, and it can occur over cis-regulatory or coding regions. It can involve horizontal transfer from another genome or duplication of elements already present in the genome. Here, we will consider different ways to add genetic complexity and how they might affect organismal complexity. I will focus on duplication rather than horizontal transfer for simplicity but similar consequences can occur with horizontal transfer as well. Smaller scale duplications can occur via errors in DNA replication, errors in meiotic crossing over, or transposon insertion. Larger scale duplications can occur via errors in meiotic crossing over, transposon insertion, or whole chromosome duplication via missegregation of chromosomes during cell division.

Small scale duplications can duplicate a single functional domain (like a bHLH domain), a single coding sequence, a single gene, or a single cis-regulatory protein binding site. In the case of the cis-regulatory binding site, the initial mutation might increase or decrease expression in target cells. Additional point mutations in the binding site could optimize for binding of a related protein. This would have the effect of driving ectopic gene expression and potentially new function. If the new site binds to a new local activator, then ectopic gene expression can occur in cells that express these activators when they also have an activating signal transduction pathway turned on. The new site alternatively could be a [Signal Pathway Response Element \(SPRE\)](#) in which case it will turn on expression ectopically only when the initial local activators are present.

Duplicating a gene (or at least a coding region) can have one of three major effects: creation of a pseudogene, DDS (duplication, divergence, subfunctionalization), or DDN (duplication, divergence, neofunctionalization). Pseudogenes do not add organismal complexity, but subfunctionalization and neofunctionalization potentially can. Under both DDS and DDN, we see a release of purifying selection due to the redundant duplicate protein (or RNA). Under DDS, a potentially pleiotropic protein is duplicated and now each duplicate can optimize for a subset of the original functions. This can lead to higher fitness and/or increased organismal complexity. Under DDN, one of the two redundant copies randomly acquires a new function that can then be optimized, as it is not under pleiotropic constraints. This new function increases organismal complexity.

Larger-scale duplications can also occur over either cis-regulatory regions or protein-coding/gene regions. The duplication of an entire enhancer element could potentially affect transcription either by acting on gene expression directly or by binding to and sequestering transcription factors present in the cell. An enhancer element can also be inserted into a new genomic location via transposon insertion or errors in crossing-over. In this case we would expect ectopic gene expression driven by this new element.

Larger-scale duplications over multiple coding regions include the duplication of the Hox-genes. This leads to multiple Hox-clusters in a single animal. An ancient duplication of the Hox-cluster gave rise to the Hox and Parahox clusters. Hox genes act in regionalizing the anteroposterior axis, mostly by acting on the ectoderm and mesoderm, of animals while Parahox genes are generally involved in endodermal and central nervous system patterning. Further Hox duplications (as seen in the whole-genome vertebrate duplications) allowed Hox genes to pattern new axes - like the vertebrate limb axis. Likewise, release of pleiotropic constraints on duplicated nervous system patterning genes may have given rise to the neural crest in vertebrates. This "fourth germ layer" plays important roles in patterning craniofacial structures as well as our enteric nervous system, adrenal gland, and other complex structures.

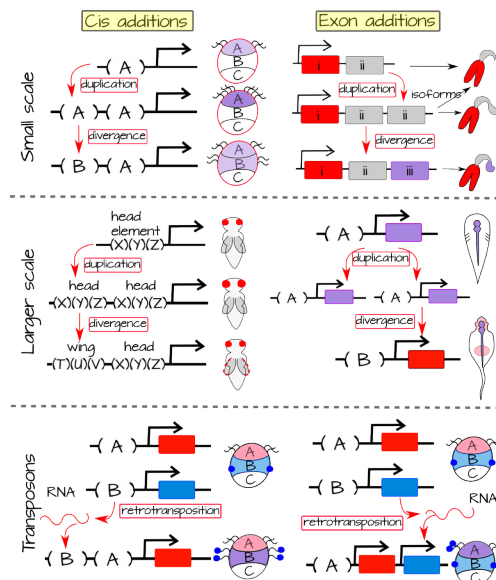


Figure 3: Types of mutations that can increase organismal complexity. An editable svg file of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/31/>

6.E: Genetic Basis of Complexity Exercises

In-class Lab

One way to increase genetic complexity is to duplicate a protein-coding gene. The [Ortholog/Paralog Mini Lab](#) explores using bioinformatics to generate hypotheses of orthology and paralogy. You will need one computer (chromebook or tablets are fine) per group for this lab.

Other Reading

For a slightly different take on homology, orthology and paralogy, check out this Microbial Genetics chapter.

[Homologs, Orthologs, and Paralogs](#)

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6.S: Genetic Basis of Complexity (References)

Further Reading and References

1. "Furcation, field-splitting, and the evolutionary origins of novelty in arthropod photoreceptors" Todd H.Oakley, David C.Plachetzki, Ajna S.Rivera 2007 <https://doi.org/10.1016/j.asd.2007.08.002>
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7: PATTERNING

We have already discussed how graded morphogens can give polarity to an embryo (e.g. Bicoid) or to a tissue (e.g. Shh and TGFbs). This covers much of developmental patterning. In this section I will summarize some other types of patterns that we see in development, both repeated patterns, like spots and stripes, as well as tissue shape and size patterning (called morphometrics). To start out, I will cover a simple mathematical model that has been used to help explain stripe and spot patterns that we see in many tissues. But it's important to keep in mind that 1) this is only a model and does not approach the true complexity of actual biological systems and 2) it does not explain all stripe and spot patterns - for example the *Drosophila* segmentation pattern, which is set up by graded morphogens.

[7.1: Turing Patterns to Generate Stripes and Spots](#)

[7.2: A Turing-like Model for Generating Stripes in Digit Development \(Rivera and Ramirez\)](#)

[7.3: Lateral Inhibition in Nervous System Patterning](#)

[7.4: Size and Shape](#)

[7.E: Patterning Class Activity and Discussion](#)

[7.R: Patterning References](#)

7.1: Turing Patterns to Generate Stripes and Spots

Alan Turing was a British mathematician who was a cryptographer and a pioneer in computer science. As a side hobby, he was also a theoretical biologist who developed algorithms to try to explain complex patterns using simple inputs and random fluctuation. His "reaction-diffusion" model uses a two-protein system to generate a pattern of regularly-spaced spots, that can be converted to stripes with a third external force. In this model, there is one activating protein that activates both itself and an inhibitory protein, that only inhibits the activator¹. By itself, transient expression of the activating protein would only produce a pattern of "both proteins off" or "spot of inhibitor on" since the activator would activate the inhibitor, thus turning off the expression of the activator (Figure 1 case). Things get more interesting when the molecules can diffuse or be transported across the tissue. In this case, the activator gets randomly turned on and it begins to diffuse away from its point source, activating itself in nearby cells. At the same time, it activates the inhibitor, which also diffuses away from the point source, inhibiting the activator. Depending on the timing on activation and diffusion or transport, this can result in the formation of an expanding ring of activator expression (Figure 1 equal rates). To get spots, however, we need two more layers of complexity. First, there must be random fluctuations in expression that turn the activator on at low levels across a tissue. Second, the activator must diffuse more slowly than the inhibitor. In this case, random spots of activator can be stabilized when they are far enough away from each other. Each of the small spots activates the expression of activator (which does not diffuse away quickly) and inhibitor (which diffuses away too quickly to completely eliminate activator expression from the initial point source). This gradient of inhibitor diffusing from each spot keeps any nearby cells from making activator. The overall result of this is a regular pattern of spots (Figure 1 bottom and side panels). The exact patterning depends on the size and shape of the tissue, the speed of activator and inhibitor diffusion, as well as any other patterning elements that might be present.

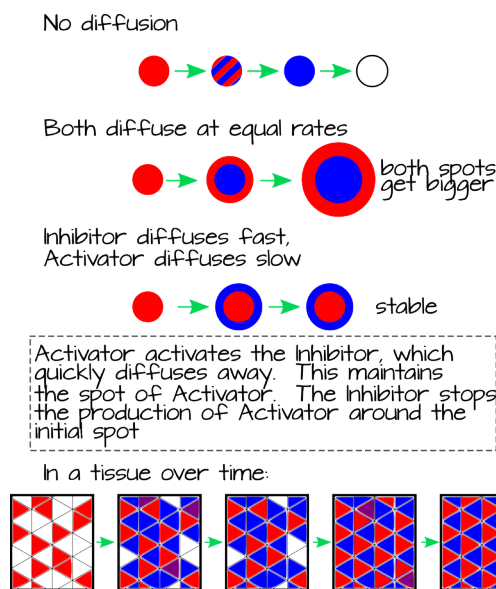


Figure 1: Turing-type patterning with an Activator (red) and an Inhibitor (blue). The Activator activates itself and the Inhibitor. The Inhibitor only inhibits the Activator. If the inhibitor diffuses more quickly than the activator, this can set up a regular pattern of spots in a tissue. In the bottom panel, the activator is randomly expressed at low levels in a subset of cells over a tissue. It self-activates to increase its own expression levels while also activating the inhibitor. The inhibitor quickly diffuses to the adjacent cells (second panel). Cells with both activator and inhibitor (purple) quickly convert to inhibitor-only as the inhibitor turns the activator off (third panel). Cells that are not exposed to the inhibitor (white) can randomly turn on the activator, turning on the diffusible inhibitor (fourth panel). This quickly sets up a stable pattern of "on" and "off" cells (fifth panel).

An editable svg version of this figure can be downloaded at: <https://scholarlycommons.pacific.edu/open-images/35/>

YouTube Video

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Can Math Explain How Animals Get Their Patterns? How Alan Turing's Reaction-Diffusion Model Simulates Patterns in Nature

In a very long and narrow tissue, there is only one direction diffusion can occur and this converts the Turing spot pattern into a stripe pattern (Figure 2). Similar forces, like directional growth and a morphogenic gradient, can also convert the spot pattern into stripes². Without an external force, the default should be spots or a meandering labrinthine pattern, depending on the properties of the activator and inhibitor. Hiscock and Megason propose four main ways to get a stripe pattern. Besides making diffusion more likely in one direction than another, a tissue can be subject to a "production gradient." This gradient is a protein or transcriptional/translational cofactor that causes higher gene expression of both the activator and inhibitor on one side of the tissue. Computational models predict that this type of gradient causes stripes to orient themselves perpendicular to the gradient (Figure 2)². Stripes will orient parallel to a "parameter gradient," where the activating and inhibitory properties of the two proteins are higher at one end of the tissue than the other. This type of modification could be produced by a gradient of a protein or cofactor that binds to the activator and both prevents it from activating gene expression and from being inhibited by the inhibitor (Figure 2)². Finally, the tissue can grow directionally. For example, your limbs developed largely by growing away from your body (distally), with a much slower rate of growth in other directions. This is due to the AER at the distal-most part of the limb bud causing cell proliferation underneath it. A computational model shows that a reaction-diffusion Turing model will generate stripes parallel to the direction of tissue growth (Figure 2)². A minilab helps us explore these models further with an [online tool](#).

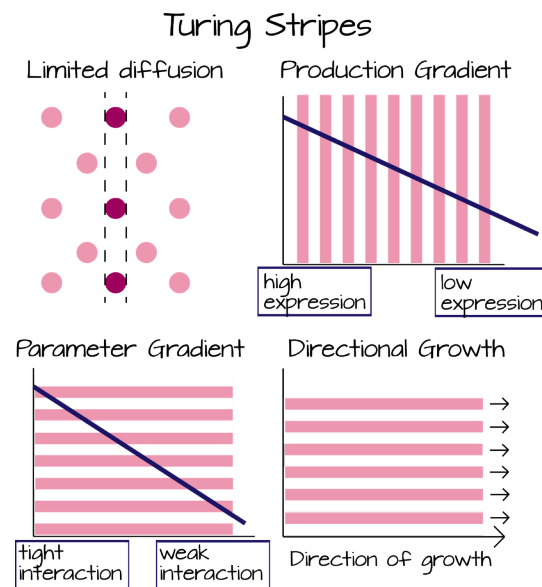


Figure 2: Turing patterning to generate Stripes by Amanda Lo and Ajna Rivera, details in text

An editable svg version of this figure can be downloaded at: <https://scholarlycommons.pacific.edu/open-images/36/>

7.2: A Turing-like Model for Generating Stripes in Digit Development (Rivera and Ramirez)

Your limbs first begin growing as a small nubbin off your torso. As cells proliferate, a small rod of skeletal tissue begins to grow right in the middle. This will become the humerus or femur. This skeletal tissue is specified early on by Sox9, a transcription factor also involved in sex-determination. As the limb tissue grows longer and wider, two parallel bones appear (the radius and ulna or the tibia and fibula). The tissue grows longer and wider still, and five parallel bones appear - the metacarpals and eventually phalanges (Figure 3).

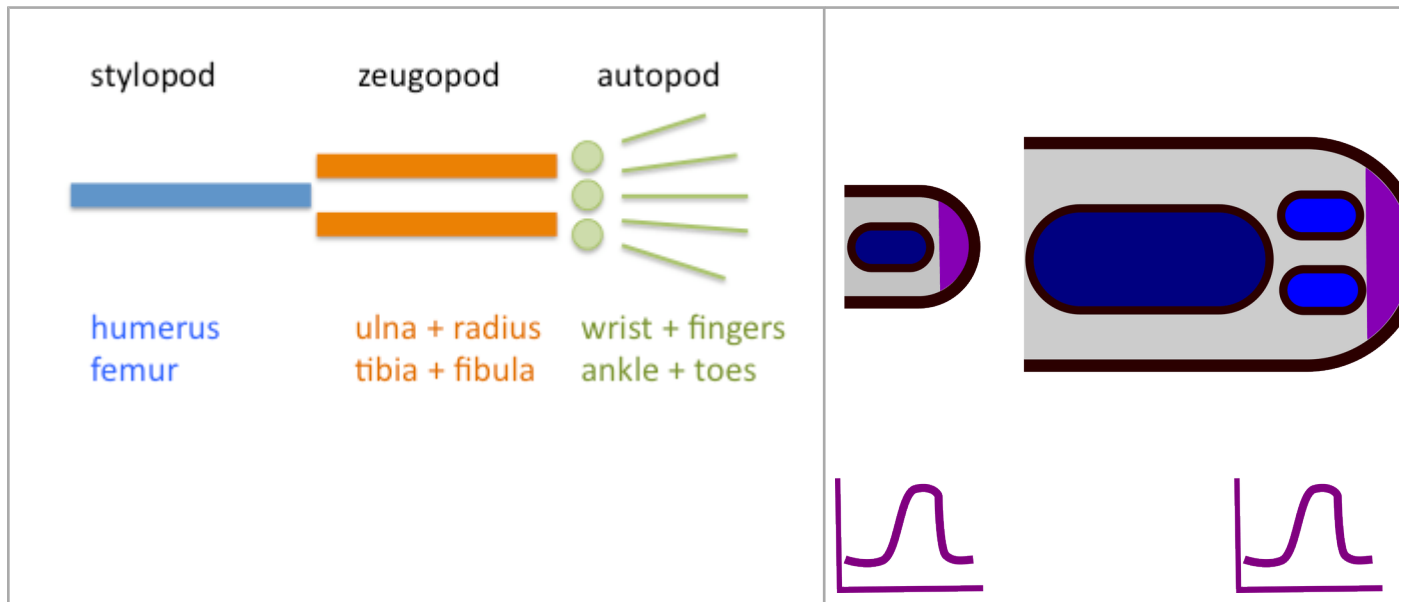


Figure 3: Parts of the Tetrapod Limb. Left panel is an image from wikimedia commons ([Peteruetz](#)) published under a [CC BY-SA 3.0](#) license. Right panel shows a basic model of tetrapod limb development. As each cartilage condensation (blue, Sox9) forms, it becomes repressive to the formation of nearby condensations. The diffusion and strength of repression give a "wavelength" shown in purple, outside the wave, condensations can form (X-axis is proximal-distal axis and Y-axis is strength of repression). The yellow region is a region of instability (high concentration of FGFs from the Apical Ectodermal Ridge) where condensations cannot form. As the limb grows larger, there is room for more condensations with the same wavelength. An editable svg file of the right panel can be downloaded at <https://scholarlycommons.pacific.edu/open-images/32/>

An early model for limb development proposed that an inhibitory diffusible morphogen is made by the cartilage condensations (that will later become limb bones). In the early limb bud, this inhibitor can diffuse across the entire tiny tissue, so only one condensation is laid down forming the large bone of the stylopod. As the limb grows longer and wider, the inhibitor cannot reach high enough concentrations to cover the entire width of the limb bud and two condensations form (the parallel bones of the zeugopod) form. In the developing autopod, the tissue is wide enough to support 5 mutually-inhibitory condensations - the five digits of the foot or hand (Figure 3)¹⁶. Several Turing models can generate a stripe pattern like this including the Activator Inhibitor model and the Substrate Depletion model (Figure 4).

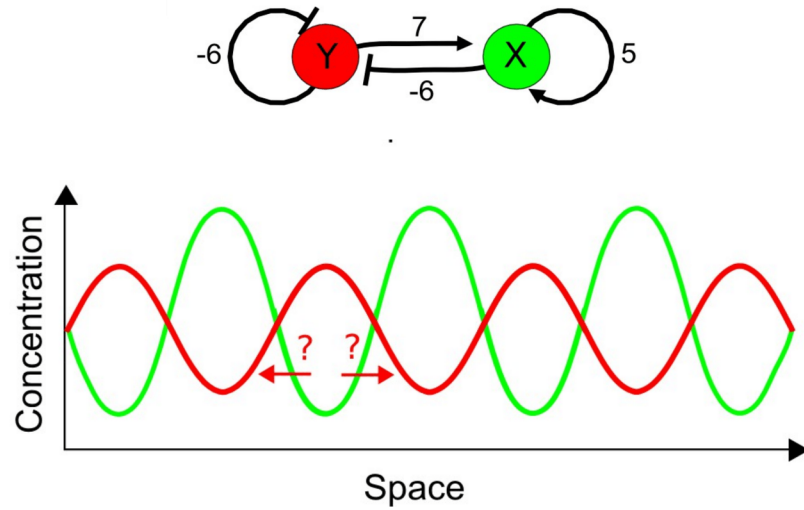
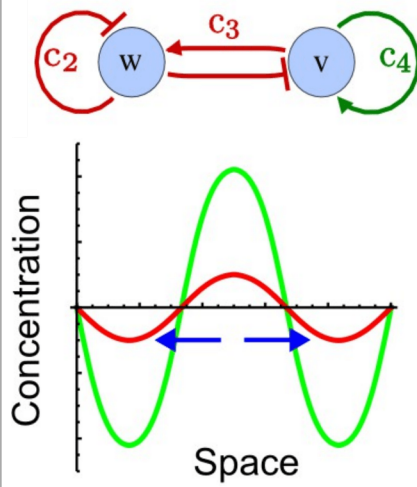


Figure 4
Turing models
digit st

The model on the left is the Activator-Inhibitor model. The model on the right is the Substrate-Depletor model. The concentration of the activator (green) and inhibitor (red) are shown as a function of space. The concentration of the substrate (green) and depletor (red) are shown as a function of space. The concentration of the activator (green) and inhibitor (red) are shown as a function of space. The concentration of the substrate (green) and depletor (red) are shown as a function of space.

The model on the right is the Substrate-Depletor model. The concentration of the substrate (green) and depletor (red) are shown as a function of space. The concentration of the substrate (green) and depletor (red) are shown as a function of space. The concentration of the substrate (green) and depletor (red) are shown as a function of space. The concentration of the substrate (green) and depletor (red) are shown as a function of space.

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Experiments on proteins expressed in either the interdigit areas or digits provided support for the Substrate Depletion Model¹⁷. However, these experiments also showed that instead of single proteins, digit patterning is actually specified via several interacting signaling pathways. Bmp, Sox9, and Wnt form the core components of the patterning that generates the "wavelength" of digit distance. All three of these genes form expression gradients, with the highest Sox9 in the middle of the digits and the highest Bmp and Wnt in the interdigits (Figure 5)¹⁷. Bmp expressed in the interdigit areas represses itself but diffuses outward to activate Sox9 in the nearby cells (the future digits). Sox9 represses Bmp, ensuring Bmp is only expressed in the interdigits. Wnt ligands are ubiquitous, but its targets (including b-catenin) are only expressed in the interdigits. Loss of b-catenin results in an expansion of Sox9 expression and an expansion of b-catenin expression reduces Sox9 expression, suggesting that Wnt/b-catenin is a repressor of Sox9^{19,20}. These interactions give a model where the digits form in a regular pattern with a wavelength specified by the strength of the Bmp/Sox9/Wnt interactions and the ability of Bmp and Wnt to diffuse (Figure 5)¹⁷.

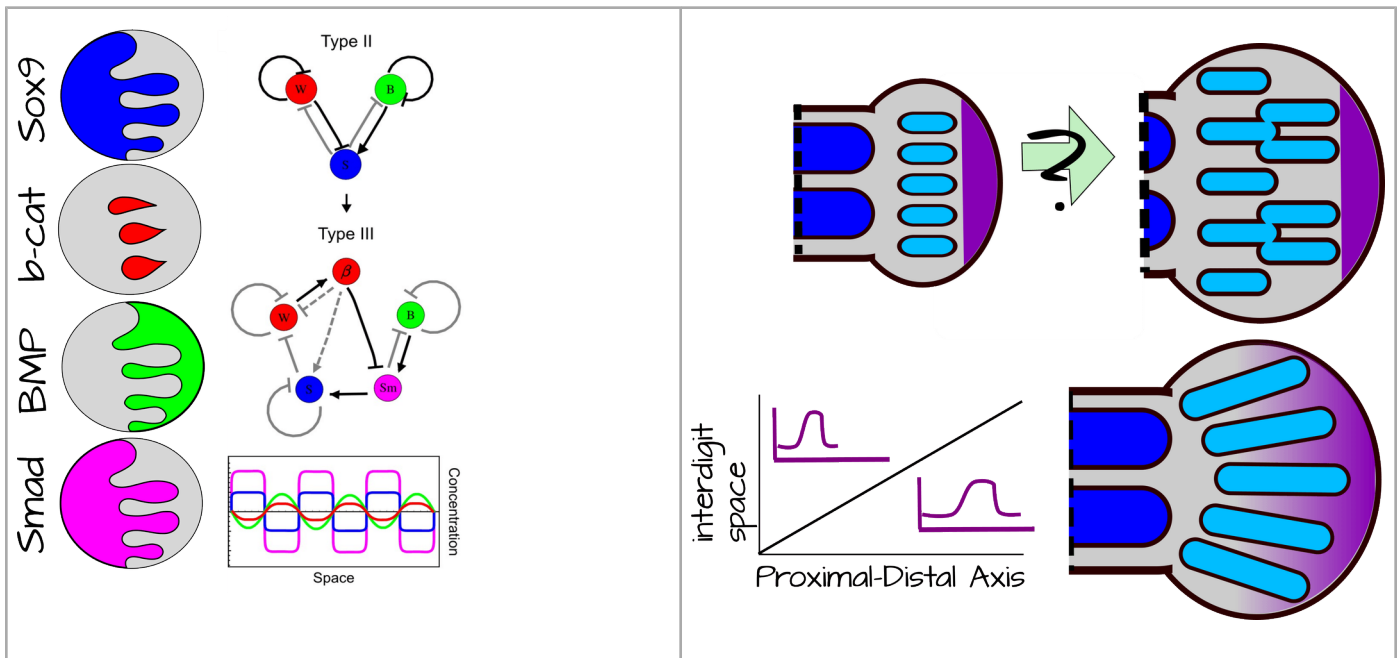


Figure 5: The Raspopovic et al.¹⁷ model for digit specification. Sox9, the primary digit marker, specifies cells to form skeletal condensations. It is expressed in the cells that will become digits in the autopod. In this model, Sox9 (blue) is the activator and it interacts with the BMP and Wnt signaling pathways. Autopods are shown as grey circles in the left column with color-coded expression patterns. Sox9 and Smad (purple, output of the BMP pathway) are expressed in the digits while BMP (green) and b-catenin (red, output of the Wnt pathway) are expressed in the interdigits.

The left column shows the Turing model for these interactions. The top model was originally proposed by Raspopovic et al.¹⁷ and subsequently refined by Marcon et al.²¹. The Marcon et al model maintains the centrality of Sox9, which represses its own expression outside the condensation. Sox 9 inhibits the Wnt pathway, which represses b-catenin and other target genes in the Sox9 zones. b-catenin represses Smad, which is a target of diffusible BMP signaling. The inhibitory action of Smad on BMP ensures that BMP is not active in the Smad-expressing cells, giving these two members of the BMP pathway complementary activation patterns.

The bottom right panel summarizes the output of the complex Marcon et al model.

Modified from Marcon et al.²¹ originally published under a CC BY 4.0 license

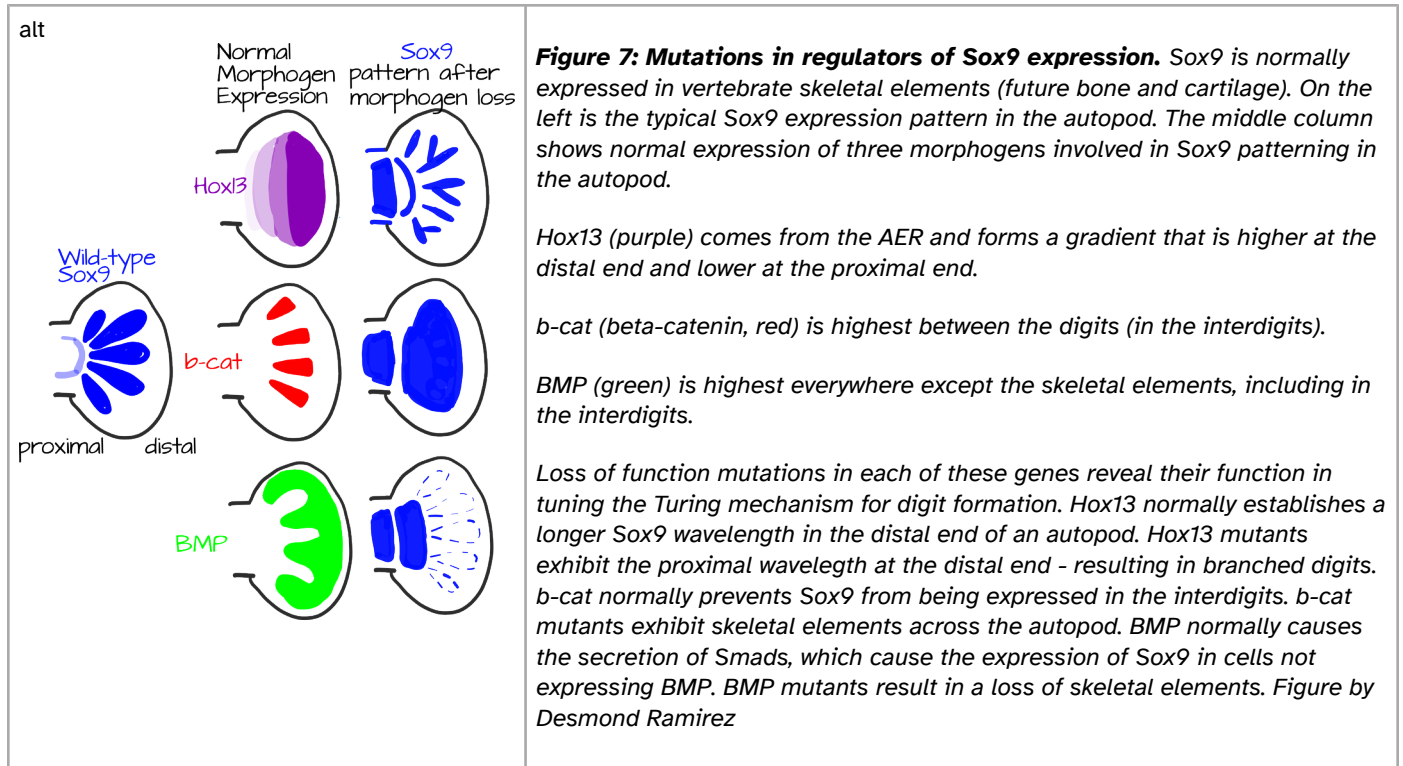
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Figure 6: Modifying the wavelength. The paddle-shaped autopod should allow for additional condensation formation as it grows. This should lead to additional bones in the distal portion of the autopod or branching of existing condensations (top panel).

Hoxd13 mutants give phenotypes like the top panel suggesting that Hoxd13 modifies the wavelength of the Sox9 condensations. Hoxd13 diffuses from the Apical Ectodermal Ridge and forms a gradient (yellow). Where Hoxd13 levels are high, the wavelength (or interdigit space) is large. Where Hoxd13 levels are low, the wavelength is small. The modulation of Hoxd13 levels and/or its ability to regulate the wavelength results in unbranched digits^{17,18}.

An editable svg version of this figure can be downloaded at: <https://scholarlycommons.pacific.edu/open-images/34/>

But there's one big problem with this. Unlike the zeugopod and stylopod, the autopod is paddle-shaped. That means that it grows wide quickly during development, making a tissue that should support more cartilage condensations at the tip than near the wrist by the proposed model (Figure 6). This problem is solved by Hoxd13. Hoxd13 is a Hox gene that helps pattern the hands, feet, urinary tract, and reproductive organs. In the hands and feet (autopods) it is expressed in a gradient, with the highest levels near the Apical Ectodermal Ridge (AER) at the tip of the limb bud. Hoxd13 stabilizes the digit number in two ways. First, it acts as a "no patterning zone." In the region with highest Hoxd13, the expression of Sox9 is unstable and new condensations cannot form. Further away from the AER in an area of lower Hoxd13, new Sox9-driven condensations can form. Even further away from the AER, where there is no Hoxd13 expression, the existing condensations are stable. No new condensations will form even as the tissue grows wider. Hoxd13 also stabilizes digit number by increasing the ability of Sox9 to inhibit Bmp and Wnt. This has the effect of making the digit wavelength wider in the distal portion of the autopod, repressing the development of additional digits there (Figure 6)¹⁸. In Hoxd13 mouse mutants, the cartilage condensations of the autopod branch in the distal portions, following the natural wavelength of the Bmp/Sox9/Wnt interaction¹⁸.



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7.3: Lateral Inhibition in Nervous System Patterning

Not all cells in a nervous system can be neurons. In fact, in our own central nervous systems about half to 3/4 of the cells are glial cells³. In *Drosophila*, neural progenitor cells (neuroblasts) are only a portion of all the neuroectoderm cells. These neuroblasts express proneural bHLH transcription factors including Achaete-Scute complex genes. The Achaete-Scute complex (Ac-Sc) is a cluster of 4 bHLH genes whose protein products form homo- and hetero-dimers to regulate the transcription of neuronal genes. Cells expressing Ac-Sc delaminate from the neuroectoderm sheet and eventually give rise to neurons⁴. Before I summarize Turing pattern-like mechanism for neuroblast specification, I first want to mention that *Drosophila* neuroblast formation differs in one very important way from a traditional Turing pattern - each neuroblast arises in isolation from other neuroblasts. This patterning is not over an entire tissue but instead is super local, occurring only over a 6-7 cell cluster with only a single cell becoming a neuron (Figure 5)⁵.

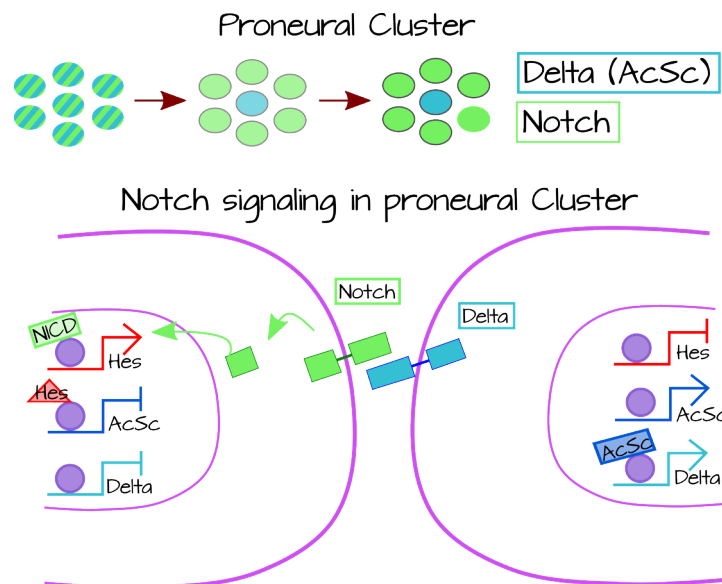


Figure 5: Notch/Delta signaling in proneural clusters in *Drosophila*. In one cell, Delta is activated by Achaete-Scute (AcSc). Delta protein is a ligand for Notch and it activates Notch signaling in cells next to it. In this next-door cell, Notch signaling activates Hairy/Enhancer-of-Split (Hes) expression, which represses AcSc. Without AcSc, Delta is not expressed. In this way, cells next to a Delta expressing cell will not express Delta themselves.

An editable svg version of this figure can be downloaded at <https://scholarlycommons.pacific.edu/open-images/37/>

After proneural cluster specification, random oscillations of Notch signaling pathway components occur until one cell randomly expresses higher levels of Delta, the Notch ligand. This activates Notch signaling in neighboring cells. The activation of the Notch signaling pathway causes the expression of Hes gene Enhancer-of-split, which is a repressor of the AcSc complex. AcSc normally increases the expression of Delta, but in these Notch positive cells where AcSc is off Delta levels begin to go down. In the neighboring Delta positive cell, there is no Notch signaling to turn off AcSc (because of the lack of Delta in all neighboring cells) and Delta levels remain high. In this way, one cell in the proneural cluster is Delta/AcSc positive and the neighboring cells are Notch/Hes positive⁶ (Figure 3). This system creates one Delta positive cell in a field via lateral inhibition. In the Turing model, Delta would be the local activator and Notch would be the repressor. Delta activates its own expression by not turning off AcSc and activates Notch in neighboring cells. Unlike the Turing model, Notch and Delta do not need to diffuse for this to work, instead the signal itself is propagated by cell-cell signaling.

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

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Graded morphogens, reaction-diffusion, and lateral inhibition are all ways to generate patterns that are either repeating (like spots and stripes) or polarize a tissue. If you have ever been to the beach or taken a physics course you know that another way to generate a repeating pattern is with an oscillator that outputs a wave function. If you haven't taken physics (or are rusty) the easiest way to think about waves and oscillators is to imagine holding a rope that is tied to a pole. As you move your arm up and down, you act as the oscillator, moving from the high position to the low position. The range of motion of your arm determines the height of the wave. You can make the waves move faster (with higher frequency) by moving your arm more quickly. The speed of the oscillator determines the speed at which the waves move. If your arm moves too slowly, the wave disintegrates quickly and never makes it to the wall.

Waves and oscillators also occur in biological systems. One of these is the segmentation oscillator in vertebrates. In this case gene expression of Notch pathway genes act as the oscillator. A "clock and wavefront" model has been proposed to describe segmental patterning in vertebrates. Segmentation of the mesoderm surrounding the notochord (the somites) occurs in an anterior to posterior fashion, with somites budding off a posterior zone of unsegmented mesoderm. As new somites bud off, old ones are pushed anteriorly, such that the anteriormost somites bud first. The "segmentation clock" is the oscillator in the presomitic mesoderm at the posterior of the animal. A "wave" of *hes*-class gene expression travels across the presomitic mesoderm (PSM). When it reaches the anterior-most point of the presomitic mesoderm it arrests and the tissue buds off the PSM. The marker telling the tissues when they are far enough anterior are opposing gradients of retinoic acid (RA) in the anterior and FGFs in the posterior. When a wave of *hes* expression reaches low/moderate levels of each gradient molecule, this signals the tissue to undergo somitogenesis (somite budding)⁷.

YouTube Video

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In the video above, Hes gene expression in the PSM is in blue and in the somites it is red. So the question you might be asking now is "How does Hes act as an oscillator but also travel in a wave?" The wave you are seeing in the video above is a wave of gene expression. The cells themselves move very little. Imagine a stadium full of people doing "the wave." If the wave takes 1 minute to get around the stadium, it can be propagated by a coordinated audience if each person raises their hands for 1 second every minute.

YouTube Video

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The people themselves aren't running around the stadium, rather they are "oscillating" between arms up and arms down. Similarly, in the PSM each cell is oscillating between Hes on and Hes off. Just like a stadium wave, cells are testing their local environment to coordinate with nearby cells. When the wave arrives at low levels of FGF and higher levels of RA it stops, just like a wave hitting a wall, and the cells with matching expression patterns bud off the PSM as a somite (Figure 6).

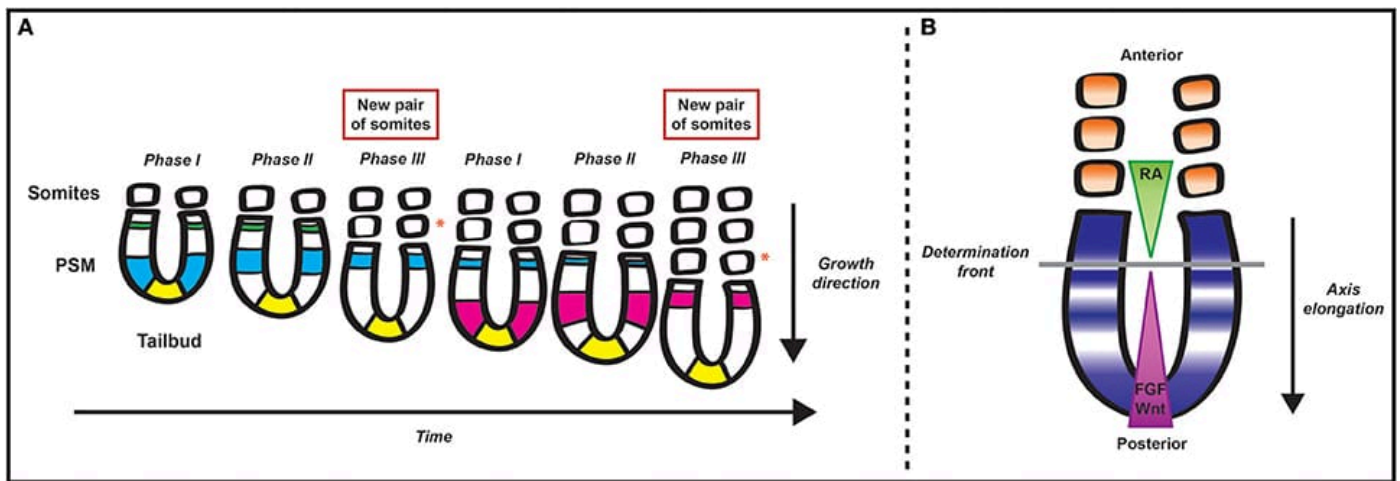


Figure from Carriere and Dale, 2017⁹ published under a [CC BY 4.0 license](#)

How does Hes act as an oscillator and how do cells coordinate with each other? We don't 100% know the answer to this, but there is a lot of evidence that Hes is a cell-intrinsic oscillator and the Notch pathway (controlling Hes expression) helps to coordinate neighboring cells. Hes genes are transcription factors and some Hes genes inhibit their own expression. When protein levels of Hes are high enough, transcription levels go down. These Hes mRNAs and proteins also have a short half life, so when transcription levels go down, they don't stay down for long. As the mRNA and proteins degrade, repression of Hes expression is released and mRNA is made again. Of course, this raises the level of Hes protein, which represses Hes expression. In this way Hes expression acts as an oscillator, as long as it maintains at least a low level of expression and degrades quickly. In mice, degradation of protein occurs within 20 minutes. Stable mutants that last for 30 minutes have dampened oscillatory behavior, just like raising and lowering your arm too slowly when making rope waves⁷. Oscillation is an intrinsic property of the cells, it can occur in cell culture after serum treatment, but this oscillation is uncoordinated between cells - after a few cycles where all cells show the same periodicity of oscillation, they begin to lose coordination and oscillate on their own wavelength^{8,9}.

YouTube Video

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As you can see in the video above, cells in the presomitic mesoderm tend to have the same state of Hes oscillation as their neighbors. This is likely coordinated through the Notch signaling pathway. As we've already seen, the Notch pathway is a juxtacrine signaling pathway with cells next door to each other communicating through cell-membrane receptors. Once activated, Notch undergoes intramembrane cleavage and the cytoplasmic half travels to the nucleus where it releases repression on Su(H) class transcription factors to activate gene expression of effectors like the Hes genes (hairly/enhancer of split genes also called Her genes). By signaling to each other, the PSM cells may exert positive feedback on their neighbors such that Notch-positive cells induce Notch signaling in next door cells¹⁰.

7.4: Size and Shape

The final type of developmental patterning that evolution can act on is the size and shape of tissues or organs. These are generally considered "morphometric" scaling issues and are classified as "allometric" changes. Morphometrics is the study of how a continuous geometry (like the outer surface of a body) can be warped. Allometry studies this in the context of evolution and development. One of the people who defined the field, D'arcy Wentworth Thompson, came up with morphometric changes that allow one known species of fish to be warped into another known species (Figure 7).

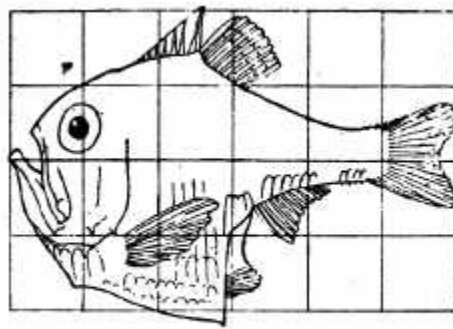


Fig. 517. *Argyropelecus Olfersi*.

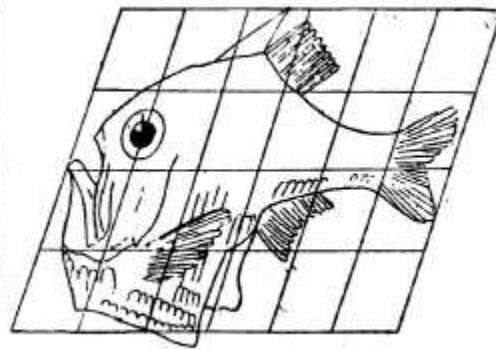


Fig. 518. *Sternoptyx diaphana*.

Figure 7: Morphometric transformation of one fish into another. From Thompson, 1917¹¹ this is a public domain image from wikimedia commons

While the extent of the role that allometry plays in evolution isn't totally clear, we know from examples that it does play some role. It's easy to see this if we look around at related species. For example, hominid skulls are very alike and often differ in ways that are easily explained by small variations in developmental growth patterns. Extra or earlier cell proliferation here, less or later cell proliferation there. While we know many genetic markers of cell proliferation (for example cell-cycle regulator Cyclin D1) increase cell-proliferation, there are many different upstream activators of proliferation depending on tissue/cell-type and developmental stage. To begin to understand the role of allometry in evolution, researchers compare tissue growth and the regulators of that growth between fairly closely related species.

Two places where both the development and evolution of size and shape changes are fairly well understood are beak shapes in birds and elytra in beetles. One of the most famous examples of evolution is *Darwin's Finches*. This group of finches underwent an adaptive radiation on the Galapagos Islands beginning about 2.3 million years ago¹². Since then, the finches have adapted to different island niches into 14 different species. Comparing beak morphology between these species uncovered two conserved allometric programs for changing cell proliferation patterns. In early development, beak width is regulated by levels of BMP4 - higher BMP4 means a deeper and wider beak. For example, ground finches have high BMP4 expression in a region that signals to the predominate skeletogenic region of the early beak - the pre-nasal cartilage (pnc)¹³. Likewise, high levels of calmodulin next to the pnc are associated with longer beaks, like the beaks of cactus finches (Figure 8).

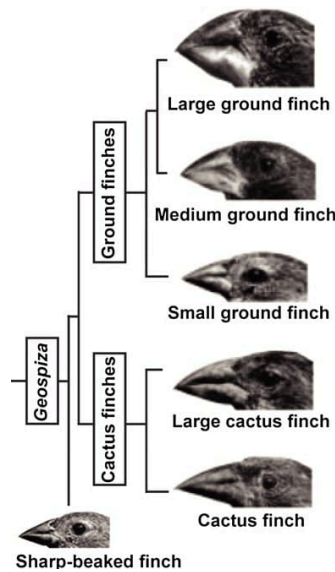


Figure from Mallarino et al, 2011¹⁴.

Slightly later in development, an upper beak structure called the pre-maxillary bone (pmx) begins to grow. This will eventually form the parts of the upper beak that structurally and functionally differ between many bird species. This part of the beak also expresses different sets of genes in different beak shapes. TGF β receptor type II (TGF β IIr), β -catenin, and Dickkopf-3 (Dkk3) are all signal transduction cascade genes that are expressed at much higher levels in the large ground finch beaks compared to smaller beaked finches. These three genes were expressed in broader domains in larger beaks than in smaller beaks when looking at all five species in the figure above (Figure 9)¹⁴.

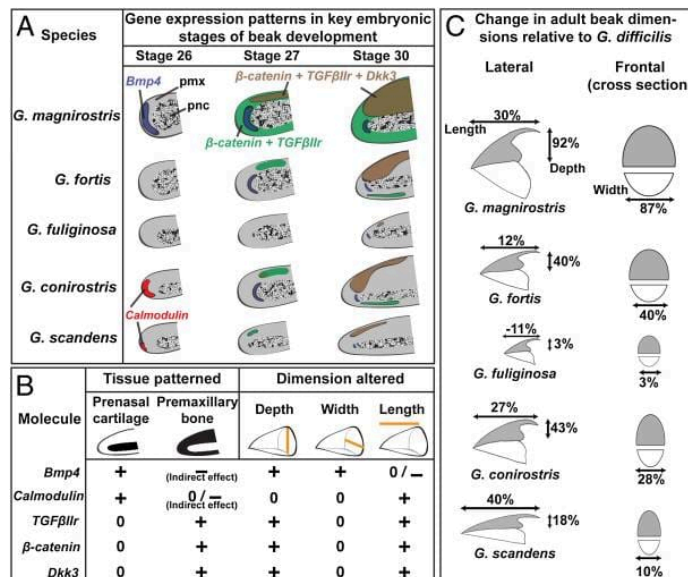


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These findings buoyed the idea that allometric growth can fuel rapid evolution by changing the scale of a particular feature. Changing the scale just refers to scaling something up or down, making it larger or smaller in one or all dimensions. For example a large square is simply a scaled-up version of a small square. A rectangle would be a square that has been scaled larger in one dimension but not the other. Researchers noticed right away that simply changing scaling of the upper beak could explain a lot of the variation in *Geospiza* and this could be explained by the coordinate system of genetics above (or even a simpler genetic system). However, scaling wasn't enough to explain the variation in other related finch species. To explain that variation, researchers found that both scaling and shearing had to be considered. Shearing refers to a geometric transformation like that seen in the fish example from Thompson et al. above. In a shear transformation, each point moves along x at a distance proportional to its y coordinate (or vice versa) giving a diagonal line from a straight line. Shearing plus scaling was enough to

explain at least the length and depth axes of the finch beaks studied (Figure 10)

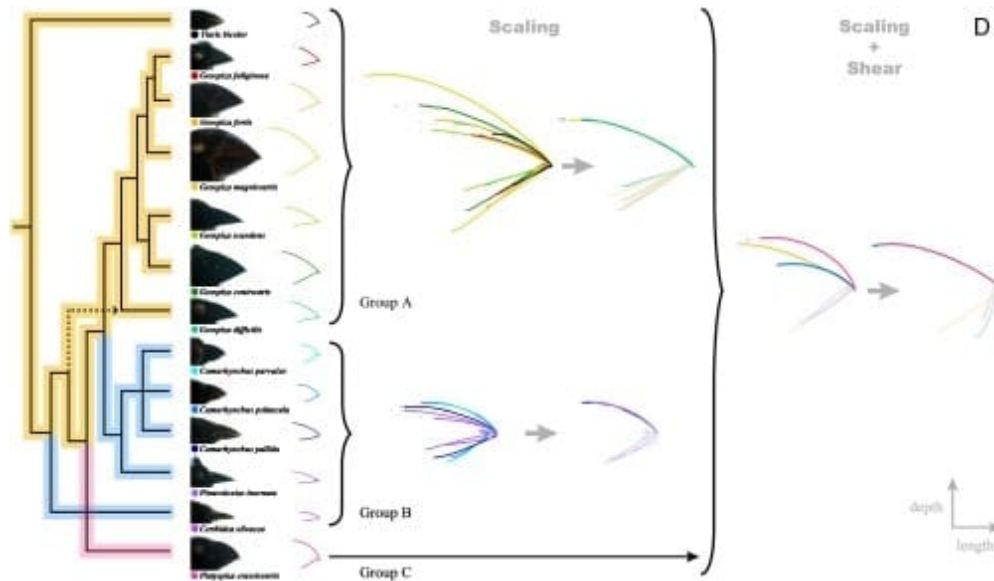


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The next question to answer is how broadly applicable is this coordinate system for beak shape transformation? Increasing or decreasing the expression levels of these genes does change chick beak shape in the ways predicted by Mallarino et al.¹⁴. This information might lead us to predict that evolution would use this coordinate system to make the wide variety of beaks that we see in living and fossil birds. However, studies of non-*Geospiza* finches show that evolution is more variable than we might imagine. If we have time, we will discuss the paper: ["Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs"](#).

7.E: Patterning Class Activity and Discussion

Assignment 1

Choose one of the stories from [How the Snake Lost It's Legs](#), and summarize the information for the class.

End your summary with at least one "future directions" question for us to consider in class.

Assignment 2

The questions below were originally written for students reading *Endless Forms* by Sean Carroll. However, the **questions in bold** are discussion questions that can be answered without these books.

The "Reading Guide" questions are appropriate for short homework answers and the "Discussion Guide" questions are appropriate for open in-class or online discussion.

Discussion Questions

1. **Do you think our "sense of wonder and beauty is diminished or enhanced by scientific understanding?"**
2. **Why do we think it is "easy" for butterflies to evolve new wing patterns but less easy to evolve new biochemical pathways (like new pigments for example)?**
3. **What kinds of lab experiments can give us insight into butterfly wing Evo-Devo?**
4. **What is the difference between morphological convergence and genetic convergence?**
5. **What changes besides protein changes in MC1R could lead to changes in animal coloration?**
6. **Can convergent evolution of a trait be driven by different genetic mutations?**
7. **Why do we know more about coloration in fruit flies than in zebras and leopards?**
8. Draw a butterfly wing with a maximum ground plan and one with fewer pattern elements.
9. What are three butterfly wing inventions?
10. How is *Bicyclus* an example of plasticity? What is the switch that regulates this plasticity?
11. Explain the genetics and selection driving melanism in rock pocket mice.
12. What is Bard's model for zebra striping?

Endless Forms Reading Guide: Chapter 8

1. What is Batesian mimicry?
2. What are the serial homologs in a butterfly wing?
3. What is a wing scale? Is it homologous or homoplasious to a fish scale?
4. What is an eyespot focus and why do we call it an organizer?

Endless Forms Reading Guide: Chapter 9

1. What is disruptive coloration?
2. What is melanin?
3. What genetic changes drive melanism? Are these cis-regulatory or exonic? Why don't they affect other developmental processes?
4. What is the significance of MC1R in humans?
5. What causes white spots on mammals?
6. What is fitness?
7. What is a selection coefficient?
8. Give two reasons why we might not see spotted mice in nature.

In-class Activity (Mini-Lab)

[Reaction Diffusion Simulator](#)

Note: If possible this is ideally run in a computer lab or with loaner laptops. Otherwise, ask students ahead of time to bring in at least one laptop per group. Students can follow the questions below or can be given minimal instructions and just "play" with the simulation.

In the Turing Model, the pattern is changed by adjusting the speed of diffusion, the strength of interaction, and the rate of degradation. In the Grey-Scott model simulation, the feed rate is related to the speed of diffusion and the death rate is related to the rate of degradation.

1. To see the a "boring" pattern, choose a feed rate of 0.006 and a death rate of 0.028. Click anywhere on the canvas.
2. Choose the present "Solitons" and click anywhere on the canvas. This feed/death rate combination produces a field of equally spaced spots. How does this relate to the Turing model?
3. Adjust the feed rate to 0.02 and the death rate to 0.058, how does this affect the spot pattern you saw in Solitons?
4. Can you adjust the feed and death rates to produce stripes?
5. What happens if you click on the canvas while a pattern is being generated. What would this represent biologically?
6. What kinds of biochemical/molecular properties might affect feed and death rates?

Suggested Journal Club Paper

[Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs.](#)

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7.R: Patterning References

Further Reading

[Elytra evolution.](#)

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8: NOVELTY

8.1: What is an Evolutionary Novelty?

8.2: Case Study - The Evolution of Insect Wings

8.E: Novelty Discussion

8.S: Novelty (Summary)

8.1: What is an Evolutionary Novelty?

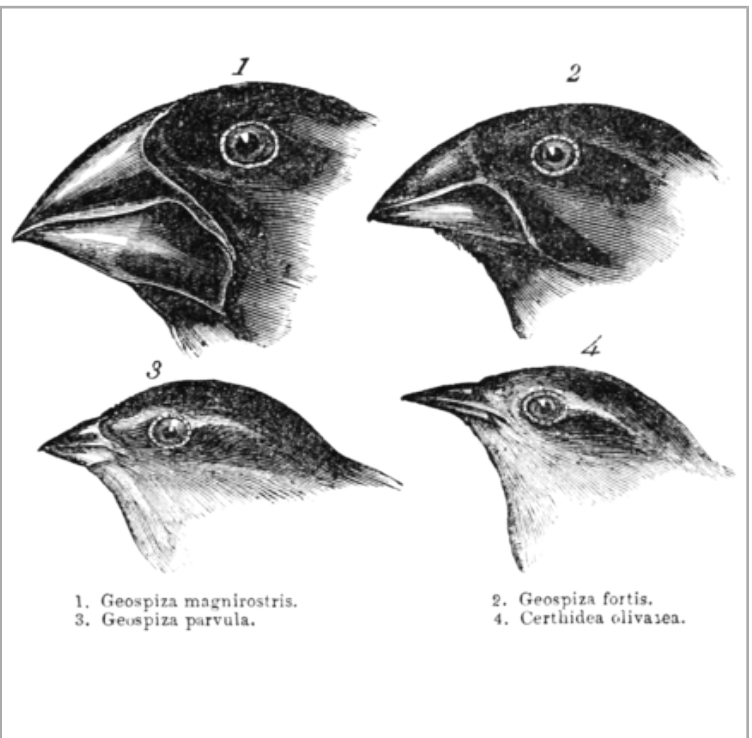
For discrete objects and characters it is easy to see what is new or novel. For example, if I went skydiving, that would be a novel experience for me since I've never been skydiving before. If we put a human on Mars, that would be a novel scientific undertaking for the human race. However, evolution often does not work with discrete characters, instead lines are blurred and fuzzy. For example, we might think of bat wings as a novelty in vertebrates since they are a synapomorphy (shared, derived, defining character) in the bat lineage. Bats invented something new - membranous wings. However, it could also be argued that this is simply a "functional innovation," that is, an elaboration of an existing structure to perform a new function. In this sense, bat wings are simply elongated forelimbs with some extra skin. So what counts as a novelty, exactly? To be direct about it, there really isn't one single definition that can be defended. The confusion seems to stem from our own biases regarding the characters we find interesting and "novel," like feathers, hands, and faces, which all had different evolutionary origins and trajectories.

Given that there isn't a single agreed upon definition of novelty, probably due to the fact that evolution doesn't follow strict patterns, how can we think about this subject at all? In 1963, Ernst Mayr, one of the founders of modern Evolutionary Biology defined a novelty as a new structure or property of an organism that allows it to perform a new function, thus opening a new "adaptive zone". In this reckoning, a novelty allows an organism to exploit a new ecological resource and should lead to an adaptive radiation¹. A major flaw in this definition, however, was pointed out in 2008 by Massimo Pigliucci. He noted that some "novelties", like the mammalian inner ear, didn't lead to adaptive radiations and thus wouldn't fall under this definition². We can broaden the definition to include any character that is unique to a taxon³, however it defies credulity to consider a character like an extra bristle on a crustacean antenna as an evolutionary novelty. Pigliucci combines these two definitions to say that novelties are unique characters that define a taxon that also have a novel function in their ecosystem. This means that they don't necessarily lead to an adaptive radiation, but they still do increase fitness compared to the ancestral state². I personally like this definition in that it gives us two clear things to look for in a novelty: 1) It's actually new and 2) It does something for the organism. However, this definition is pretty broad and fuzzy. Under it flight feathers in birds, a novelty that allowed them to take to the skies and inhabit the most remote spots on earth, are placed at the same level as a functional protein mutation in a bacterium that gives it 10% greater capacity to digest pesticides.

What is an adaptive radiation?

An adaptive radiation is the rapid evolution of a lineage to fill multiple open ecological niches. One of the most famous adaptive radiations is the evolution of the Galapagos Finches (also called "Darwin's Finches"). When an ancestral population of finches reached the Galapagos Islands the islands had no other similar species with which to compete. The multiple open niches, like feeding on flowering cacti, hard seeds, small seeds, and insects, imposed disruptive selection on the birds. That is, finches with beaks better adapted to eating insects were selected for as were finches with beaks better adapted to eating hard seeds. Finches with intermediate phenotypes (mediocre at eating either food source) were selected against. Over long periods of time, this led to speciation of the finches into at least 12 distinct species each exploiting a slightly different food resource.

Image From "[Voyage of the Beagle](#)", Public Domain



One of the luminaries in the field of evolutionary novelty, Gunter Wagner, has created a definition of novelty that can help to direct research programs. His definition is more functional and specific than other definitions. By his reckoning there are two types of

novelties:

- Type I, a new body part that is not strictly homologous to other body parts and
- Type II, a novel variant on a body part that allows for a new function.

A Type II novelty would include the evolution of fins in marine mammals - this is just a reconfiguration of an existing body part. In the case of Type II novelties the homology to ancestral body parts is clear, in a Type I it shouldn't be⁴. By this definition, we have to decide what we consider to be compelling novelty and what we consider to be compelling evidence. For example, we might decide that mammary glands - derived from hair glands - are novel since we personally feel they have enough differences from hair glands to be independent evolutionary units, but decide that the shiny scales of garfish (ganoid scales) are too similar to standard fish scales to count. In the latter case, we ignore the novel compounds that create the shininess.

Wagner has addressed the problem of using our own perspective to decide what counts as a novelty or not by defining novelties as being associated with novel core Gene-Regulatory-Networks (GRNs). Positional information can vary between species (see the Bicoid example!) but this evolutionarily variable positional information is read out by an evolutionarily stable core-GRN. This core-GRN specifies the tissue by activating "realizer-genes", sometimes called "structural genes". These are the genes that do the business of giving an organ its functional properties. This realizer-gene output, like the positional information, is also changeable. The realizer-genes can be modified by adaptations to local environments^{4,5}. Since the core-GRN is the stable component of the genetics underlying the development of the novel organ, a novel core-GRN thus is associated with a truly novel organ.

While I truly like this definition because of its simplicity - to understand how novelty arises we just go after those novel core-GRNs that define the novelty - it does have its weaknesses. A technical weakness is that we cannot be sure of the genes that make up a GRN with our current technology. For example, it's easily possible to miss a gene when sequencing transcriptomes, or for plastic developmental programs to compensate for a gene we CRISPR out of the genome. A more philosophical weakness is that in our own hubris it is very easy to shift the boundaries of what we consider to be the core-GRN and equally easy to ignore evidence of the same or similar core-GRNs in clearly non-homologous tissues (like the Notch/Hes interaction being a core-GRN in non-homologous segmentation in some annelids, arthropods, and vertebrates). That said, there does appear to be mounting evidence that, at least in some cases, we can associate a novel organ with a novel core-GRN⁴.

For now, we will try taking the best of all these definitions to examine some interesting characters that have evolved in various groups. We can even try to define them as novel (or not) by these definitions. We will look at comparative developmental biology and genetics of several key "innovations" (a term that has to do with function rather than distinctiveness). In this chapter, we will look at one ectodermal novelty, insect wings.

8.2: Case Study - The Evolution of Insect Wings

Insect wings are an incredibly important novelty associated with the radiation of the insects into one of the most diverse clades on the planet. They occupy land, water, and air and eat almost every food source imaginable. While their origin seems almost "out of the blue," careful developmental and paleontological studies have revealed key insights into their evolutionary history. Unlike insect legs, which form on the ventral side of the body, wings are dorsal appendages. This alone suggests a separate evolutionary origin from legs since they are not serially homologous. But, like legs, wings are found only on the thoracic segments - suggesting that Hox genes limit the expression of wing organizer genes. The insect body plan has three thoracic segments - T1, T2, and T3. Of these, only T2 and T3 build wings. In *Drosophila*, the T3 wings are reduced and form tiny halteres, which are proprioceptive organs. In beetles like *Tribolium*, which has been the focus of several wing development studies, the T2 wing forms an elytron (plural: elytra), a hard protective covering (Figure 1).



Figure 1: Types of insect wings. A dragonfly has pairs of wings on T2 and T3. A crane fly has a pair of wings on T2 and a pair of halteres on T3. A ladybug has a pair of elytra on T2 and a pair of wings on T3. Wings, halteres, and elytra are serially homologous structures. Dragonfly image from pixabay.com by user francok35 under a CC0 license, crane fly image from Wikimedia Commons user Nono64 under a CC 3.0 BY-SA license ladybug image by Clinton and Charles Robertson published under a CC 2.0 BY-SA license.

The Hox gene *Scr* represses wing development in T1, lowering *Scr* expression in *Tribolium* beetles gives an extra T1 outgrowth that resembles an elytron⁶. Looking more closely at the T1 tissue that forms the extra elytron in *Scr*- beetles, researchers in the Tomoyasu lab found that it is made up of two separate tissues, both of which normally express a gene called *Vestigial*⁶. *Vestigial* is normally expressed in early wing tissues (called "wing discs") but is also expressed in a few other tissues in the insect body. The Tomoyasu lab was very intrigued by the fact that two non-wing *Vestigial* expressing tissues were repressed from coming together to form a wing by *Scr*. These two tissues are called the pleural plate (the ventral-most tissue, referred to as "pleural") and the carinated margin (the dorsal-most tissue, referred to as "tergal"). The Tomoyasu lab hypothesized that wings evolved from these two body wall tissues fusing together and growing out of the body (Figure 2). Some evidence from this comes from comparing insects to crustaceans (Note here that I use "crustacean" to refer to all non-insect pancrustaceans but "crustacean" is not a true evolutionary clade).

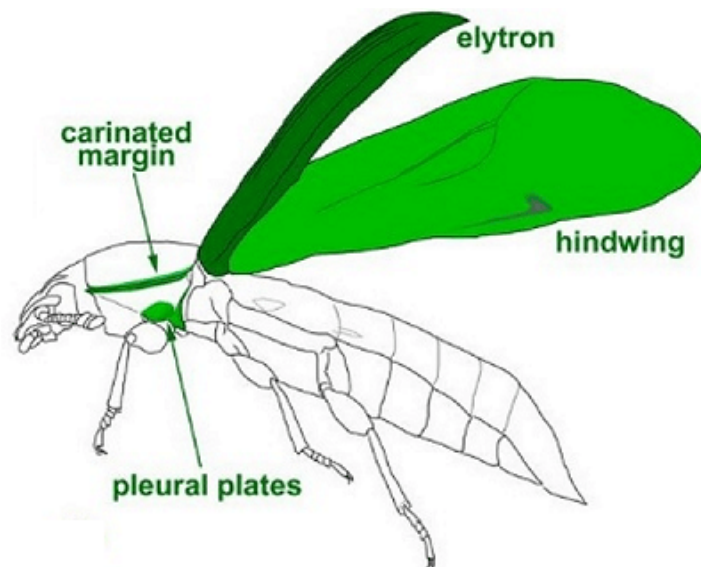


Figure from Clark-Hatchell et al, 2013⁶. In the ancestor to insects and crustaceans, the pleural plate likely formed the gill branch of the limbs, rather than part of the body wall or wing. Original figure published under a PNAS license.

Insects and crustaceans form a monophyletic clade called Pancrustacea and they share the same overall body plan. However, insects have derived limbs that are similar across the clade, while crustaceans in general have much more diversity. Ancestrally, pancrustaceans were marine organisms and had branched limbs, with one branch forming structures like gills. Insects obviously don't use gills, since they live on land and not in water, so the gill-forming structures in crustaceans are thought to form the pleural plates in insects. If the Tomoyasu hypothesis is correct, then insects have an ancestral gill forming structure joining up with a portion of the dorsal body wall (tergal location) to form a new appendage - the wing (Figure 2).

The Tomoyasu lab garnered more evidence for this when they looked for other serial homologs of wing tissue in abdominal segments. They found repeated Vestigial gene expression in dorsal body wall structures called "gin traps". These are defensive structures found in *Tribolium* pupae but disappear by adulthood. They express other wing-specifying genes like Vestigial, Apterous (Ap) Nubbin, and Disheveled (Dsh). Interestingly, they are formed on the abdomen, a part of the body in insects that is famous for not growing limbs. The lab also found a second tissue that expresses wing-specifying genes, the ventral carinated margin (Figures 1 and 2). The lab used the wing and gin-trap marker Nubbin to follow the development of both the carinated margin and the gin trap⁷.

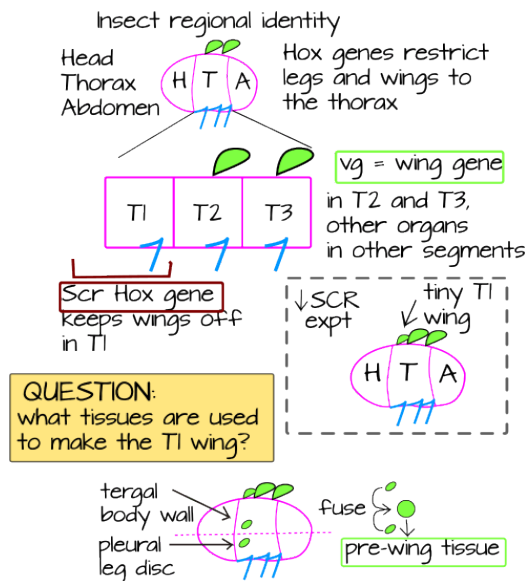


Figure 3: Determining wing identity, details in text. An editable svg version of this figure is downloadable at <https://scholarlycommons.pacific.edu/open-images/1/>

When the researchers experimentally lowered levels of abdominal Hox genes *Ubx* and *AbdA*, they found that instead of gin traps, they got a fusion of the two Nubbin-expressing tissues. This fused tissue later formed a wing-like outgrowth in an abdominal segment (Figure 3). This suggests an evolutionary scenario wherein the pancrustacean ancestor had two Vestigial/Nubbin expressing tissues in its abdominal and thoracic segments. The tergal tissue would become part of the body wall and the pleural tissue would join the ventral legs to become the gill-branch. When insects moved to land, however, the gill-forming tissue was up for grabs since gills were no longer selected for. In the abdomen, the gill-forming tissue becomes part of the body wall, but in the thorax, it fuses with the Vestigial/Nubbin expressing tergal tissue to form wings. In the abdomen, where wing formation is suppressed, the tergal Vestigial/Nubbin tissue is free to take on a new fate. In the case of *Tribolium*, it forms a defensive structure in the abdomen, the pupal gin-traps (Figures 3 and Figure 4). In the thorax it forms the carinated margin. In this way, the wings are a novelty, but can also be thought of as serial homologs to the pleural plates+carinated margin in the thorax as well as to the gin-trap+pleural tissue in the abdomen.

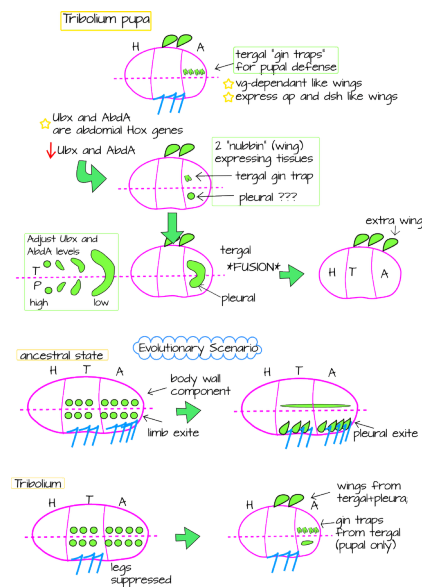


Figure 4: Evolution of Insect Wings, details in text. An editable svg version of this figure is downloadable at <https://scholarlycommons.pacific.edu/open-images/38/>

Variations on a theme

Case study: bat wings

- <http://genesdev.cshlp.org/content/22/2/141.full>
- <http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005738#pgen.1005738.ref012>
- www.ncbi.nlm.nih.gov/pmc/articles/PMC1458926/

Brand new stuff:

Case study: placenta

- <https://www.nature.com/articles/s41559-017-0072>

8.E: Novelty Discussion

What counts as novelty?

(Note: This is intended as an online Discussion Board homework, it could also be modified for use in a classroom discussion)

Todd Oakley (an expert in the genetic basis of novel features in evolution) sparked an interesting Twitter and Amazon book review discussion on how to define novelty.

Please read his blog post [linked here](#) and discuss the following questions with your classmates:

1. How would you define novelty?
2. Is there a fundamental difference between microevolution and macroevolution?
3. Do you agree with McShea and Brandon's rule of macroevolution (that complexity and diversity increase unless opposed)? Why or why not?
4. What does Dr. Oakley mean when he says that novelty happens at the macroevolutionary scale but not at the microevolutionary scale?

8.S: Novelty (Summary)

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3. "Intraspecific Variation in Developmental Characters: The Origin of Evolutionary Novelty" (2000), Wallace Arthur, American Zoologist, www.jstor.org/stable/3884463.
4. "Homology, Genes, and Evolutionary Innovation" (2014), Gunter Wagner, Princeton University Press
5. "Evolutionary innovations and novelties: Let us get down to business!" (2015), Zoologischer Anzeiger - A Journal of Comparative Zoology, Gunter Wagner, <https://doi.org/10.1016/j.jcz.2015.04.006>.
6. "Insights into insect wing origin provided by functional analysis of vestigial in the red flour beetle, *Tribolium castaneum*." (2013), PNAS, Courtney M. Clark-Hachtel, David M. Linz, and Yoshinori Tomoyasu, <https://doi.org/10.1073/pnas.1304332110>.
7. "Dual evolutionary origin of insect wings supported by an investigation of the abdominal wing serial homologs in *Tribolium*" (2017), PNAS, David M. Linz and Yoshinori Tomoyasu, <https://doi.org/10.1073/pnas.1711281115>.

9: Evolvability and Plasticity

Evolvability has emerged as the connection between population-level evolution and macroevolution. Although both of these processes are fundamentally the same (both occurring through mutation, drift, and selection), they happen at such different scales that we typically use very different tools and mindsets to study each of them. For example, population-level evolution is often modeled with Hardy-Weinberg related equations examining the fitness and prevalence of particular alleles. On the other hand, we typically study macroevolutionary change by comparing distinct species using the fossil record and molecular genetics. The question is, then, how do we go from here to there? How do these micro-level processes eventually result in macro-level patterns? Evolvability is one useful way to approach this. A population is "evolvable" if it can cope with changing environments via adaptation. That is, **if genetic mutations in the population have a good chance of increasing fitness.**

While at the surface level, this seems simplistic, we can follow it to deeper levels to see how the evolvability of a population affects developmental processes whose evolution underlies macroevolutionary changes. Gerhart and Kirschner tackle this in their paper the [Theory of Facilitated Variation](#). In this paper they identify major routes by which developmental genetic changes can "facilitate" (or help increase) phenotypic variation without compromising fitness (i.e. they increase evolvability).

These routes, termed weak regulatory linkage, exploratory processes, and compartmentation, are summarized below:

Ability to engage in weak regulatory linkage

Linkage occurs between processes that are connected to each other or to the same specific conditions. For example, when you are cold you shiver and get goosebumps. These two processes are connected to each other via a condition (cold) and also via the sympathetic nervous system. Likewise, developmental processes can be linked via shared gene regulatory networks and/or via shared regulatory components (for example genes that are expressed in response to the same transcription factor, or signal transduction pathways that respond to the same signaling molecule).

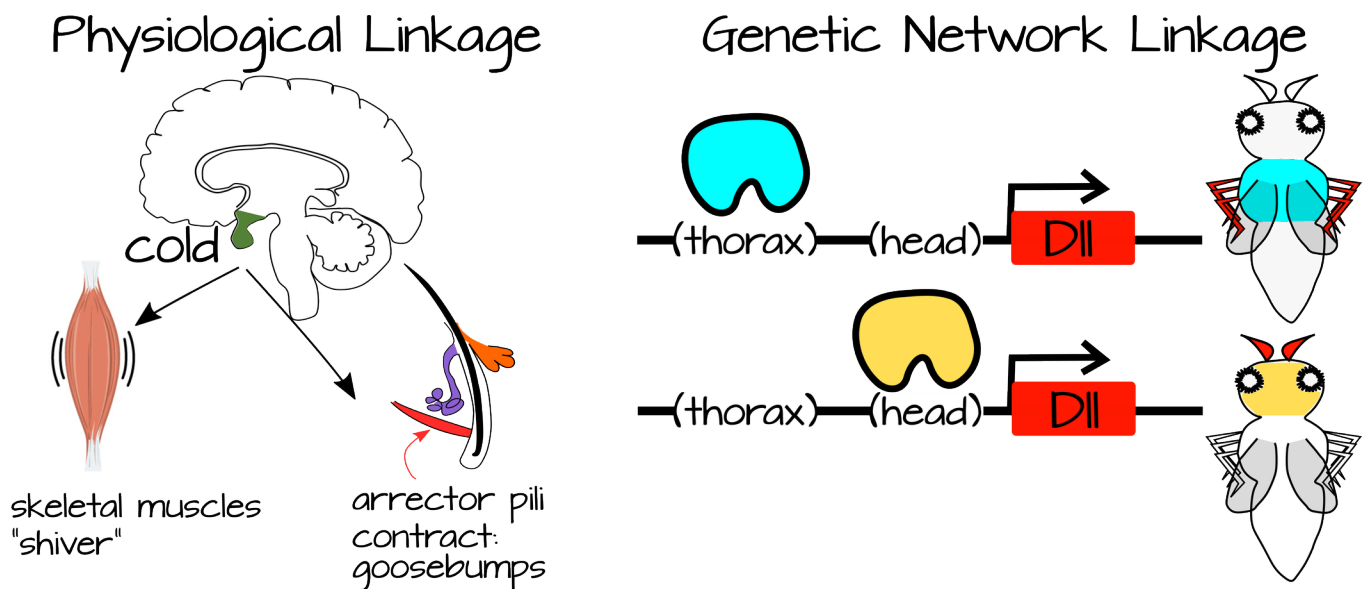


Figure 1: Regulatory Linkage. A single, simple, input may trigger multiple complex outputs. On the left, the hypothalamus integrates cold sensations from the sensory system. It sends out hormonal and nervous system signals to different body parts to trigger thermoregulatory processes. The skeletal muscles will contract quickly to produce heat, causing shivering. Arrector pili muscles in the skin will contract to cause hair follicles to stand up. On the right, Distalless (Dll, red) transcription is regulated by thoracic (blue) and head (yellow) transcription factors.

Its expression directs appendage development in both tissues - antennae and mouthparts in the head, and legs in the thorax. Brain illustration by Alain Gallien <http://svt.ac-dijon.fr/schemassvt/>, skeletal muscle illustration by Open Staxx College <http://cnx.org/content/col11496/1.6>, both images from Wikimedia Commons and published under a CC BY license. An editable svg version of this figure is

downloadable at <https://scholarlycommons.pacific.edu/open-images/39/>

Weak regulatory linkage refers to linked processes that are regulated by simple inputs that do not provide much information to the processes. We have seen examples of this in Habits of Highly Effective Signalling Pathways, where a transcription factor

releases repression on a set of downstream genes. These genes self-activate via local activators as soon as the inhibition is released suggesting there could potentially be multiple ways to de-inhibit them. This is considered weak regulatory linkage because the regulation by the transcription factor is "weak." When local activators are present, the default for the process is "on". The transcription factor is only needed to release inhibition. Once this inhibition is released, a cascade of gene expression can occur, resulting in a complex process with large developmental or physiological consequences. In this way a complex process can be turned off or turned on in new places with small-scale regulatory changes. For example, the transcription factor expression can be turned up or down, or other proteins can act to modify the ability of the transcription factor to bind to its target DNA.

In general, weak regulatory linkage occurs when a simple signal can trigger multiple complex processes depending on the cellular context. Gerhart and Kirschner note that this increases the plasticity of a system since small changes to the regulatory factors (transcription factors for example) can change the functional output of a complex system. The complex system itself is largely self-regulating, requiring only a trigger from the regulatory factors. In this way, development can occur more slowly or more quickly in certain conditions, choices between two tissue states (e.g. gonad type) can be modified by environmental factors, etc.

Exploratory processes

Exploratory processes are search and find processes like following a chemical cue gradient to a point source. We see this type of process in development when we examine how axons form during neurogenesis and how vasculature arises to feed organs and tissues. We see it in adults in the vertebrate adaptive immune system and foraging behavior in chemically-guided organisms like ants. We say that this type of process shows robustness because it adapts quickly to local environmental changes. For example, with strict XY grid patterning of vasculature, growth over developmental time would need to be under strict control and size/shape variance in organs would not be tolerated. Small blood vessels, however, do not grow due to a coordinate system, rather they grow based on a "supply and demand" system with outgrowths to regions of low oxygen. Cells secrete a protein signal (VEGF) when they are low on oxygen, promoting blood vessel growth towards them².

Interactive Content

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

[View this Interactive Content online](#)



Video: Angiogenesis. The video above illustrates the process of blood vessel formation (angiogenesis) in response to low oxygen levels. In this video, high oxygen levels are red and the lowest oxygen levels are blue, see scale on right. Hypoxic (low oxygen) tissues secrete VEGF protein, which signals blood vessels to grow sprouts in the direction of the VEGF gradient. VEGF secreting tissues are hasmarked. This system ensures that tissues get the oxygen and nutrients they need depending on their metabolic rate. Video from Secomb et al, 2013⁵ Published under a [CC BY 4.0 license](#)

Exploratory processes are also adaptable over evolutionary time since they allow for size and shape variance. This is important within populations, where individuals may vary in size or shape. But it is also important over longer periods of evolutionary time as these flexible patterning processes will form a useable system based on their simple set of rules for growth. For example, transplanted leg discs in insects become innervated as do ectopic limbs in chick embryos^{3,4}. In this way, exploratory processes facilitate evolution by helping to build a viable body when other morphological components have evolved into more fit conformations.

Compartmentation

In plants, the ability to separate the the light reactions from the Calvin Cycle of photosynthesis either temporally (as in CAM plants) or spatially (as in C4 plants) has lead to incredible success and diversity in these plant types as they are no longer reliant on keeping their stomata open during the hottest and dryest (but also sunniest) parts of the day. In our own cells, we can see compartmentalization of our mitochondria, with the intermembrane space about 10 times more acidic than the lumen. This allows for the proton motive force to be concentrated and also protects the pH of the rest of the cell. Our cells also physically separate cell-cell signaling from transcriptional regulation - allowing modification of signaling pathways dependent on cell history

(development) and environment.

When one compartment of an organism (or cell) can act semi-independently of another, we expect higher variation. That is, each compartment can run its developmental program via activation and inhibition of specific GRNs without disrupting the development of other compartments. In this way, gene expression changes that affect only one or a few compartments might be limited in effect. Evolution thus may be able to act on compartments individually, as long as these are expression changes like transcript number, protein isoforms, or post-translational modification. Protein-coding mutations, on the other hand, could potentially act on many compartments at once. Evolution can potentially act on individual compartments via gene expression changes, while exploratory processes can maintain the robustness of signaling and nutrient pathways across the entire body.

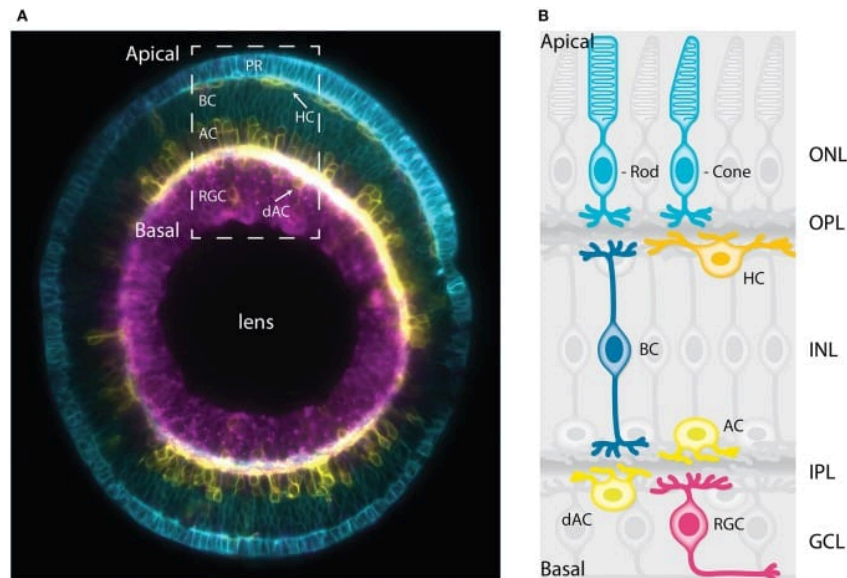


Figure 2: Compartmentation in the nervous system. The central nervous system of vertebrates is highly compartmentalized. During development, "lamina" (layers of neural cells) form via asymmetric cell divisions and cell migration. On the left is a picture of a zebrafish retina with different types of neurons stained different colors. Different types of neurons inhabit different lamina (layers). This compartmentation by function is known to support processing of different types of visual information (color, depth, motion, etc) in parallel to greatly speed up visual processing in the cortex of the brain. Thus, a spatial separation of functions is strongly selected on in the retina. On the right is a cartoon of the different types of neurons found at each layer of the vertebrate retina. Rod and cone cells are the photoreceptors and retinal ganglion cells (RGC) send information to the brain via the optic nerve. Figure from [Amini et al, 2018](#) originally published under a CC-BY 4.0 license.

Evolvability in animals relies on two competing types of development:

1. Processes resistant to evolutionary change. These include exploratory processes that use external signals to find the most efficient or most effective morphology, leading to retention of the process of. But these can also include processes that are conserved over long evolutionary periods of time due to physical or genetic constraints.
2. Changable processes. These include processes that change over time because they are either compartmentalized or are co-opted onto other robust developmental programs. Compartmentation (a process resistant to change) allows for physical separation of developmental processes, and weak regulatory linkage allows for co-option of processes to new times and places (for example compartments).

In this way, evolutionary change happens on a background of robust processes. Some of these robust processes are malleable in developmental time (like vascularization), while others are constraints - limiting what is possible in a particular evolutionary lineage. One example we have already considered is tetrapod limb evolution. While there are physical and genetic constraints on this limiting the number of bone condensations per developmental section, these constraints also create compartments - the stylopod, zeugopod, and autopod as well as the 5 digits. Size variation and adaptations to the compartments is supported by robust exploratory processes, linking bone development to supporting musculature, vasculature, and innervation.

Further Reading

Deep homology (genetic constraints): <https://www.sdbonline.org/sites/fly/lewhelddeep/deephomology.htm>

Bauplans (developmental constraints): <https://evolution-outreach.springeropen.com/articles/10.1007/s12052-012-0424-z>

Genetics is modulated by environment, this modulation can be short-lived (like getting a tan) or long-lived (like getting insulin-resistant diabetes). It can even span generations (thrifty epigenotype). How does this "plasticity" arise in genetics and how does this change how evolution works?

<http://science.sciencemag.org/content/357/6350/eaan0221.long>: If you get your 23andme genotype done, it will only tell you what your risk is for certain disease and disorders. Why can't we be sure?

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Thumbnail is a graph of standing variation in a population (10,000 polymorphic sites from Human Chromosome 1) by Graham Coop, published on Wikimedia Commons under a [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/) license.

10: CASE STUDIES

This section examines the evolution and development of specific novel traits including ectodermal appendages (like hair and sweat glands), lactose tolerance in adults, and eyespots in butterflies (thumbnail image).

Several of these sections were written by students as they took Evolutionary Developmental Biology as part of their final projects.

[10.1: Case Study - Ectodermal Appendages](#)

[10.2: Bird Dactyly](#)

[10.3: Butterfly Wing Spots](#)

[10.4: Lactase Persistence](#)

[10.5: Gene Duplication and Folivory \(leaf eating\)](#)

10.1: Case Study - Ectodermal Appendages

Ectodermal appendages are organs associated with the skin like feathers and hair. We typically only call vertebrate skin elaborations "ectodermal appendages" even though other animal groups have skin organs (like glands) as well. In this section we will just focus on the vertebrate appendages with a special focus on the mammary glands. Details on this as well as a summary of the development of other skin organs can be found here: [Mechanisms of ectodermal organogenesis](#).

Ectodermal appendages (or organs) have common developmental patterning and are thought to have a common evolutionary organ as well, with co-option of elements from different appendage types occurring frequently. These appendages are first obvious as a thickening of the epidermis to form a "placode." This is followed by cellular proliferation of the epidermis and condensation of underlying mesenchymal cells of the dermis to form a bud with a thick mesenchymal base. The ectodermally derived bud can now undergo one of multiple trajectories, which it chooses based on signals from the mesoderm - the mesenchyme of the dermis as well as the somitic mesoderm (Figure 1). We roughly classify these appendages into two main groups: the oral appendages, including teeth and salivary glands, and the skin appendages, including feathers, scales, hair, mammary glands, sweat glands, and oil glands.

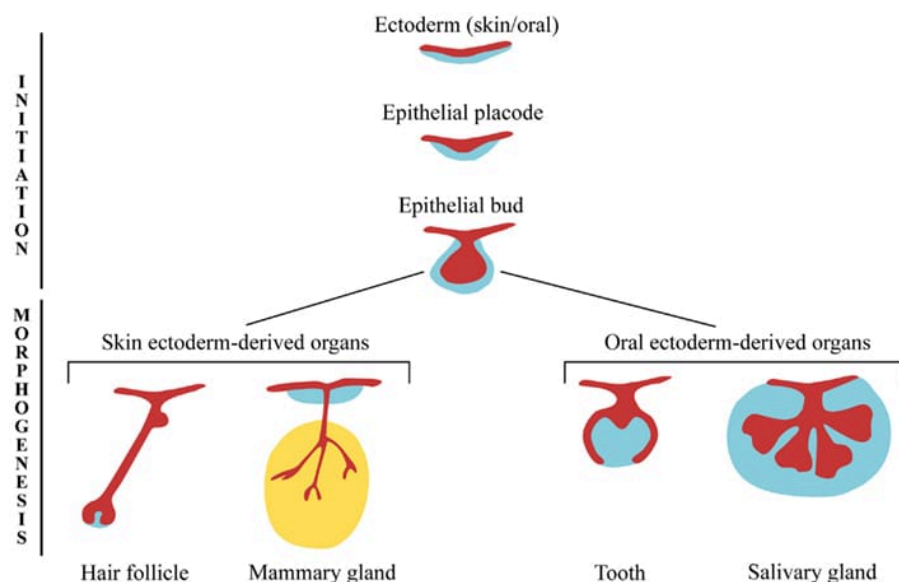


Figure 1: Overview of ectodermal appendage development: From Jimenez-Rojo et al, 2012¹ published under a [CC BY-NC 3.0](#) license. Mammary gland development differs slightly from general ectodermal appendage development in that placode formation is preceded by mammary line (ML) formation. Two lateral lines extending along the trunk are later split up into small placodes.

Early on, scientists noticed something interesting - once an ectodermal placode was in place, it could be induced to form one of many tissues. For example, mammary bud mesenchyme can stimulate the formation of mammary glands in dorsal epithelium². One interesting experiment showed that mouse dental mesenchyme could even induce teeth in chick oral epithelium, despite the fact that birds do not ordinarily grow teeth³. As discussed earlier, ectodermal appendage development follows the trajectory of general to specific (Figure 2) with the early placodes being less specified than later buds.

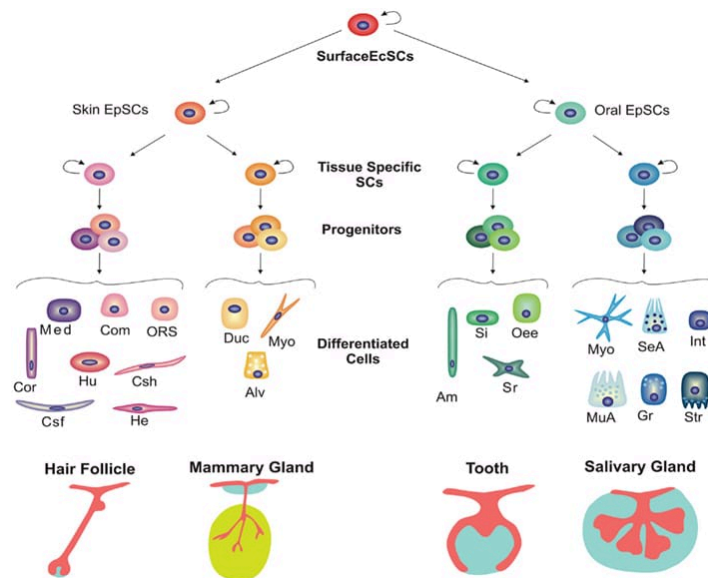


Figure 2: Specification of cell types in ectodermal appendages¹ published under a [CC BY-NC 3.0](#) license. Ectodermal Stem Cells (EcSCs) give rise to Epidermal Stem Cells (EpSCs) in the skin and oral epithelium. Regionalization and Tissue Specific signaling from the mesoderm specifies these EpSCs as a specific appendage. Once specified, the stem cells produce several types of progenitor cells that will differentiate into the cell types of the organ. The mammalian hair follicle is made up of 8 different tissue-specific epithelial cell types, while the mammary gland is made up of only three tissue specific cell types, myoepithelium, ductal, and secretory (alveolar) cells. Glandular skin appendages are typically made up of three layers - the acellular basal lamina on the outside, contractile myoepithelium in the middle, and luminal lining cells on the outside. In the alveoli, the luminal cells secrete milk. The ductal cells are luminal cells that line the ducts leading from the alveoli to the nipples.

Evolution and Diversity of Tetrapod Skin Appendages

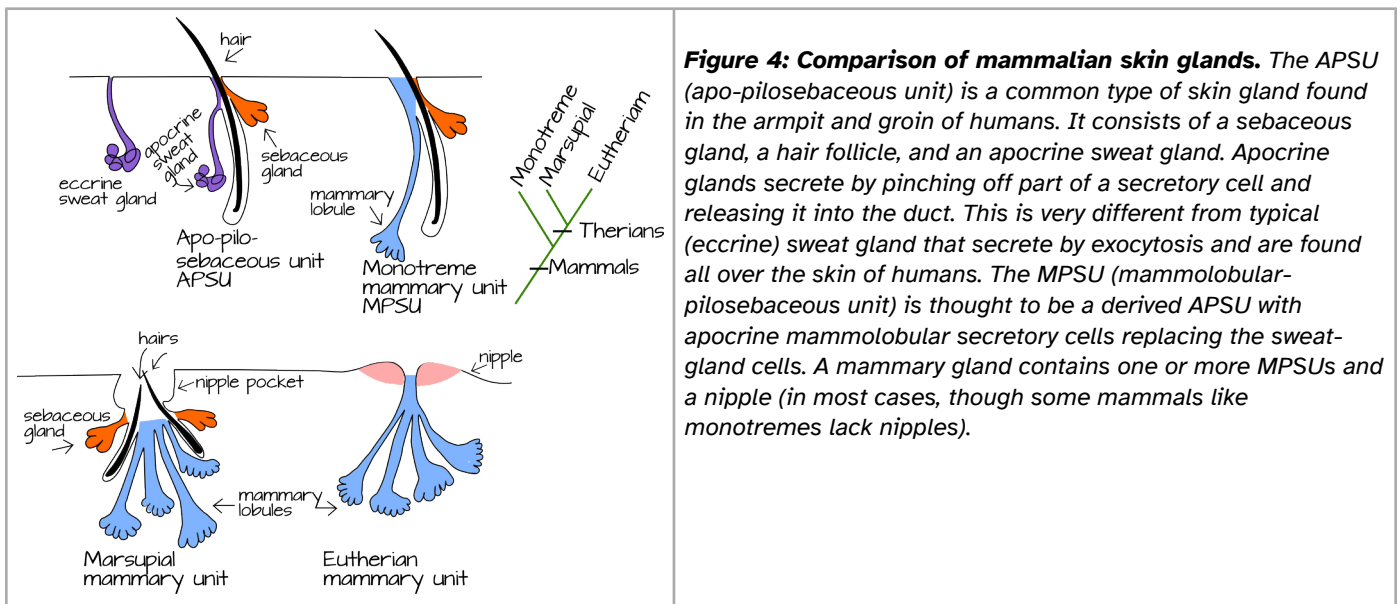
Fishes have their own amazing and diverse skin appendages including the "lateral line" organs that detect electrical fields, pressure and water flow changes⁴. However, here we will focus on the ectodermal appendages of the tetrapods with a focus on the mammary glands of Mammalia. The ancestral tetrapod is hypothesized to have had mucus glands in its skin, similar to those in living amphibians. These mucus glands keep the skin moist and prevent the internal parts of the animal from drying out. Additionally, mucus glands and other skin glands are used by brooding modern amphibians to keep their eggshell-less eggs from drying out in terrestrial ecosystems and may have played a similar role in early tetrapods (Figure 3).



Figure 3: A Northern Dusky salamander guarding her terrestrial eggs from Dave Huth [CC BY-NC 2.0](#)

About 350 million years ago, one group of tetrapods, the *Amniota*, evolved a new type of skin protein that prevented their skin from drying out: keratin. All amniotes (including mammals, birds, lizards, etc) have alpha-keratin and the sauropsids (birds and other reptiles) additionally have beta-keratin, which they use to build feathers and scales. Unlike egg-laying mammals, sauropsids have calcified eggshells which reduce water loss. The egg-laying mammals (the monotremes like platypus), have parchment shelled eggs that can dry out quickly. This is thought to be the ancestral condition for the group containing the mammals, the synapsids, and glandular skin secretions from the brooding parent (like mucus secretions in salamanders) are thought to have helped prevent egg desiccation in ancestral synapsids including mammals⁵.

The ancestral mammal thus inherited glandular skin that was multifunctional: it likely secreted oil, sweat, and other dilute secretions (possibly a form of sweat) to keep its eggs hydrated. We can get some hints as to how these skin glands diversified into multiple ectodermal appendages by comparing the three living clades of mammals: the egg-laying monotremes, the therian marsupials, and the therian placentals (also known as the eutherians). Mammals have four main types of skin glands, these are the wax producing glands of the ear (which we will ignore for now), the sebaceous glands that produce oil, the sweat glands, and the mammary glands. The last three of these glands have special anatomical relationships with each other. A typical hair follicle is associated with a sebaceous gland and can also be associated with an apocrine sweat gland. The mammary glands in some species are associated with a hair follicle as well as a sebaceous gland. This has led to the hypothesis that mammary glands are at least partly derived from apocrine sweat glands (Figure 4).



Overview of mammary gland development

Despite the diversity in their final forms, mammary glands develop along largely similar pathways across mammals. As shown in Figure 1, they are induced by underlying mesenchyme and other signals to form two parallel mammary lines, which are likely induced by the Gli3-induced secretion of FGF10 by a subset of the somitic mesoderm, driving the expression of Wnt10b in the overlying ectoderm and specifying mammary cell fate⁶. Monotremes retain mammary lines as patches of milk-secreting cells. Cross-talk between the epidermis and dermis (mesenchymal) generate a mammary bud, which grows downward into the mesenchyme to form a mammary sprout. In therians, the mammary buds and sprouts receive BMP4 signaling from the underlying mesenchyme to continue to prevent them from taking the default hair-follicle fate⁶. In other parts of the skin, Noggin and Shh inhibit BMP4 and allow the default hair fate⁶. The epithelial mammary buds express PTHrP (**Parathyroid Hormone related Peptide**), which activates its receptor in the mesenchyme to induce condensation and differentiation of the growing mammary primary sprout. Thus there is cross-talk between the epidermis and dermis (mesenchyme) to generate the mammary bud and induce growth into the primary sprout. In some placental mammals like mice the primary sprout grows deeply into hypodermal fat tissue where it begins to branch into alveolar glandular tissue. In other mammals it branches more shallowly into secondary sprouts, which themselves branch into alveolar glandular tissue⁸.

Where did the hair go?

If mammary glands evolved from hair follicles and their associated glands, what happened to the hair? While many placental mammals have areolar hair (Figure 5), most do not have hair associated with the part of the nipple that secretes milk. Additionally, no placental mammals examined develop a hair follicle during or before formation of a mammary sprout. I will get back to the placental mammal issue in a moment, but first I'd like to spend a little time looking at our hairy-aerola cousins.



Figure 5: Placental mammal nipple hair. (Public Domain; [Xpoirotx](#))

Monotremes, those basally branching mammals, do maintain hair association with mammary glands (Figure 4). Oftedal proposes that this is the ancestral mammalian case and that mammary hairs, rather than a nipple, have a dual use: they not only wick milk from skin glands into the mouth of an infant, but they also wick moist secretions onto desiccation-prone eggs. The evolution of internal embryonic development and live-birth in the therian mammals changed the evolutionary pressure on the milk-producing glands. They lost the selective pressure to keep eggs hydrated and instead were selected on solely for nourishment of infants. This may have driven the milk ducts to condense and flow into a single nipple per mammary gland, rather than a dispersed hair patch (Figure 6)^{5,8}.

Evidence from mammary gland development in therian mammals lends some support to this hypothesis. In marsupials, early mammary gland development proceeds as a physically condensed version of monotreme development. In monotremes, a single mammary primary sprout develops into a hair follicle, a sebaceous gland, and mammary secretory and ductal cells (the multi-component MPSU). In marsupials, a single primary sprout forms multiple MPSUs, each with its own hair follicle, sebaceous gland, and mammary cells. However, the hair follicles eventually atrophy and die, leaving behind a large pocket into which milk and sebum can empty (Figure 4)⁸. Placental mammals (eutherians), like us, are more variable. Eutherians can make one or many primary sprouts per mammary bulb and (as mentioned above) may make or lack secondary sprouts. Species without secondary sprouts do not have hair follicles and sebaceous associated with growing mammary glands, but species with secondary sprouts do. This includes humans and horses. Our MPSU hair follicles and sebaceous glands atrophy and die during embryogenesis so we are left with an MPSU made of a single component: the mammary alveoli and ducts⁸.

By comparing the development of the mammary glands of these different groups, Oftedal and Dhouailly have proposed a scenario for mammary gland evolution by co-option and heterochrony. Briefly, mammary glands use existing skin placode developmental mechanisms to begin to form, in particular the existing patterning used by the apo-pilosebaceous glands, then switch to a program of branching morphogenesis (a developmental patterning mechanism found in the kidney, lungs, and salivary glands in tetrapods but also in bird feathers and insect tracheal development). This creates the ductal tree of the mammary gland as the apocrine portion of the apo-pilosebaceous unit, instead of a sweat gland⁸. The origin of the overlying nipple is still unclear but is developmentally induced by the developing mammary gland.

The genetics of mammary gland evolution: co-option and novelty

Earlier, I briefly discussed the fate choice that skin makes between hair and mammary gland - turning on BMP4 signaling in mesenchymal cells via FGF10 signaling from part of the somitic mesoderm turns off the default hair program and turns on the

mammary program. FGF/Wnt/BMP interactions are used in forming other skin in overlying ectoderm is used in patterning other skin appendages and may be a general cassette for ectodermal patterning⁹. In this way, mammary glands placode and bud formation are likely driven by similar signaling as other ectodermal appendages with some modifications. Once the mammary placode/bud begins to grow, it expresses a common epidermal organ gene: PTHrP. PTHrP has a more ancestral function in vertebrates in endochondrial bone formation and tooth eruption. As teeth are an ectodermal epithelial structure, it is likely that PTHrP's role in mammary gland development was co-opted from tooth development. Evidence for co-option of tooth genes comes from another gene family key in tooth development -the calcium binding protein ODAM. ODAM was duplicated during tetrapod evolution to form the caseins, the calcium transport proteins in milk¹⁰.

FGF10 and Wnt10b are not the only players in mammary specification. Several Hox genes are implicated in ectodermal organ specification, with Hoxc8 initiating mouse mammary placode development and Hoxb3, b6, b9, d9, d10, and d8, among others, expressed during later development, including during postnatal mammary development^{11,12}. Of these, Hoxd9 and d8 are most commonly expressed in mouse mammary bulbs. These two genes are also involved in both limb and hair development in mice.

While Hoxd9 and d8 are located next to each other on the chromosome and largely regulated by two giant cis-regulatory elements HoxD-T-DOM and HoxD-C-DOM, they do not exhibit completely identical patterns of expression, suggesting that there are specific regulators that act differentially on their individual promoters. Recently, scientists in the Beccari lab in Geneva dissected the regulatory elements driving Hox d8 and d9 expression and found how they managed to go from being expressed in the limb and hair to being expressed in the mammary bulb. While Hoxd8 mammary bulb expression depended on a 13kb local enhancer region, the Hoxd9 mammary bulb expression depended on distant cis regulatory regions in T-DOM¹².

The Hoxd9 mammary bulb cis regulatory element is particularly intriguing because it seems to partly rely on the existing cis regulatory elements that drive limb expression of Hoxd9. Limb expression is driven by cis regulatory elements within T-DOM and during limb development, a giant 1500 kb region of the chromosome folds over to contact Hoxd9. The mammary bud enhancer for Hoxd9 is also found within this 1500 kb region, and it is thought that this enhancer takes advantage of the attraction between the limb bud enhancer and the Hox cluster to get access to Hoxd9 during mammary bulb development (Figure 7)¹². Taken together, this begins to paint a picture of the evolution of mammary placodes and bulbs - they use existing transcription factor and patterning genes in new combinations. At least one of the genes involved gets expressed in the developing mammary tissue by co-opting and modifying existing enhancer elements.

Once the embryonic mammary glands are built, they still need to secrete milk. While the similarities between milk-producing glands and apocrine sweat glands are obvious at anatomical and developmental levels, the content of the two substances produced is substantially different. One milk product, the caseins, is made from mutated ODAM tooth protein (see above). The major protein essential in processing the fat droplets found in milk, XOR, is usually an antimicrobial protein but plays a second role in milk biosynthesis. This is a clear case of heterotopy (expression of a gene in a new domain). Lysozyme took both paths - it was co-opted from the immune system as an antimicrobial protein in the mammary glands, and it also underwent a gene duplication to form a whey protein used to lactose¹³. Even though milk composition is complex and varies from species to species (even within species as an infant gets older), we can trace the evolutionary history of these components and see how evolution could have acted to produce this secretion.

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Thumbnail: Closeup image of peacock feathers, which is one example of Ectodermal Appendages. (Public Domain).

10.2: Bird Dactyly

Birds are in the class Aves. They evolved from theropod dinosaurs and the first fossil bird discovered was *Archaeopteryx*. A few characteristics that make an animal a bird include wings, toothless beaks, and feathers. Bird feet have four digits but these can be arranged in different ways. The most common digit arrangement is three in the front and one in the back as seen in perching birds and raptors like the Savanna Hawk (Figure 1). The difference between the perching foot and the raptor foot is not in the digit layout but in the claws. In the raptor foot, the claws are more curved. They can also have two in the front and two in the back as seen with the grasping bird in the image. Another variation in bird feet is webbed digits which occur in swimming birds like ducks.

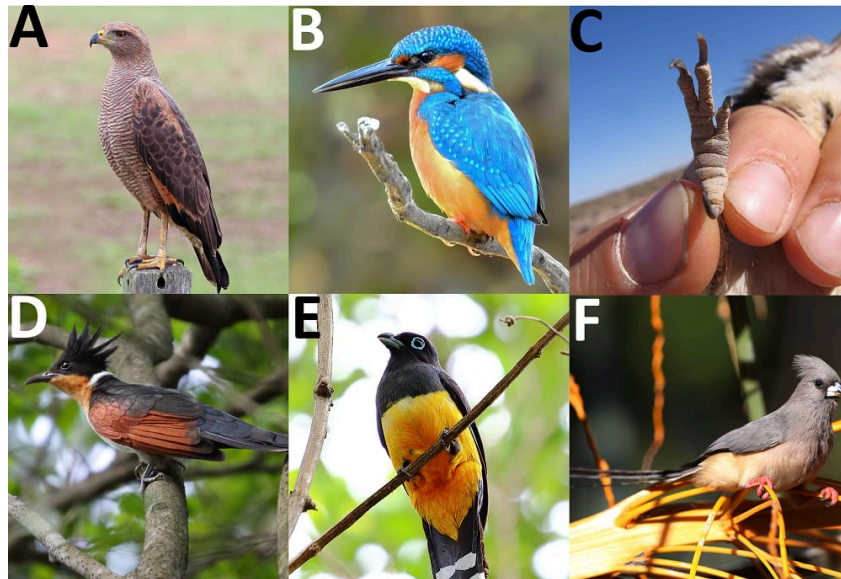
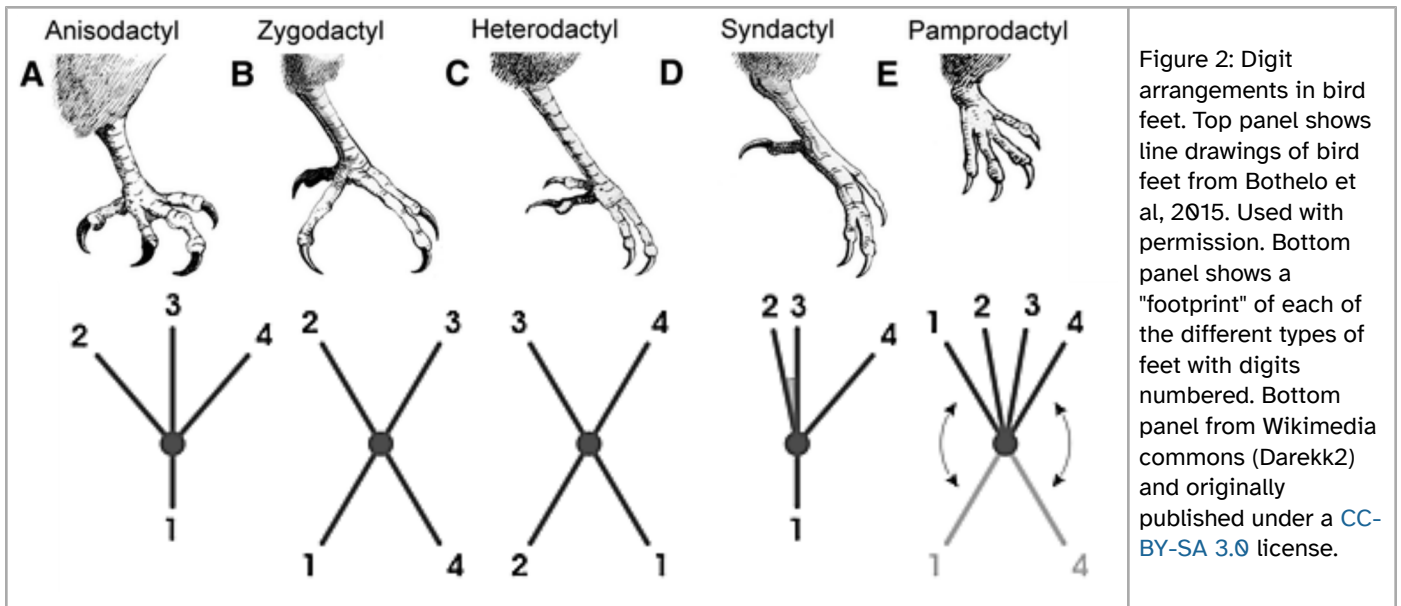


Figure 1: Birds with different types of digit arrangements on their feet A. Savanna hawk (anisodactyl) by Charles J Sharp, published under a [CC BY-SA 4.0 license](#). B. Common kingfisher (syndactyl) by Shantanu Kuveskar, published under a [CC BY-SA 4.0 license](#). C. Sacred kingfisher foot by PookieFugglestein published under a [CC0 1.0 license](#). D. Chestnut Winged Cuckoo (zygodactyl) by William Lee published under a [CC BY-SA 3.0 license](#). E. Black-headed trogon (heterodactyl) by Ryan Candee published under a [CC BY 2.0 license](#). F. White-backed mousebird (pamprodactyl) by Derek Keats published [CC-BY 2.0 license](#) note forward position of all digits.

There are a few different digit arrangements commonly seen in bird feet (Figure 2). This most-common arrangement is called anisodactyly. It is where digits 2, 3, and 4 face anteriorly and the hallux faces posteriorly (Figure 2). Syndactyl feet have the same arrangement as anisodactyl, but digits 2, 3, and 4 are encased by skin, this is seen in kingfisher feet (Figure 1). There are also more extreme cases of webbing, especially in ducks and other water-fowl. A zygodactyl foot is when two digits, 2 and 3, are facing anteriorly and the other two digits, 1 and 4 are facing posteriorly, one example of this is in cuckoos and owls (Figure 1). A heterodactyl foot is similar to a zygodactyl foot but digits 3 and 4 face anteriorly and digits 1 and 2 face posteriorly, this foot is very rare and only seen in trogons (Figure 1). And finally a pamprodactyl foot is when digits 1 and 4 can rotate so that all four digits can face anteriorly. Mousebirds and swifts are famous for their pamprodactyl feet (Figure 2). When their digits face the same way, swifts (not pictured) are able to use them as hooks.



Birds arose from theropod dinosaurs with 4 toed feet (Figure 3). The anisodactyl foot formed when the hallux was rotated posteriorly. It also used to be slightly elevated and smaller than the other digits. It is now no longer elevated and longer than the second digit. This resulted in digits 2, 3, and 4 facing anteriorly and digit 1 posteriorly as shown in the previous images. This is a modified version of the ancestral foot with few changes. The zygodactyl foot formed when both digits 1 and 4 rotated posteriorly (Figure 4). This is a more drastic change than the anisodactyl foot.

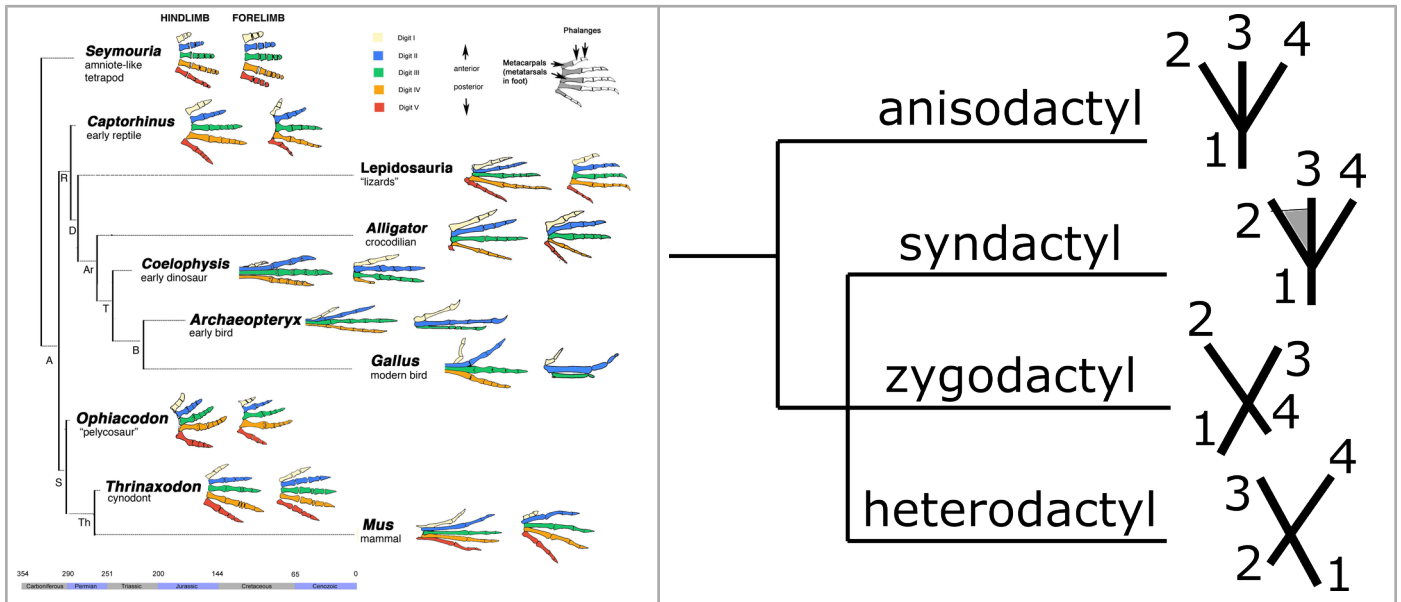


Figure 3: The evolution of digit morphology. Left panel: Homologous digits are color coded. Forelimbs are on the right and Hindlimbs are on the left. Most vertebrates have five digits on each autopod, but the reptile lineage shows asymmetric digit loss. Birds, like Archaeopteryx and chick (Gallus gallus) have four digits on their hindlimbs and three in their wings. Within the bird lineage different digit arrangements have evolved. Image from Vargas et al, 2008 published under a CC BY license. Right panel: the evolution of digit arrangements within the bird lineage. The order in which each type of dactyly evolved is unknown and at least one has evolved multiple times. Figure by Jessica Niccum, Alexis Amador, and Ajna Rivera

Botelho et al (2014) examined the development of the zygodactyl foot seen in birds like budgies. In this study, they found that in the beginning stages, budgerigars had similar development to that of a chicken (which has anisodactyl feet). Unlike chickens, however, after about 35 hours, the fourth digit rotated medio- laterally. After 36 hours, the fourth digit became flexed resembling a semi-zygodactyl foot, like owls have. After 37 hours, the fourth digit further rotated to resemble zygodactyl foot orientation

(Figure 4). This suggests that digit rotation near the end of foot development is responsible for the evolution of zygodactyly, and zygodactyl feet do not grow digits in a new location. Because of this late-stage difference, the researchers examined the possibility that muscle formation and activation was responsible for digit rotation.

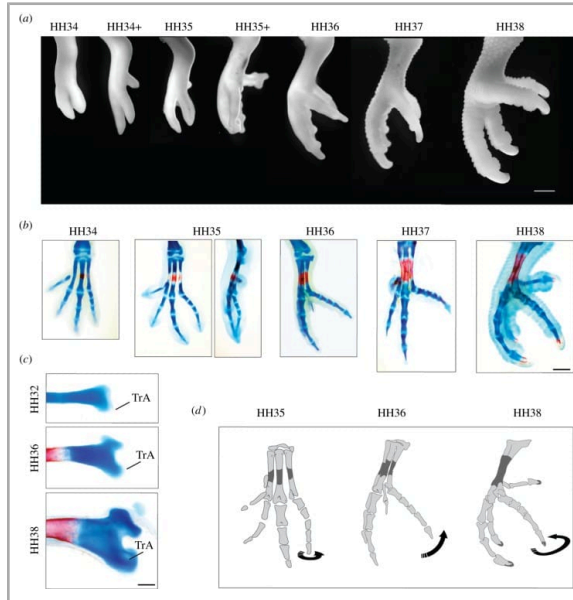


Figure 4: Development of a zygodactyl foot. A. External anatomy of a developing budgerigar foot at successive stages (HHXX). B. Skeletal structures in budgerigar foot development, cartilage is stained blue and bones are red. C. TrA is trochlea accessoria, the distal tip of metatarsal 4. The hook shape seen at HH38 is characteristic of zygodactyl birds. D. Diagram of the change in digit 4 orientation during development. Figure from Botelho et al 2014. Used with permission.

Much of the development of various digit conformations is tied to the development of muscle formation and activation in the early stages. In anisodactyl feet, both EBDIV and ABDIV (extensor and adductor brevis muscles) are well developed. In zygodactyly such as budgies, however, the EBDIV is almost completely absent. The researchers found that the EBDIV becomes reduced when it separates from the other muscles in zygodactyl feet. The ABDIV, however, is well developed. The ABDIV causes the flexion of the 4th digit. This flexion combined with the constraint from EBDIV results in the zygodactyl orientation (Figures 4 and 5).

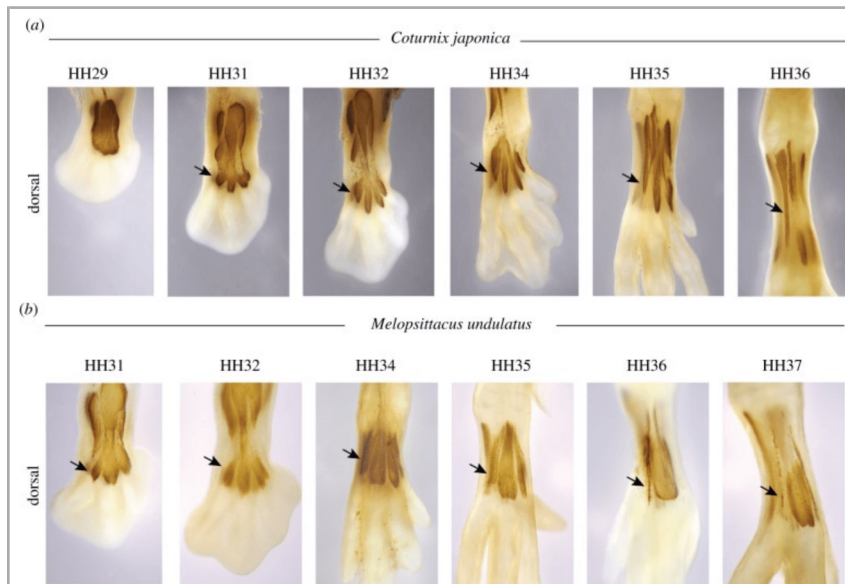


Figure 5: Anisodactyl vs. zygodactyl foot development. A. Shows the development of a quail foot while B. Shows the development of a budgerigar foot.

Brown is antibody staining against myosin2, which is found in muscles. Arrows point out the musculus extensor brevis digiti 4, which degenerates in the budgerigar and allows the musculus abductor digiti 4 to pull the fourth digit medio-laterally.

Figure from Botelho et al, 2014. Used with permission.

To our knowledge, development of other bird hindlimb digit confirmations haven't been studied. Based on what we know about anisodactyl and zygodactyl foot development, we hypothesize a similar evolutionary and developmental trajectory for heterodactyl feet. The Heterodactyl foot looks similar to the Zygodactyl foot but digit 2 is rotated instead of digit 4. We think the development could be the same as the Zygodactyl foot but the muscular changes affect the 2nd digit. Trogon birds have heterodactyl feet (Figures 1 and 2). They have two digits anteriorly, digits 3 and 4, and two posteriorly, digits 1 and 2.

Syndactyl feet, like those of kingfishers (Figure 1) have fusion of two digits via webbing. Under this webbing, they have the same

digit conformation as anisodactyl feet. Research on bat wings has shown that the webbing between the digits is due to expression of a *Bmp* antagonist. In other animals like mice, *Bmps* cause the death of the interdigital mesenchyme. So, a *Bmp* antagonist in syndactyl birds could potentially prevent the apoptosis of the mesenchyme surrounding the digits, leading to syndactyl feet.

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10.3: Butterfly Wing Spots

Butterfly wing patterns are organized along Ground Plans, which are compartments for different intervein pattern elements (Figure 1). These pattern elements include eyespots, ellipses, and midlines. The Ground plan, at least for nymphalids, has three symmetry bands: the basal system closest to the body, the central system, the border system, and the margin, which is farthest from the body. Eyespots occur the border band. Eyespots serve a role in predator avoidance and sexual signaling. They come in different sizes, shapes, numbers, and colors (Figure 5). They can also differ within a species based off geography, season, and sex. There are three stages of eyespot development: establishment of eyespot foci in late larval wings, establishment of color rings in early pupae, and pigment synthesis in late pupae.



Figure 1: Blue Morpho Groundplan by Kinsei Imada. Veins are in blue and pigment spots are in white. Colored regions indicate clonal boundaries. Cells within a clonal boundary are thought to come from the same "mother" cell. Clonal boundaries were estimated by comparing gynandromorphic butterflies to wild type butterflies. Gynandromorphic butterflies have some male cells and some female cells. Cells from a male "mother cell" will all be male and cells from a female "mother cell" will all be female. Since male and female cells have different pigment patterns, gynandromorphic butterflies allow us to figure out clonal boundaries.

Stages of eyespot development

Establishment of eyespot foci in late larval wings is the first stage of eyespot development. The location of eyespot foci are determined by the genes *Antennapedia* (Antp), *Notch* (N), and *Distalless* (Dll) in *Bicyclus anynana*. These interact with the transcription factor *Spalt*. *Distalless* has different functions in different butterflies. In *B. anynana*, it promotes eyespot development but in *Junonia coenia* and *Vanessa cardui*, two other nymphalid butterflies, it represses it, suggesting that evolution may act even at early stages of eyespot development. Establishment of eyespot foci occurs in four steps: margin and intervein expression, midline patterning, focal determination, and focal maturation.

Notch functions in defining dorsal-ventral boundaries, it defines intervein tissue through lateral inhibition with its ligand *Delta*. *Notch* upregulation followed by the activation of *Distalless* is an early event for the development of eyespots. *Notch* is upregulated in discrete focal pattern and *Distalless* is upregulated in intervein midlines (Figure 2). *Notch* expression tends to increase over time because of a positive feedback mechanism. The focal *Notch* upregulation precedes *Distalless* activation with lag time of 1.5 stages which is about 12-24 hours.

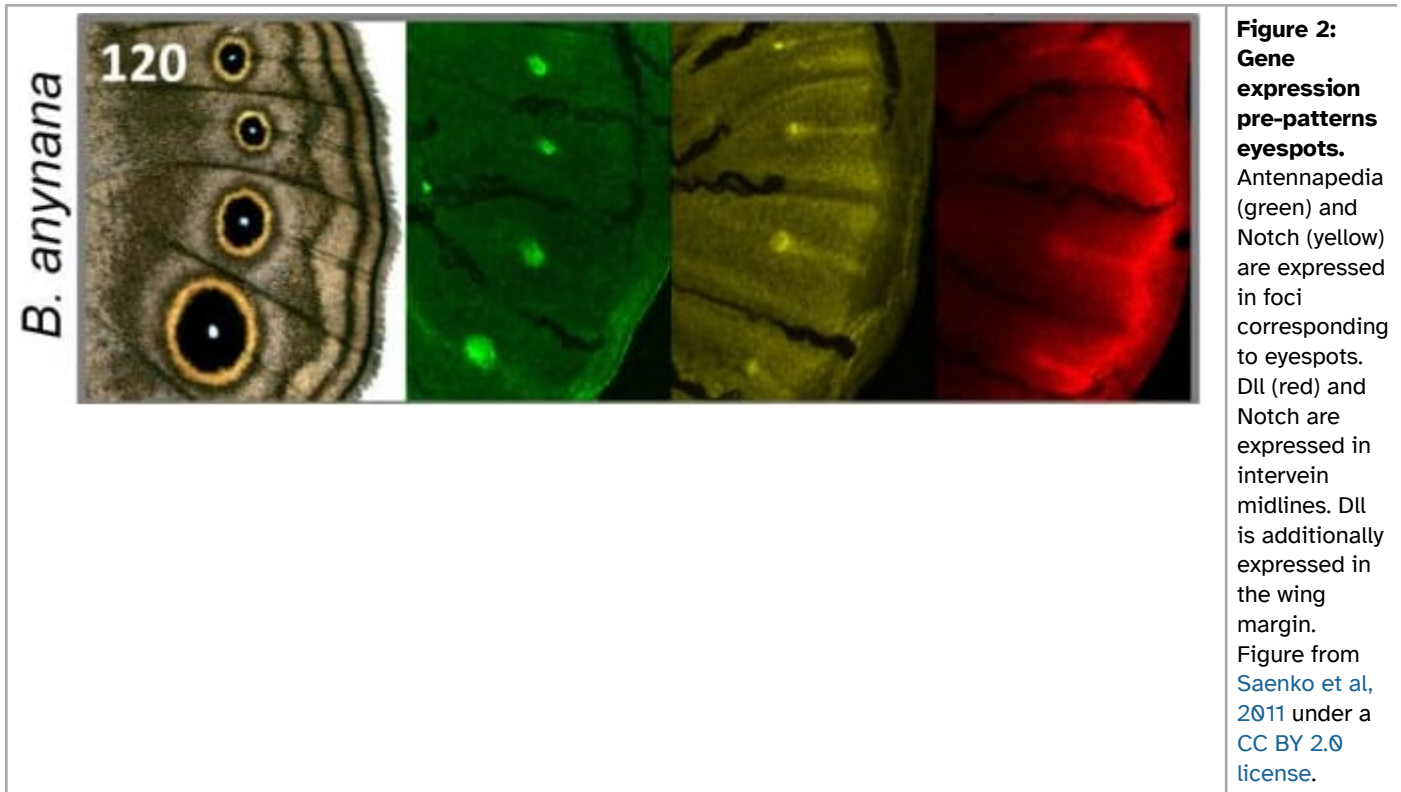


Figure 2 shows the spatial and temporal patterns of Antp, Notch and Distalless in late last instar wing marginal discs of three different butterflies. It shows that there is a perfect correlation between presence of eyespots and late last instar Notch and Distalless focal expression. In genetic experiments, researchers reduced levels of Notch or Distalless, which reduced or eliminates two specific eyespots in the hindwing.

Establishment of color rings is the second step of eyespot development. There is currently no confirmed model, but the most popular theory is that there is a morphogenic gradient coming from the eyespot foci. Epidermal scale cells at different distances respond to signal using transcription factors called Engrailed and Spalt. This stage occurs during pupal development. Though not confirmed how this happens, research shows that the expression of both Distalless and Spalt corresponds with adult black scales, and the expression of both Distalless and Engrailed corresponds with gold scales.

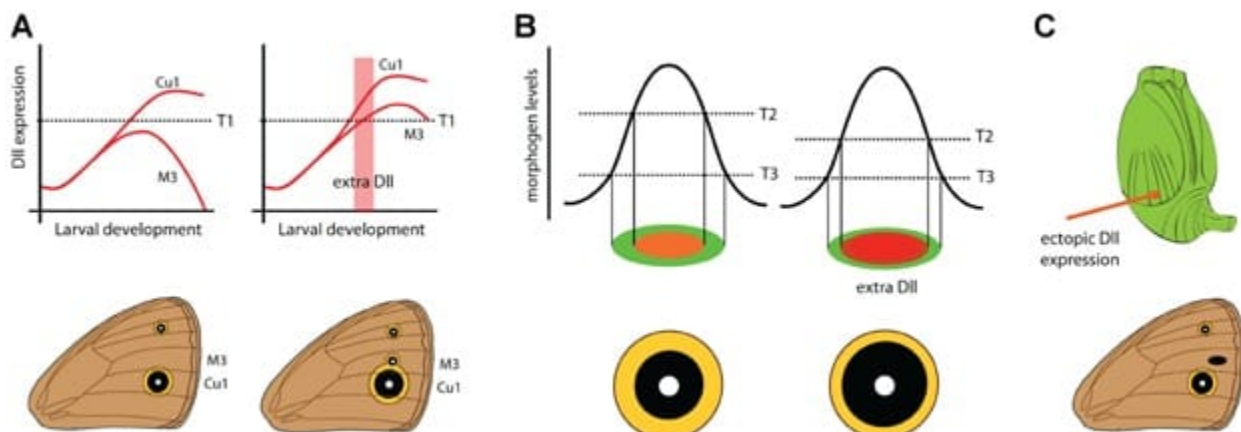


Figure from Monteiro et al, 2013 published under a CC BY-NC-ND 4.0 license.

Pigment synthesis is the third step of butterfly eyespot development. Figure 4 shows the biochemical pathway of pigment synthesis in *B. anynana*. As mentioned before, Spalt and Distalless corresponded to black scales, and Engrailed and Distalless corresponded to gold scales. Melanin biosynthesis pathways allow for the pigmentation of these eyespots.

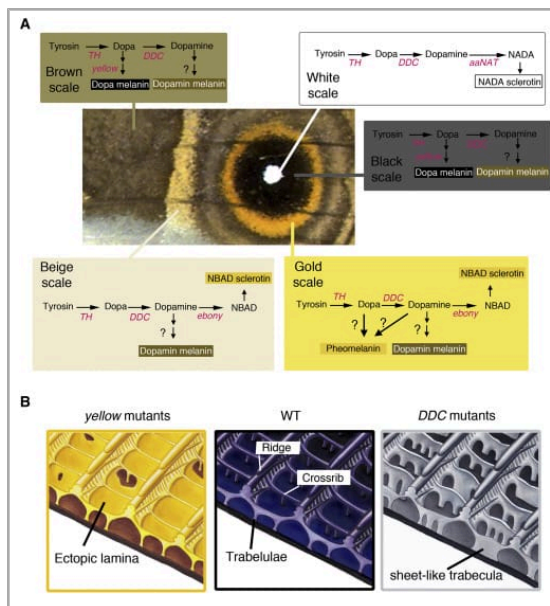


Figure 4: Melanin biosynthesis pathways for pigment synthesis in ***B. anynana***. Figure from Matsuoka et al, 2018 and published under a [CC BY 4.0 license](#).

There are five products of melanin biosynthetic pathways, dopa-melanin, dopamine-melanin, pheomelanin, NBAD, and NADA. Dopa-melanin and dopamine-melanin (the eumelanins) are black and brown pigments, respectively. Pheomelanin is a reddish-yellow pigment, NBAD sclerotin has a yellow color, and NADA sclerotin is colorless. Black scales have a combination of dopa-melanin and dopamine-melanin, which are black and brown pigments. Gold scales have a combination of pheomelanin, dopamine-melanin, and NBAD sclerotin, which are reddish-yellow, brown, and yellow pigments.

Plasticity in eyespot development

Bicyclus anynana eyespots vary in size based on season. Environmental temperature during larval and pupal stages of development leads to changes in ecdysone hormone levels, which regulates eyespot size. This plasticity has likely evolved due to seasonal predation. During the wet season, *B. anynana* have higher levels of ecdysone and larger eyespots, which help them avoid predation. During the dry season, ecdysone levels drop as do eyespot sizes. Hindwing eyespots display higher levels of plasticity in overall size, center size, and center brightness than forewing eyespots, since the ecdysone receptors in hindwing eyespots are much more sensitive than those in forewing eyespots. The result of this is that slight changes in ecdysone levels affect hindwing eyespot development in a much higher degree than forewing eyespot development.

The evolution of eyespots

Eyespots are present in Nymphalidae. They have convergently evolved in butterflies and moths. In butterflies, they first originated along the dorsal midline, as dorsal fin, then appeared as a pair of anterior pectoral fins, and finally in a more posterior region of the body as pelvic fins. Eyespots replaced the simpler pattern elements that already existed at those locations. The simple colored spot became a multicolored and multi-ringed eyespot.

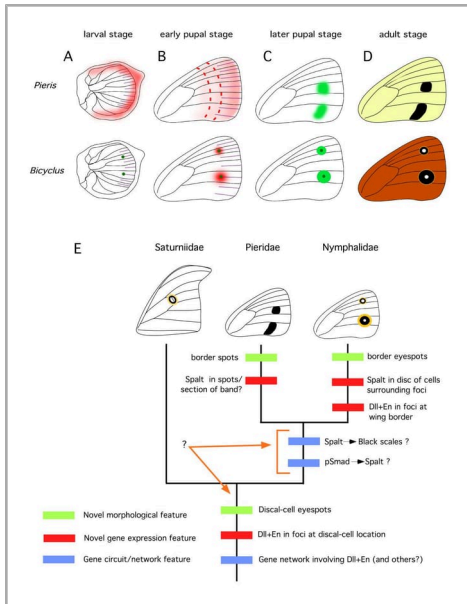


Figure 5: A hypothesis for the evolution of eyespots. Novel features (gene expression, gene networks, and morphological features) are marked where they evolved along the lepidopteran lineage. This hypothesis posits the co-option of discal-cell eyespots (as seen in saturniid butterflies) into border spots and border eyespots following the invention of Spalt regulation of melanin production in scales. Figure from [Monteiro et al, 2006](#) originally published under a [CC BY-2.0 license](#).

The three selective pressures on eyespots are camouflage, anti-predatory defense, and sexual display. Eyespots are a form of camouflage because they look like vertebrate eyes. The selective pressure of anti-predatory defense and sexual display are often at odds with each other, *Bicyclus* species overcome this with signal partitioning. Signal partitioning is when visual signals are separated to different parts of the body, and signal displays are restricted to appropriate spatio-temporal conditions. *Bicyclus* species do this by folding their wings, which hides the dorsal wing surface and exposes the ventral wing surface. To deal with the selective pressure of anti-predatory defense, eyespots are generally on the border of the wing. They are far from the body, so the predators attack the wing instead of the body, allowing the butterfly to escape. Anti-predatory defense places huge selective pressure on large ventral eyespots on the forewing and hindwing because to scare off potential predators, *Bicyclus* species fold their wings. By exposing the ventral wing surface, there is selection for larger ventral eyespots. On the other hand, during sexual display, males open up their wings to expose the dorsal wing surface. Females prefer intact dorsal forewing eyespots in *B. anynana*, since it indicates that the eyespots have UV reflective pupils. Sexual display thus select for more intact dorsal forewing eyespots. Figure 6 shows the differences in ventral and dorsal wing surfaces in four different *Bicyclus* species. Ventral wing surfaces are shown on the left, and dorsal wing surfaces are shown on the right. Ventral eyespots are much bigger than the dorsal eyespots, which shows that signal partitioning does occur with anti-predatory defense selecting for larger ventral eyespots and sexual display selecting for more intact dorsal forewing eyespots. Dorsal and forewing characters are also more likely to change through evolutionary time compared to ventral and hindwing characters because mate selection is a stronger selection pressure than anti-predatory defense.

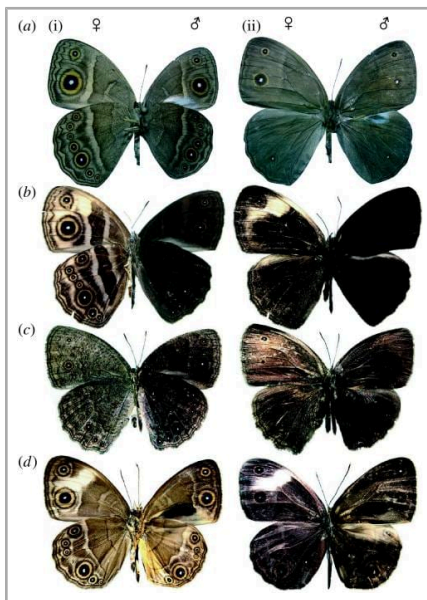


Figure 6: Ventral and dorsal eyespots in four different *Bicyclus* species. Ventral forewings and hindwings are shown on the left, and dorsal forewings and hindwings are shown on the right. Figure from [Oliver et al, 2009](#). Used with permission.

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10.4: Lactase Persistence

Think for a minute about how many people you know are lactose intolerant. While most people think in terms of lactose intolerance, evolutionarily, lactose intolerance is the ancestral state. In mammals, the ability to digest lactose has been selected for in infants. In humans, some people also have the ability to digest lactose as an adult. This is called "lactose persistence." There are four different forms of lactase "deficiency": primary, secondary, developmental, and congenital. Primary lactase deficiency is the loss of lactase from childhood into adulthood, this is common among humans with about 70% of the world's adult population unable to digest lactose. Secondary lactase deficiency is the loss of lactose digestion due to injury to the body (most commonly, the intestines). Developmental deficiency is the lack of lactase due to premature birth, and congenital is the lack of lactase from birth onward. The focus of this article will be on primary lactase deficiency.

Lactose tolerance

Lactose tolerance considers the presence of lactase within the small intestine. It is located on the brush border of the small intestine enterocyte. Lactase is an enzyme that is responsible for breaking up lactose into glucose and galactose, which would be used for glycolysis and energy production in the body. However if lactase is absent in the small intestine, lactose ends up being processed in the large intestine, allowing bacteria to consume it, produce gases and acid, causing traditional symptoms of lactose intolerance such as bloating, flatulence, and diarrhea (Figure 1).

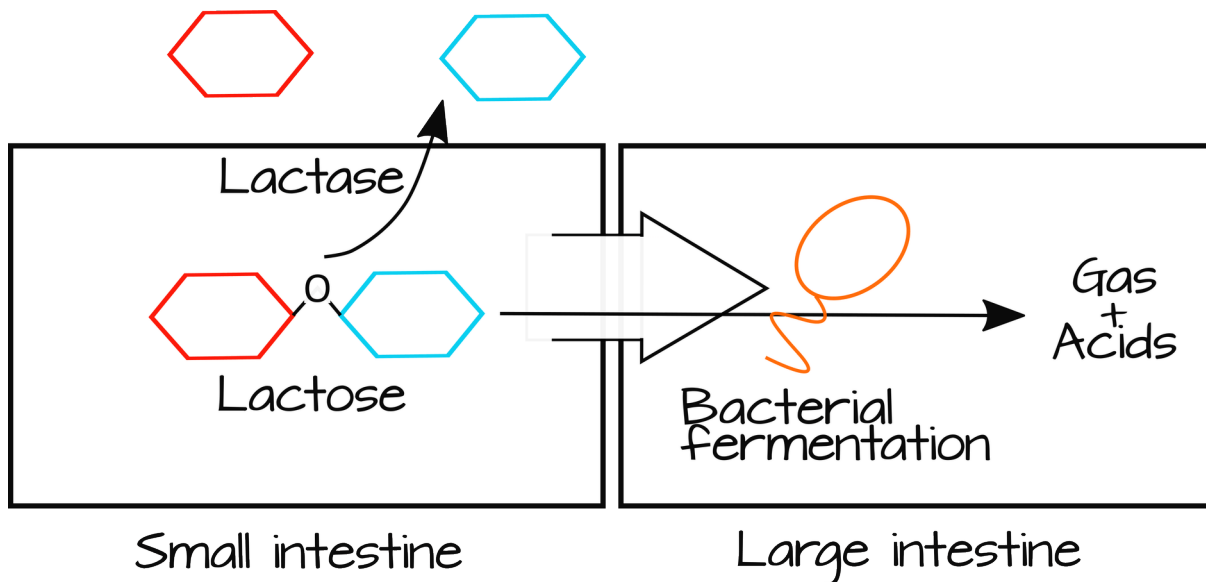


Figure 1: Lactose Processing. Lactose is processed in the small intestine by lactase, it is broken down into two simple sugars and exported from the gut. If lactase is not present, lactose moves through the gut to the large intestine, where it is fermented by bacteria. The side products of this fermentation are gas and acids.

Understanding the genetics of lactase persistence is vital to knowing how it is inherited between populations. Lactose intolerance is inherited in an autosomal recessive pattern, that means that one copy of the lactase persistence allele is enough to confer the ability to digest lactose as an adult. The lactase enzyme is encoded by the LCT gene. This gene has a set of protein-binding regions that help regulate its expression. On the same chromosome is the MCM6 gene, encoding a helicase. Within MCM6 is an additional enhancer region for the LCT gene. Mutations in this enhancer region can confer lactase persistence. The common mutations are named after their distance upstream of the LCT start codon and the nucleotide at that location: -13907*G, -13910*T, -13915*G, -14009*G, and -14010*C.

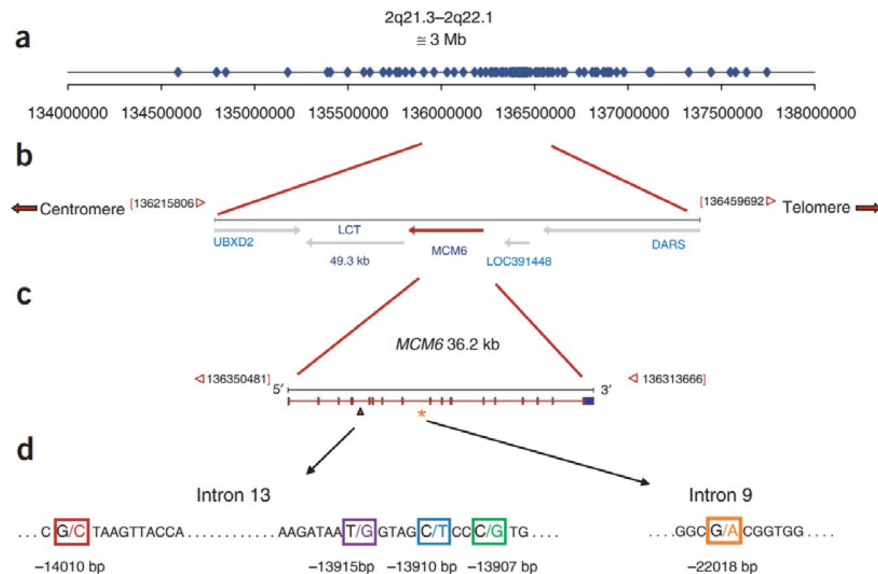


Figure 2: Mutations in the MCM6 gene that affect Lactase expression. This chromosome map incrementally zooms in on the chromosome. (a) Distribution of 123 SNPs; (b) map of the LCT and MCM6 gene region; (c) map of the MCM6 gene; and (d) location of lactase persistence-associated SNPs within introns 9 and 13 of the MCM6 gene in African and European populations. This figure is from Deng et al, 2015 and was published under a CC BY 4.0 license.

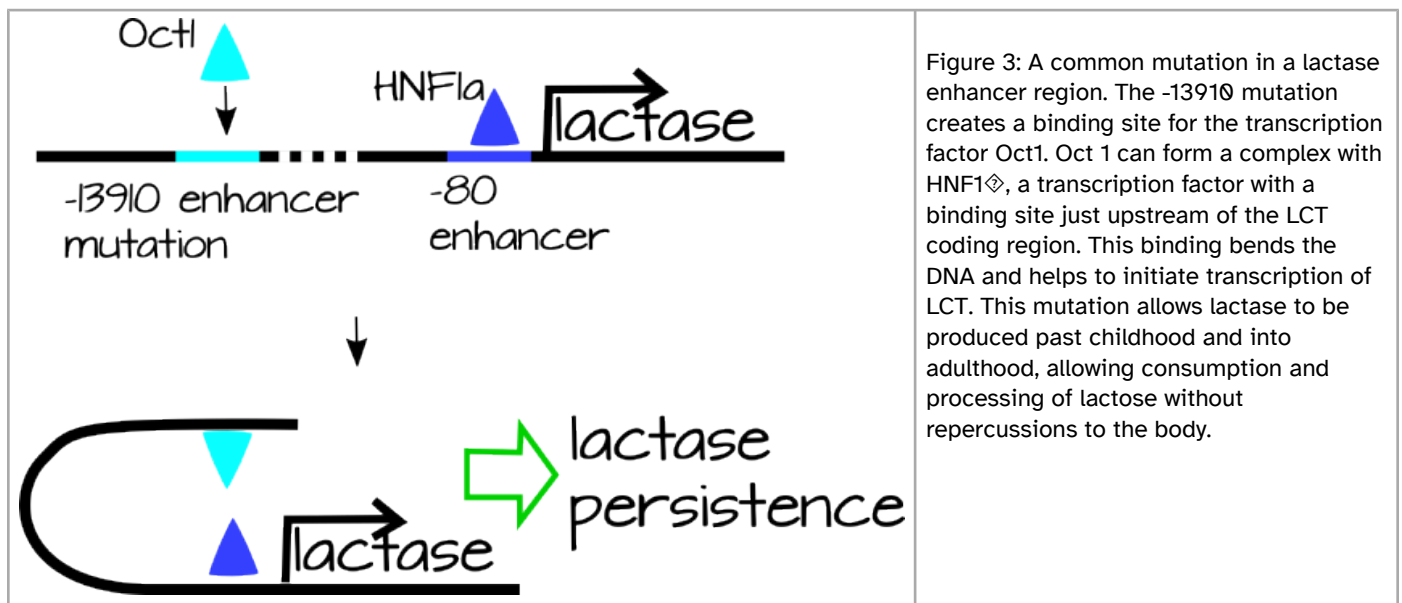
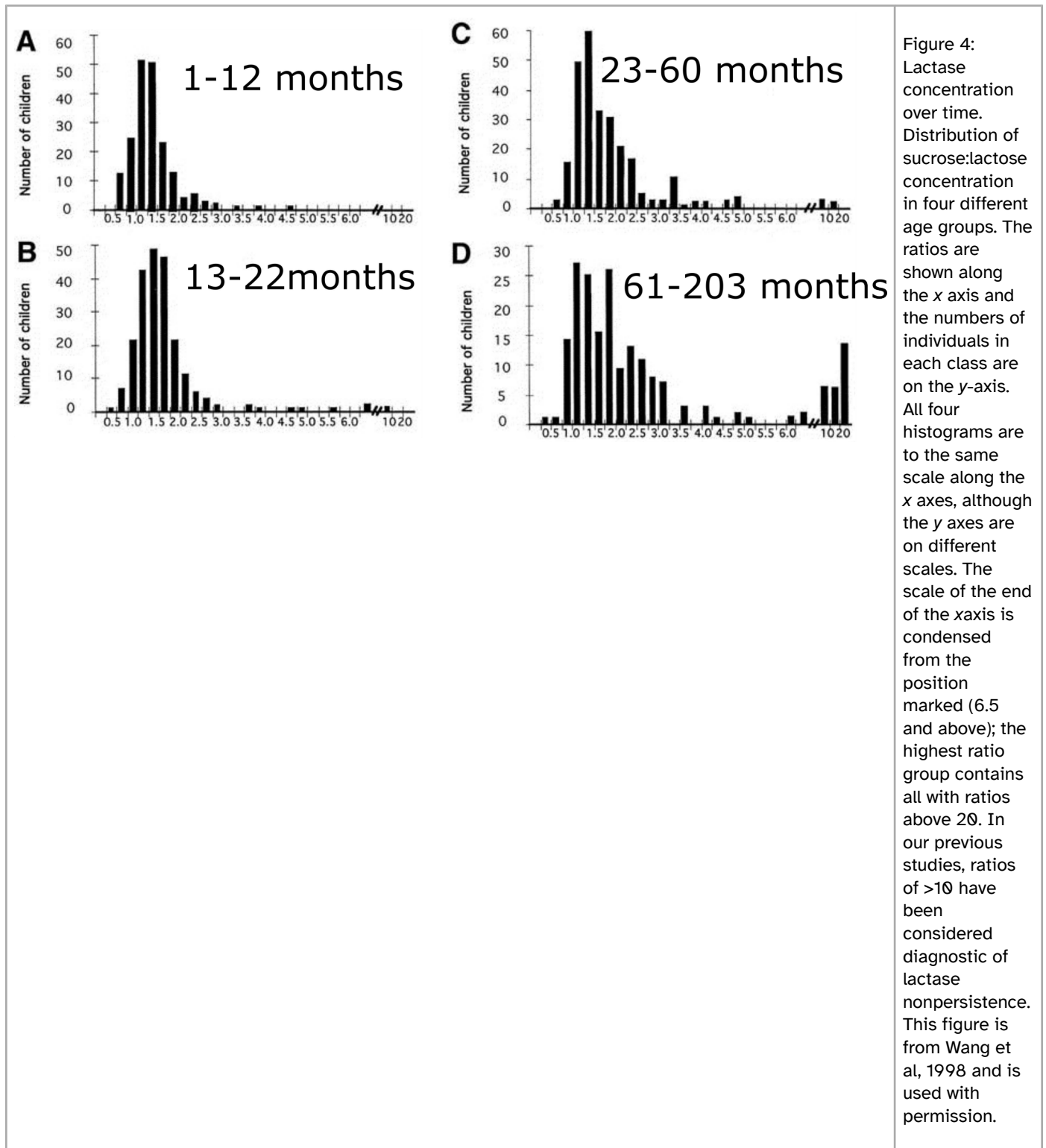


Figure 3: A common mutation in a lactase enhancer region. The -13910 mutation creates a binding site for the transcription factor Oct1. Oct 1 can form a complex with HNF1α, a transcription factor with a binding site just upstream of the LCT coding region. This binding bends the DNA and helps to initiate transcription of LCT. This mutation allows lactase to be produced past childhood and into adulthood, allowing consumption and processing of lactose without repercussions to the body.

The evolution and development of lactose intolerance

Lactose intolerance usually begins between childhood and early adulthood. It is measured by comparing sucrase and lactase concentrations in small intestine biopsies. Sucrase processes sucrose (table sugar) in the small intestine and is present throughout a person's lifetime, its concentration is used to normalize lactase concentration. Figure 3 compares lactase:sucrose concentration in different age groups. In infancy, lactase and sucrase levels are around the same concentrations in most individuals, indicating that there is an abundant amount of lactase present. This is vital during infancy as babies require milk from their mothers for nutrition and energy. At older ages, lactase levels slowly wane compared to sucrase. This is the period where children are weaning off their mother's milk and will no longer need the lactase to digest this source of lactose. In children 5-17 years old, there is a wide spread of lactase concentrations, with many individuals exhibiting very low levels. In many people, the body has turned off the transcription and formation of lactase as it is no longer naturally needed to digest mother's milk. While the exact mechanisms pertaining this change in expression is not known, it is possible that epigenetic modifications are affecting the MCM6 gene. This data is important in demonstrating that humans naturally lose lactose persistence as they grow out of infancy,

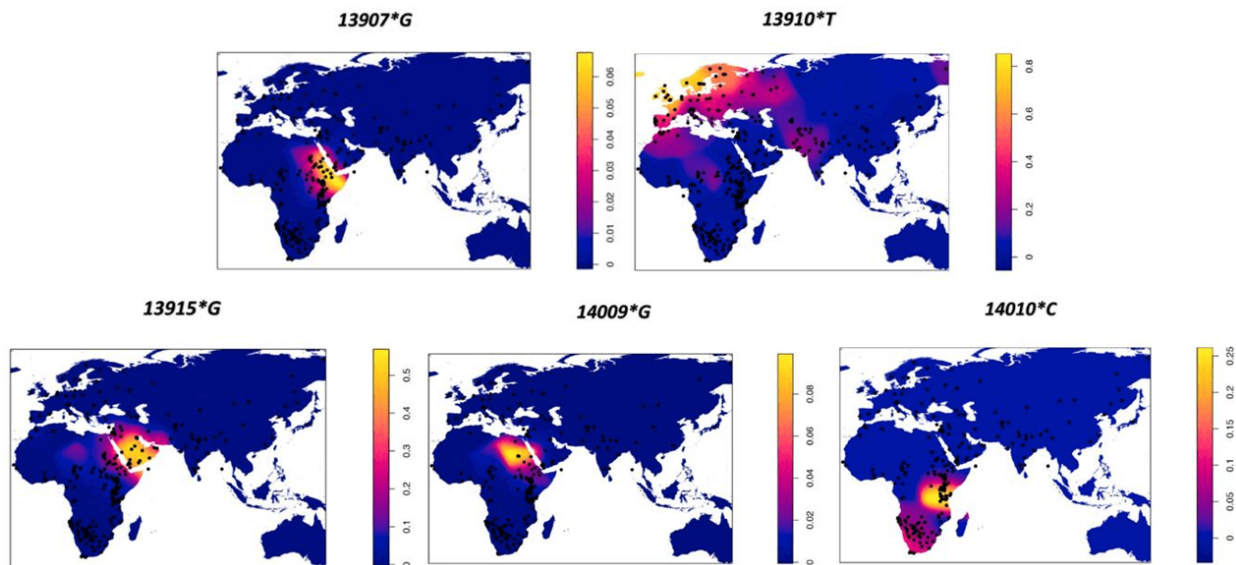
similar to other mammals.



Lactose intolerance in different human groups.

Human Group	Individuals Examined	Intolerance (%)
Dutch	N/A	1
Europeans in Australia	160	4
British	N/A	5–15
Central Italians	65	19
Indians	N/A	20
Australian Aborigines	44	85
African Bantu	59	89
Asian Americans	N/A	90
Chinese	71	95
Southeast Asians	N/A	98
Thais	134	98
Native Americans	24	100

Lactase persistence has evolved several times in humans (see Table to the left from [Silanikove et al, 2015](#), published under a [CC BY 4.0 license](#)). This is associated with ruminant domestication. Not all populations with domestic ruminants (for example sheep and goats in Southern Europe and water buffalos and yaks in Asia) have high rates of lactose tolerance, however. This may be due to the prevalence of cheese, yogurt, and butter production in these populations. Cheese, yogurt, and butter are all low in lactose. The convergent evolution of lactase persistence has been driven by different mutations in enhancer regions. The most common is the European 13910*T mutation (Figure 3). This mutation is seen at the highest levels in Northern Europe and at lower levels in Southern Europe, Africa and the Middle East (Figure 5). The spread of the mutation is thought to be due to migration of populations from the North. However, it is suspected that the population in India is due to the British colonizing and reproducing with the Indian population. Other mutations (Figure 5) are more regionally localized and are all thought to be associated with pastoralism (livestock domestication).



Figures used with permission.

Future directions

As of right now, there's no complete hypothesis for the development of lactase persistence. It's commonly thought that persistence evolved due to a change in food preservation and diet. Especially seen for the 13910*T mutation, a change in climate may have favored adaptations for changes in diet. As people migrated North from the Fertile Crescent, the climate got colder, presenting an environment not suitable for growth of some of their crops. However, the colder environment allowed for further

preservation of milk from domesticated cows. The lack of available food and the readily resource of milk favored people who had the ability to digest milk. Milk is nutritious in proteins, fats, and sugars, giving people additional calories and nutrients.

Most data on lactase persistence is from the Old World where populations are more homogenous. However in the New World, populations found in the USA and Latin America may be more diverse, with many people of mixed racial descent. This presents the possibility of developing some form of tolerance no longer on a binary scale but more on a gradient. It is possible that while some populations would still become intolerant as adults, the period of childhood tolerance could be longer than current data shows. This research will not only guide people into further understanding their ability to process lactose, but also allows people to be more aware of how their current diet might affect the genes and traits they pass on.

10.5: Gene Duplication and Folivory (leaf eating)

Gene Duplication and Folivory: A Novel Trait -- Daniel Song and Ajna Rivera

Earlier in this book, we considered gene duplication as a genetic mechanism for increasing organismal diversity in evolutionary time. In this case-study, we will examine duplication of RNase genes associated with the evolution of leaf eating (folivory) and gut fermentation. Many mammalian clades have evolved gut fermentation and folivory independently, Monkeys have likely evolved it in parallel many times, perhaps not surprisingly given their arboreal lifestyle. Efficient gut fermentation allows monkeys exploit new food resources, leaves, which opens up a new ecological niche. In this way, not only does the complexity of the organism increase (more genes, novel physiological function, new food resource), but so does the complexity of the ecosystem with new ecosystem interactions now possible.



Figure 1: **Left:** A guereza colobine (Colobus guereza) monkey from Africa eating leaves. Photo credit: Thomas Bresson, originally published on Wikimedia Commons under a CC BY 3.0 license **Middle:** A douc langur colobine monkey from Asia may have independently evolved folivory. Photo credit: Ucumari photography originally published on Flickr under a CC BY-NC-ND 2.0 license **Right:** a phylogeny of the colobine monkeys. RNase duplication may have occurred once (yellow star) or twice (green stars), an expanded dataset favors the green star scenario.

Leaves are abundant, why don't all animals eat them?

Leaves (including blades of grass) have abundant energy-rich carbohydrates, but these carbohydrates are largely inaccessible to animal guts. Unlike carbs in roots and fruits, leaf carbohydrates are largely stored as the structural polysaccharides cellulose and hemicellulose. These have β -1,4-glycosidic linkages between their glucose monomers that animal digestive systems cannot break down. Despite this, many animals like colobine monkeys and cows get much of their energy from digesting leaves. Behavioral, physiological, and anatomical adaptations make this possible. For example, some folivorous animals are known to rest more than their relatives (like koalas) and some use their senses to choose leaves with a higher protein to fiber content (like howler monkeys).

Folivorous animals are also able to extract more energy from dietary fiber (cellulose) than typical mammals. They do this by hosting symbiotic cellulase-producing bacteria in their guts. These anaerobic bacterial symbionts ferment semi-digested fiber in specialized gut compartments. These gut compartments act as fermentation vats. The side products from fermentation as well as the bacteria themselves can then be used by the animal for nutrients.

These specialized gut structures can take various forms and have evolved several times. For example, koalas and rabbits have independently evolved an enlarged cecum (where a human appendix is located) and colon to house their symbiotic bacteria. Because these adaptations occur near the end of the GI tract, koalas and rabbits are considered to be "hindgut fermenters." On the other hand, ruminants like cows and giraffes have fermentation vats called rumens directly linked to their esophagus (Figure 2). They are called "foregut fermenters." Colobine monkeys are similar to ruminants in that they have partitioned stomachs,

although their stomachs are not chambered like a ruminant stomach. Colobines additionally have at least some ability to house cellulase-digesting bacteria in their hindguts (Figure 2).

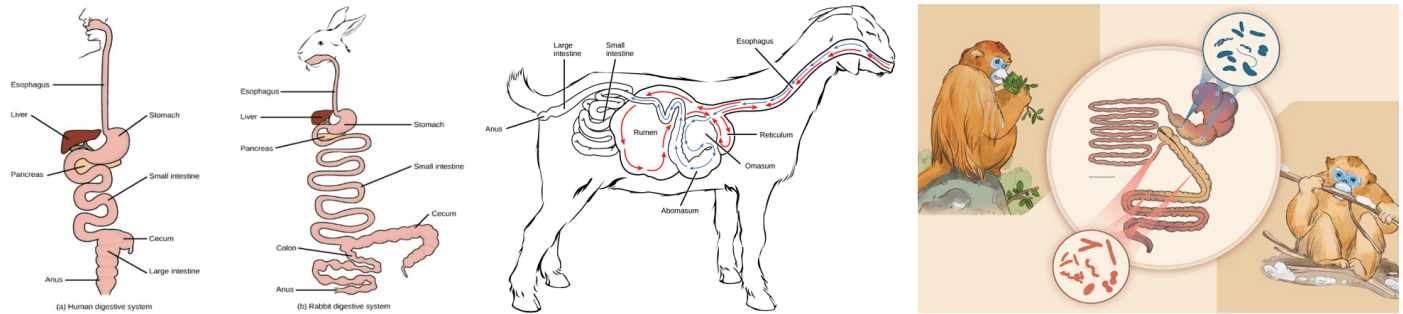


Figure 2: **Left:** Comparison of the familiar human digestive system with a hindgut digester (rabbit). Fermentation occurs in the elaborated cecum of the small intestine. Some hindgut fermenters have fermentation in the colon as well. Image credit: Dr N B Shridhar originally published on Wikimedia under a CC BY-SA 4.0 International license. **Middle:** Ruminants, like goats and cows, are foregut fermenters. Ruminants have a separate fermentation vat called the rumen. Food is chewed, passed through the rumen for fermentation, then returns to the mouth for additional chewing. Food then goes through additional stomach processing before entering the small intestine. Image credit: originally published in Open Stax Biology under a CC-BY 4.0 license (authors Connie Rye, Robert Wise, Vladimir Jurukovski, Jean DeSaix, Jung Choi, and Yael Avissar). **Right:** Colobines are thought to primarily ferment in their sacculated stomach (foregut fermentation) but also house cellulase-producing bacteria in their colon (hindgut fermentation). Unlike ruminants, colobines do not have a completely separate structure for foregut fermentation. Image credit: Liu et al, 2022 published under a CC BY-SA-ND 4.0 license.

Review: Gene Duplication and Evolution

Genetic duplication can occur on different scales, as small as an exon duplication to duplication of contiguous genes on a chromosome. There are various mutagenic mechanisms that result in gene duplication including whole-chromosome duplications via nondisjunction, tandem duplications via unequal crossover, retrotransposons, and duplications after rearrangements and repair of breaks. Duplication creates a new set of raw materials that can be tinkered with to take on new functions while the original set maintains its original intended purpose. Neofunctionalization occurs when positive selection acts upon the new duplicated set of genes and one or both gain a new function. If this new function is either fit in the environment or neutral, the duplication may be maintained by evolution.

Why maintain duplicated RNases?

Pancreatic RNases are ribonucleases found extracellularly in vertebrates. They were likely early components of the vertebrate immune system but underwent multiple duplications to form a superfamily with a wide array of properties. Among other things, they are involved in angiogenesis, host defense, and stimulation of the activity of endothelial cells. Fermenting mammals have a particular need for high expression levels of gut RNases because their fermenting bacteria keep much of their nitrogen in RNA. This nitrogen of course initially comes from the food the mammal eats, if the fermenting mammal does not recycle the RNA nitrogen, it will lose much of the nitrogen from its food. Therefore, gut RNases play an important role in nitrogen recycling in fermenting mammals.

In ruminants (like cows), RNaseA (a type of RNase1) specializes in bacterial RNA digestion and is produced in the pancreas then delivered to the small intestine. The ruminant small intestine has a slightly alkaline pH, similar to extracellular fluid. Therefore the duplicated ruminant RNase acts at the same pH as the ancestral mammalian RNase. It has however gained a different function: an enhanced ability to degrade double stranded RNA.

Colobine monkeys have a different problem to solve in their intestines. A high amount of fatty acids released during fermentation makes their intestines slightly acidic. The duplicate colobine gut RNases have high activity at slightly acidic pH, unlike ancestral mammalian RNases. Interestingly, colobine RNases lack a high dsRNA digesting function. Mutational studies suggest that this is because the mutations in ruminants that allowed for this function are precluded by other functional mutations in colobine intestinal RNases. This is a potential example of molecular constraints on protein function: the protein can either have high

dsRNA digesting activity or it can work at lower pHs but not both. In both ruminants and colobines we can see a **pattern of gene duplication followed by neofunctionalization** that likely allowed these fermenting mammals to maintain their ancestral RNase function as well as the gain a novel function in digestion.

Like colobines, howler monkeys are also folivorous and appear to have duplicated pancreatic RNases. Although howler monkeys are hindgut fermenters, they are like colobines in that their fermentation process produces abundant fatty acids and thus a low-pH environment. Perhaps not surprisingly, the multiple species of howler monkeys studied have one copy of RNase1 that is similar to the version found in non-folivorous primates and at least one copy that has undergone rapid evolution and neofunctionalization.

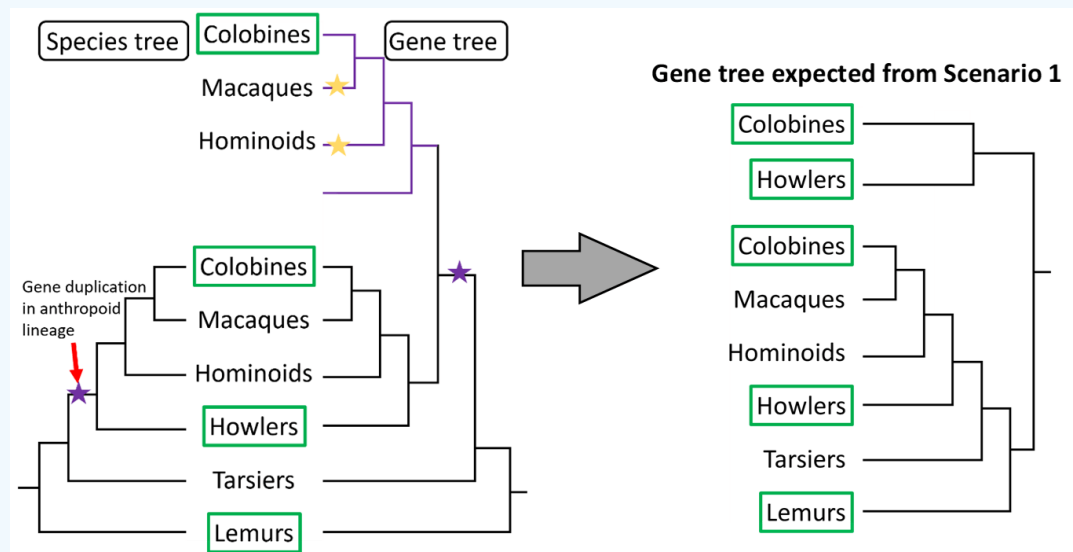
Evidence for duplication of RNases associated with folivory

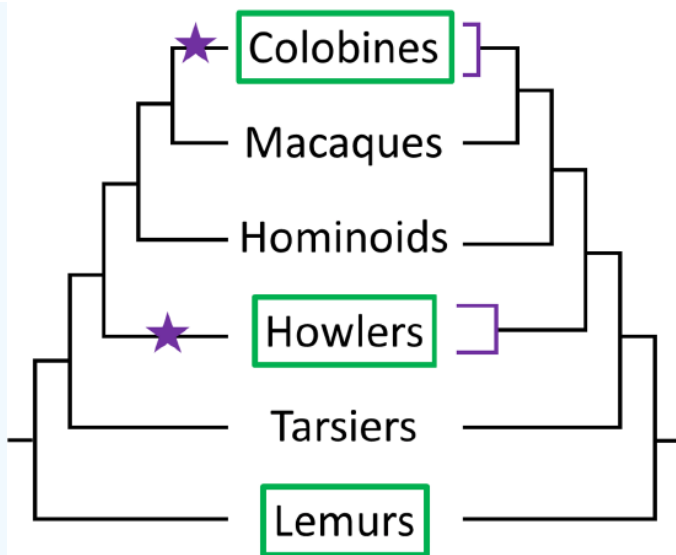
Because RNases are widely duplicated in the vertebrate lineage, it can be difficult to assess whether they specifically duplicated and underwent neofunctionalization in one specific lineage versus another. For example, if we find that both howler and colobine monkeys each have two copies of RNase1, how would we know whether this duplication occurred in their common ancestor or if it occurred more recently and was maintained by selection for fermentation activity (Box 1)? Two different lines of evidence can be used: First, phylogenetic trees can be made from the sequence data and reconciliation analysis can be performed on the sequence tree (Box 1). Second, researchers can perform direct sequence comparison and site-directed mutagenesis to infer whether key functional mutations are the same in the different lineages.

Box 1: Patterns of gene evolution can be inferred from genetic sequences and species phylogenetics

The figure below compares two scenarios for gene duplication, both of which could explain the pattern of two RNase genes in both the colobine and howler lineages. Green boxes denote lineages with at least some folivorous members.

In **Scenario 1** (below), a duplication (purple star) of RNase occurred in the last common ancestor of colobines and howlers. The two RNase sequences were inherited by all descendants of this lineage (purple lines denote second copy) and subsequently lost in the macaques and hominoids. Once the sequences duplicate, each copy follows the species divergence pattern. Note that the purple (duplicate) Gene Tree pattern looks exactly like the anthropoid Species Subtree. Species Tree after Pozzi et al, 2014.





In **Scenario 2** (left), separate RNase duplication events occur in the colobine and howler lineages (purple stars) giving rise to lineage-specific duplicates (purple lines on right). In this scenario, the resultant colobine RNases are more similar to each other than to either howler RNase. Whereas in Scenario 1 there was one duplication and 2 loss events, in Scenario 2 there are two duplications and no loss events. From only this information, there is no way to know whether duplications or losses are more common and, therefore, which scenario should be favored.

However, using total evidence (coding and non-coding sequence) of primate RNases, Zhang (2009) ran a phylogenetic analysis and found that howler and colobine RNases did not form a **monophyletic clade** (a group that shares a common ancestor), as would be expected from Scenario 1. Instead, the howler RNases formed a monophyletic clade with each other, as did the colobine RNases, as predicted by Scenario 2.

For additional information on constructing phylogenetic trees, see:

[Molecular Analyses and Modern Phylogenetic Trees](#)

Does evolution repeat itself?

Folivory has evolved many times in mammals, leaves are abundant and found year round in many ecosystems, but molecular and physical adaptations to folivory have only been studied in a handful of mammalian species. Sequence changes to RNases have been associated with shifts to folivory in colobines, artiodactyls (specifically the ruminants), hippos, lemurs, sloths, and howler monkeys. Of these, gene duplication has specifically been studied in colobines, lemurs, ruminants, and howler monkeys. The colobine and howler monkey story is perhaps the most intriguing, because it gives insight into the mechanisms of convergent evolution. Specifically, howler and colobine monkey RNases appear to have convergently evolved the same amino acid changes to adapt to the same nutritional resource (Figure 3, see below for more details). It is unlikely that these changes are homologous because howler and colobine monkeys both have close relatives without these functional changes and without the duplicated copies of RNase (Figure 4).

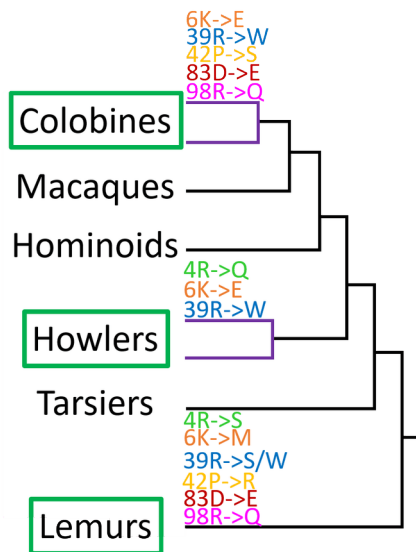


Figure 3: Convergent amino acid changes in transition to folivory

Common amino acid changes in folivorous primates are mapped onto the Scenario 2 (evidence based) RNase gene tree. Purple branches (colobines and howlers) indicate duplicate sequences. After duplication, one copy (the "standard" copy) retains ancestral function and its sequence is similar to the ancestral sequence. The other copy (the "neofunctionalized" copy) is free to evolve and accumulates mutations. Coding mutations can lead to changes in amino acid sequence. If these sequence changes lead to a more useful (or at least not less useful) protein, then they may be retained (positive selection).

Amino acid changes in folivorous lineages are shown **above** the lineage line. Changes are included if they are found in at least half of the folivorous species examined from that lineage and are also found in at least one other folivorous lineage. The original position and amino acid code is on the left, the new amino acid at that position is on the right. Note that rapid evolution occurs in only one copy of the colobine and howler lineages. Lemurs, another group of mostly folivorous primates, has only a single copy of RNaseA, but exhibits many of the same amino acid changes as the colobine and howler monkeys.

How often have primate RNases duplicated?

Even though gene duplication may seem like a rare event, its rate in evolutionary history is not well known and likely differs between lineages. While the phylogenetic evidence strongly suggests independent duplications in the howler and colobine lineages (Box 1 and Figure 1), RNase may have also duplicated within the colobines themselves. An analysis comparing douc langur (*Pygathrix nemaeus*, an African colobine) RNase sequences to guereza (*Colobus guereza*, an Asian colobine) showed that douc langur had two RNase1 (pancreatic RNase) genes and guereza had three. Each species maintained a "typical" RNase1 with similar sequence and optimal pH to Rhesus monkey and human RNase1. The "atypical" RNase1s all showed signs of positive selection, had a lower optimal pH, and had lost the ability to efficiently degrade dsRNA.

All of the neofunctionalized proteins have amino acid substitutions that give them the lower optimal pH (and lower their isoelectric point). At first glance this might suggest that these proteins originated in a duplication event in an ancestral colobine. However, another 10 amino acid substitutions differ between the two species. Phylogenetic analysis using whole sequences gave ambiguous results depending on the methodology. This could be due to the accelerated evolution in the neofunctionalized proteins. However, the non-coding sequences do not show accelerated evolution. When these were used in a phylogenetic analysis examining 12 colobine species, all African colobine pancreatic RNase sequences fell into a single moderately-well supported clade to the exclusion of the Asian colobine sequences. Whole-sequence analysis produced well supported Asian and African clades (Figure 5). If a duplication occurred in an ancestral colobine, the ancestral colobine RNase1 sequences should fall in a single clade to the exclusion of the neofunctionalized sequences.

	pI	1	4	6	32	39	42	83	98	122
<i>Homo sapiens RNASE1</i>	9.10	K	R	K	R	R	P	D	R	A
<i>Propithecus verreauxi</i>	5.27	-	S	M	-	S	R	E	K	-
<i>Propithecus coquerelli</i>	5.27	-	S	M	-	S	R	E	K	-
<i>Propithecus tattersalli</i>	4.98	T	S	M	-	S	R	E	Q	-
<i>Propithecus candidus</i>	4.98	N	S	M	-	S	R	E	Q	-
<i>Propithecus diadematus</i>	4.98	N	S	M	-	S	R	E	Q	-
<i>Propithecus edwardsi</i>	4.98	N	S	M	-	S	R	E	Q	-
<i>Avahi laniger</i>	5.12	-	S	M	-	S	R	E	Q	-
<i>Indri indri</i>	5.77	-	S	M	-	S	R	E	Q	-
<i>Mirza coquerelli</i>	8.83	-	M	-	-	-	-	-	-	-
<i>Microcebus murinus</i>	8.99	-	P	M	-	-	-	-	-	-
<i>Cheirogaleus medius</i>	8.66	-	M	-	-	-	-	-	-	-
<i>Haplorhina griseus</i>	8.68	G	-	M	-	-	-	-	-	-
<i>Polemus simus</i>	8.99	G	-	M	-	-	-	-	-	-
<i>Lemur catta</i>	8.85	-	M	-	-	-	-	-	-	-
<i>Eulemur macaco</i>	8.83	-	M	-	-	-	-	-	-	-
<i>Eulemur flavifrons</i>	8.83	-	M	-	-	-	-	-	-	-
<i>Eulemur fulvus</i>	8.83	-	M	-	-	-	-	-	-	-
<i>Eulemur rufifrons</i>	8.83	-	M	-	-	-	-	-	-	-
<i>Lepilemur mustelinus</i>	8.45	-	M	-	-	-	-	-	-	-
<i>Lepilemur grecockorum</i>	8.45	-	M	-	-	-	-	-	-	-
<i>Daubentonius madagascariensis</i>	8.98	-	S	M	-	-	-	-	-	-
<i>Otolemur garnettii</i>	9.10	-	P	M	-	-	-	-	-	-
<i>Pan troglodytes RNASE1</i>	8.96	-	-	-	-	-	-	-	-	-
<i>Macaca mulatta RNASE1</i>	9.24	-	-	-	-	-	-	-	-	-
<i>Colobus guereza RNASE1 N</i>	9.24	-	-	-	-	-	-	-	-	-
<i>Procolobus badius RNASE1 N</i>	9.24	-	-	-	-	-	-	-	-	-
<i>Nasalis larvatus RNASE1</i>	9.22	-	-	-	-	-	-	-	-	-
<i>Pygathrix nemaeus RNASE1</i>	9.20	-	-	-	-	-	-	-	-	-
<i>Rhinopithecus roosevelti RNASE1</i>	9.22	-	-	-	-	-	-	-	-	-
<i>Semnopithecus entellus RNASE1</i>	9.10	-	-	-	-	-	-	-	-	-
<i>Trachypithecus francoisi RNASE1</i>	9.10	-	-	-	-	-	-	-	-	-
<i>Colobus guereza RNASE1 Pb</i>	7.57	-	Q	E	-	W	E	E	D	-
<i>Procolobus badius RNASE1 P1a</i>	6.40	-	S	A	E	W	S	E	Q	-
<i>Nasalis larvatus RNASE1 Pa</i>	7.57	-	G	E	L	W	S	E	Q	-
<i>Pygathrix nemaeus RNASE1B</i>	6.93	-	G	Q	E	L	W	S	E	-
<i>Rhinopithecus roosevelti RNASE1B</i>	8.43	-	G	Q	E	L	W	S	E	-
<i>Semnopithecus entellus RNASE1 P</i>	7.58	-	G	E	L	S	E	E	D	-
<i>Logothrix lagotricha RNASE1</i>	8.12	-	-	-	-	-	-	-	-	-
<i>Alouatta palliata RNASE1</i>	8.45	-	-	-	-	-	-	-	-	-
<i>Alouatta seniculus RNASE1</i>	8.66	-	-	-	-	-	-	-	-	-
<i>Alouatta palliata RNASE1B</i>	6.56	-	Q	E	-	W	E	E	D	-
<i>Alouatta seniculus RNASE1B</i>	6.55	-	Q	E	-	W	E	E	D	-

Figure 4: Comparison of 9 key adaptive sites in primate RNase1s. Other studies have shown that most primate RNase1s are very similar to the human ortholog (top row, dots) at the illustrated residues, suggesting purifying selection. Folivorous lemurs (green highlights) do not have duplicated RNase1 genes. However, their single copy shows accelerated evolution. The new copies of RNase1 in colobines and howler monkeys (grey highlights) show accelerated evolution at the same sites as lemur RNase1s. The non-neofunctionalized colobine and howler RNase1s are very similar to the human, macaque, and chimp sequences.

Folivorous species are indicated with a green leaf. Residues are color coded by function. pI indicates the estimated isoelectric point of each protein. Higher pIs are found in proteins that are more positively charged at a neutral pH. Lemur RNases and the nonfunctionalized colobine and howler RNases tend to exhibit lower pIs than RNases with ancestral sequences. This may be associated with their novel function in a more acidic (low pH) environment.

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Multiple parallel evolution in primates

Because of the similarities in both fermentation and gut environments between colobines and howlers, researchers compared the neofunctionalized RNase1 sequences between these clades. When compared to cow and human RNase1 sequence, all monkey sequences were more similar to humans. This suggests there was not sequence convergence between cows and monkeys to support bacterial fermentation. When comparing all primate sequences, the non-neofunctionalized RNases are very similar to each other and to the human sequence. The neofunctionalized proteins, however, have some specific residues in common, even across clades. For example all neofunctionalized RNases and only 2 standard RNases show a conversion of R → W at residue 39 (human sequence). Many neofunctionalized RNases show conversion of R → Q at residue 4 and K → E at residue 6, these residues are not found in any of the standard sequences (Figures 3 and 4). Because howler monkeys and colobines are so evolutionarily distant and their closer relatives lack these changes, these novel proteins represent convergent evolution of both function and sequence.

Neofunctionalization without duplication?

Lemurs, non-monkey primates, also have several folivorous lineages, which are hindgut fermenters like howler monkeys. However, unlike howlers and colobines there is no evidence of RNase duplication in the lemurs. However, the single pancreatic RNase in lemur genomes has undergone rapid evolution at the same sites seen in the neofunctionalized colobine and howler RNases (Figures 3 and 4). Several of these sites were predicted to affect catalytic activity in other primates. In particular, the 83D → E substitution found in colobines and known to greatly affect RNase activity is also found in the single-copy sifaka lemur sequences, though intriguingly not in other folivorous lemur sequences. It is possible that recent evolution of folivory, dietary flexibility, or even non-involvement of RNase with folivory in lemurs is responsible for this pattern and the lack of duplicate genes.

Other RNase expansions

Carnivora (Felines, bears, canids, etc.) , a class with a short and simple digestive system compared to other mammals, has also had multiple gene expansions within the Mustelids (ferrets, badgers, wolverines, etc.). Carnivora do not typically have a great deal of microbial digestion because they largely eat meat, which is fully digestible by native enzymes. Molecular and computational analysis suggest that these expansions are associated with novel physiological functions. Gene expansions of RNase1 in insectivorous bats are thought to be associated with immune, rather than digestive functions. Intriguingly, bat lineages with other feeding patterns did not show expansion.

Summary

Leaf eating (folivory) has evolved numerous times in mammalian lineages. The evolution of a fermentation vat in the gut that houses cellulose-digesting bacteria comes along with a cost: loss of nitrogen to bacterial RNA. Several lineages have duplicated and neofunctionalized RNases to recycle this nitrogen back into the organism instead of losing it as waste. This duplication has occurred convergently in howler monkeys and colobine monkeys. Additionally, there is some evidence to suggest that it has happened at least twice in colobine lineages. The evidence for convergent evolution comes from comparing both coding and non-coding gene sequences. The coding gene sequences show some convergent amino-acid substitutions in sites that affect the isoelectric point and enzymatic activity of the RNases. More recently evolved is folivory in lemurs (non-monkey primates). The lemur single RNases show accelerated evolution but not duplication. Future work will likely examine additional folivorous groups as well as the function of RNases in groups with gene expansions for non-digestive functions.

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MINI-LABS

- 1: Ortholog/Paralog Lab
- 2: Comparative Vertebrate Anatomy Observations
3. Butterfly Wings Lab: Boundaries and Pattern Formation
Reaction Diffusion Simulator

1: Ortholog/Paralog Lab

For this assignment you will turn in your alignment, your tree, the names of a pair of orthologs, the names of a pair of paralogs, and a short description of the significance of the gene you chose. Additionally, you will have an "Appendix" with notes on the steps (if anything happened at a step that was not described in the instructions, if you noticed anything interesting).

INSTRUCTIONS

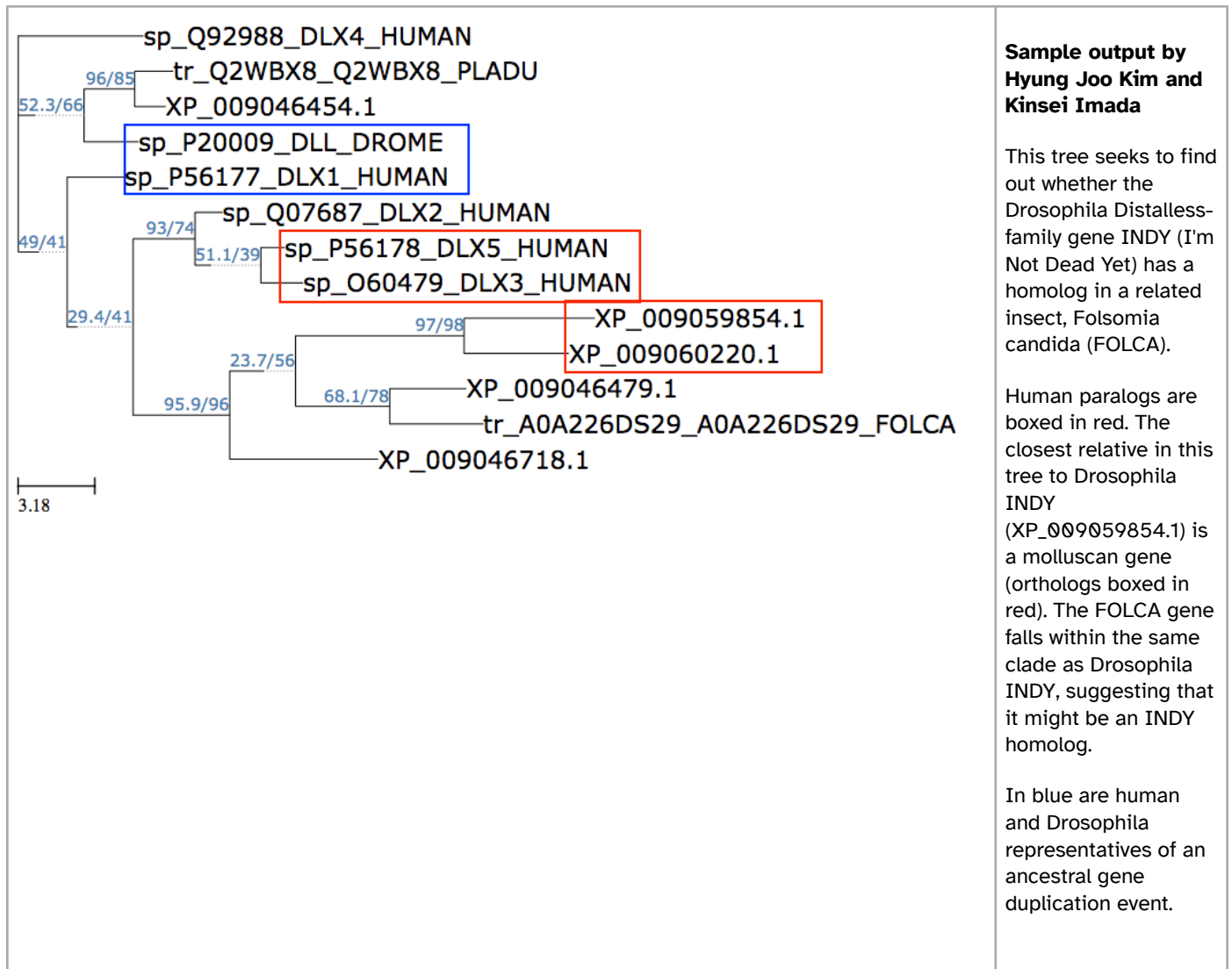
Getting your set of sequences

1. Get your "bait" sequences.
 1. Go to Uniprot and type in YFG name + a model species (for example if you are interested in centipede genes ultimately, you would put in *Drosophila*). Read a bit about your gene and scroll down to FASTA. Click on this and it will take you to the FASTA formatted protein sequence.
 1. If there is more than one "version" of YFG for that species, get the FASTA for all of them - they are likely homologs. For example, looking at *Dlx* (the *Distalless* in vertebrates) I find 5 version in humans. So I would get the FASTA for each of these. Put these all in the same text document.
2. Get your "model" sequences. Follow the same instructions for your other two (or three) models. If you are having trouble selecting your models, you can ask me for help! Add these to the FASTA text document.
3. Get your "test" sequences
 1. Blast your "bait" sequence in NCBI blastp. Limit your search to your test species, it helps if you know the scientific name (Wikipedia has these). Choose the sequences with an evalue less than $1e-10$. If there are too many, just pick the top 5. If there aren't any with an evalue this low, pick the top 3. Add these to the FASTA text document.
4. Get your "outgroup" sequence
 1. This should be a sequence that is similar to but not homologous to YFG. To find one, go back to UniProt and click on BLAST. Paste in your bait sequence and choose the UniRef50 database from the pulldown menu and click run. Wait.
 2. Scroll down the results list until you start seeing gene names that differ from YFG. Click on a high scoring one of these and get the FASTA sequence, add this to your FASTA text document at the very top.

Making your tree

The next step is to align your sequences and make a tree. Aligning sequences places the most similar parts of each sequence in vertical columns. It makes it easier to visually see whether the sequences are really very similar or not so much. You can also sometimes see things like conserved domains in an alignment. The other useful thing about alignments is that they can be used to score similarity by statistical algorithms. These programs use alignments to infer phylogenetic relationships.

1. Paste your FASTA formatted sequence into <https://www.ebi.ac.uk/Tools/msa/muscle/> and choose Pearson/FASTA as your output. The output is important because we need an output file that the next program can read.
2. The results page has a bunch of different options. Save the basic text version and then click around to visualize your alignment in different ways. Do you see anything interesting? Any patterns?
3. Upload this "alignment file" to <http://iqtree.cibiv.univie.ac.at>, and click submit job. IQtree by default will test different models of molecular evolution on your data and see which one fits. We aren't going to use super fancy models, so we don't need to add in things like a gamma distribution or free rate heterogeneity. As IQtree runs you can ponder the difference between paralogs and orthologs and/or start writing up a description of your gene and what happened at each step you did to find out about it's genetic complexity in your non-model organism.



2: Comparative Vertebrate Anatomy Observations

In this lab, you will consider the morphological processes of evolution and development by examining developing vertebrate embryos.

Before you start please adjust your oculars so you can see two full circles, you may need to slightly adjust how far your eyes are from the microscope.. This can take 5-10 minutes so be patient.

Please choose one of the following:

1. Chick or Frog embryo, single stage (you will “reconstruct” the 3D morphology by comparing sections of a single embryo).
2. Chick or Frog embryo across time (you will “reconstruct” development by examining different stages)
3. Chick and Frog comparisons (you will compare Chick and Frog morphology at a similar developmental timepoint)

Take some time to get acquainted with your slides and your microscope. Make sure you can see through both oculars and you are focusing up and down for each section.

Start to make a few sketches of what you see on plain white paper. Practice drawing the features that stand out to you and seem salient. You may use the books provided, online resources, or people in the classroom for help in figuring out different features.

Note: I offer an extra credit for an art piece based on sketches (examples below) and provide watercolors, watercolor paper, and colored pencils for the students. I grade it on detail, quality, and overall aesthetics.

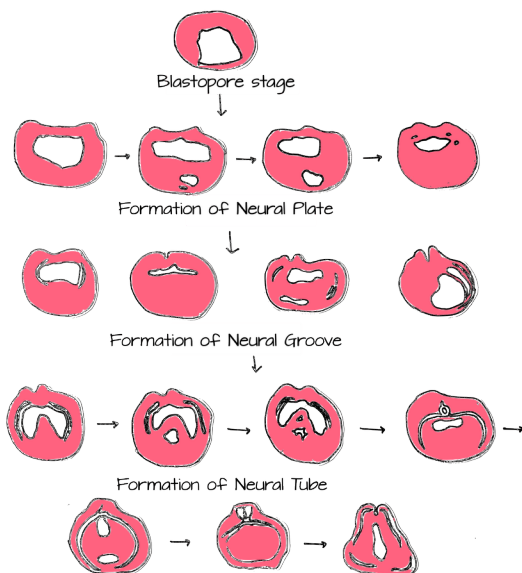
Resources:

[Early Frog Development](#)

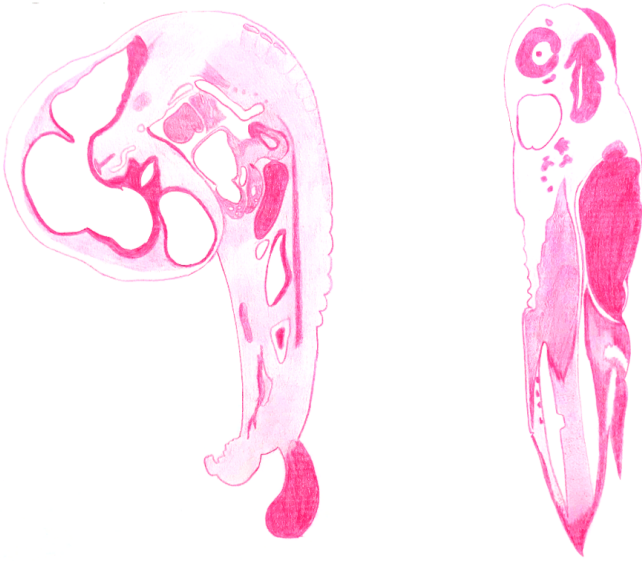
[Late Frog Development](#)

[Chick Development](#)

Sections of a chick embryo at five stages by Kristina Vu



Frog vs. Chick comparative embryology during late-organogenesis by Yongjin Byun



3. Butterfly Wings Lab: Boundaries and Pattern Formation

Butterfly Wings Lab

Nipam Patel, the author of the picture below, is interested in using gynandromorphic Morpho butterflies to understand wing development in insects. Much of his research focuses on developmental mechanisms of boundary formation across evolution. Boundary formation (like we see in brain lamination or in segmentation) is a key process in compartmentalizing an embryo. As we read before, compartmentalization can increase evolvability by separating cellular functions and reducing pleiotropy.



Figure 1 from [ScienceFriday.com](https://www.sciencefriday.com) ([Links to an external site.](#))[Links to an external site.](#)

In Figure 1, a series of Blue Morpho butterflies is pictured. The top left butterfly is a wild-type male and the top center butterfly is a wild-type female. All of the rest of the butterflies are gynandromorphic with some male cells and some female cells in their wings. We will use these natural sports to make a hypothesis about boundary formation in butterfly wings.

Genetic mosaics and eyespots

Genetic mosaics are classical models for studying developmental processes. Mutations in key developmental genes often results in early embryonic lethality. Sometimes, a genetic mosaic organism can be generated that has only a small subset of cells or a single tissue/organ that exhibits the mutation. In these cases, the role of the gene in just that tissue or organ can be studied much later in development¹. *Drosophila* wing compartments and the finding that the transcription factor *Engrailed* is involved in boundary formation were both discovered using genetic mosaics^{2,3}. *Drosophila* wings tend to be fairly simple in terms of wing vein patterning and pigmentation, compared to butterfly wings (see Figure 3 below). However both wings undergo very similar early development and share a basic "ground plan", the set of instructions that both defines the major regions of the wing (veins and margin) as well as sets the boundaries for the initiation of color pattern elements⁴. These pigmented pattern elements (notably eyespots) can be repeated across the wing with each element exhibiting a similar morphology due to similar developmental patterning⁵. Because of this, eyespots are considered to be serial homologs like limbs or ectodermal appendages.

Color elements are a useful assessor of boundaries since they are easy to see and vary both within a population and between species. Since the color elements are serially homologous, a single wing offers multiple opportunities to observe the effect of boundaries on pattern formation. By creating or finding genetic mosaics that exhibit abnormal wing patterning, we can start to understand how compartments both restrict individual phenotypic variance (by providing a ground plan) and allow for greater population variance (by reducing inductive effects).

2. Do you think this ground plan is homologous to the *Drosophila* and *V. braziliensis* ground plans? Why or why not?
3. Imagine you are a butterfly researcher trying to justify a collecting trip to an exotic location to collect butterflies. How would you pitch your research to the greater scientific/healthcare community?

Class Discussion Readings and Questions

Read an [overview of Gynandromorphism in animals](#)

Read an [overview of tetragametism](#)(genetic gynandromorphism)

Read [a case study of a genetic "gynandromorphic" human](#), you can skip the Materials, Methods, and Results sections, please focus on the Introduction, Case Report, and Discussion.

Choose one or more of the questions below to answer

1. What is gynandromorphism, how can we tell when an organism is gynandromorphic? How did doctors know that a phenotypically normal girl was gynandromorphic (in the third reading)?
2. Why do scientists and doctors seek out gynandromorphic individuals to study? How are they useful for understanding biology? Can scientists create gynandromorphs or other types of mosaic/chimeric animals?
3. What are the different mechanisms that can give a 46XY/46XX karyotype in an amniocentesis? Be sure to describe both the false positive as well as different developmental mechanisms for generating true positives.
4. Gynandromorphic (46XX/46XY) humans often have abnormal genitalia and gonads that are either a mix of ovarian and testicular tissue (called ovotestes) or one ovary and one testis. What information does this give us regarding the development of gonads and genitalia in humans?

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Reaction Diffusion Simulator

Reaction Diffusion Simulator

Note: If possible this is ideally run in a computer lab or with loaner laptops. Otherwise, ask students ahead of time to bring in at least one laptop per group. Students can follow the questions below or can be given minimal instructions and just "play" with the simulation.

In the Turing Model, the pattern is changed by adjusting the speed of diffusion, the strength of interaction, and the rate of degradation. In the Grey-Scott model simulation, the feed rate is related to the speed of diffusion and the death rate is related to the rate of degradation.

1. To see the a "boring" pattern, choose a feed rate of 0.006 and a death rate of 0.028. Click anywhere on the canvas.
2. Choose the present "Solitons" and click anywhere on the canvas. This feed/death rate combination produces a field of equally spaced spots. How does this relate to the Turing model?
3. Adjust the feed rate to 0.02 and the death rate to 0.058, how does this affect the spot pattern you saw in Solitons?
4. Can you adjust the feed and death rates to produce stripes?
5. What happens if you click on the canvas while a pattern is being generated. What would this represent biologically?
6. What kinds of biochemical/molecular properties might affect feed and death rates?

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GLOSSARY

Introduction

Anomaly

An anomaly is anything that deviates from the standard/what is expected. Biological anomalies help us understand what is biologically possible, rather than just what is biologically “normal.”

Morphology

The set of all biological characteristics of an organism. This includes anatomy, physiology, and behavior.

Developmental Biology

Describes/studies anatomy, physiology, and genetics of an organism over time. Typically it refers to embryological development (from a single cell to an independent organism), and/or juvenile development (from a new independent organism to an adult). However, development occurs throughout an organism’s lifespan, for example stem cell differentiation. We can also think about the development of single celled organisms as they “mature” in response to environmental changes and reproduce.

SNP/RFLP testing

Genetic testing that looks for small “polymorphisms” (DNA sequence differences) in individuals. SNP stands for “Single Nucleotide Polymorphism,” a single nucleotide location that can vary in sequence in a population. For example some people might have a T and some might have a C at the same location. We say this location is “polymorphic.” RFLP stands for “Restriction Fragment Length Polymorphism,” a DNA locus with a “length” polymorphism after cutting with a specific endonuclease (enzyme that cuts DNA). The difference in length of a DNA fragment among individuals may be caused by a small mutation that removes or adds a cut site or a larger scale deletion or insertion of DNA.

Teratogenic anomalies

Anomalies caused by a teratogen, an environmental agent that affects development.

Scalable

The same patterning process can be used to create larger or smaller versions of the same body plan.

Oocyte

An unfertilized egg cell (note: depending on the species, this can specifically refer to an immature egg cell, but here I use it to refer to any unfertilized egg cell regardless of maturity).

Organizer

A tissue, cell, or molecule that creates a chemical/protein gradient that affects cells close to it or far away from it differentially to form an organized group.

Concentration gradient

A gradient formed by a high number of molecules on one side and a low number on the other with intermediate numbers in between.

Diffusion gradient

A concentration gradient formed by diffusion – the random movement of molecules away from a point source.

mRNA

Messenger RNA, this acts as the intermediary step between DNA and protein.

SSD model

Synthesis, Solute, Diffusion model for the Bicoid protein concentration gradient in *Drosophila melanogaster*. This model has Bicoid protein diffusing from a point source to create a diffusion gradient.

Bicoid/Nanos

Proteins that “organize” head and tail tissues in *Drosophila melanogaster* and other flies. They form opposing concentration gradients which have cascading effects that end up in an embryo with distinct regions along its anterior/posterior axis.

Transcription

The process of making RNA using a DNA template

Translation

The process of making protein (or a peptide) from an mRNA template by joining together amino acids in a ribosome.

Gene Expression

Making a functional product from a DNA template (i.e. transcription and/or translation). While most cells in a multicellular organism have all of the organisms genes represented in their DNA, only a small subset of these genes get made into product (expressed) in an individual cell.

RNA interference

A small RNA can interfere with translation if it is a reverse complement to an mRNA.

Untranslated Regions

Portions of the genome that do not code for protein.

Phosphorylation

Adding a phosphate group (PO₄) to a protein. This changes the function of the protein.

Allosteric inhibition/activation

Inhibiting or activating a protein by interfering with a site outside the “active site.” The active site is where the substrate binds.

Homo and heterodimers

A dimer is two peptides/proteins bound together to perform a new function. A homodimer is made up of two of the same protein. A heterodimer is made up of two different proteins.

Glycosylation

Adding a sugar/carbohydrate group to a protein. This changes the function of the protein.

Proteolytic cleavage

Cleaving a protein between two amino acids. This can destroy the function of a protein or activate a function (e.g. pepsinogen -> pepsin).

Fertilization

Chemo-attractants

Chemicals that attract a particular type of cell or organism. For example, chemicals that attract axons during neurogenesis, or chemicals secreted by egg cells that attract sperm from the same species.

Cortical rotation

The rotation of the cell cortex (outer layer) in relation to the cell inner-layer. This is mediated by the cytoskeleton and only happens in certain species.

Ventralized embryo

An embryo that is primarily made up of ventral tissues (i.e. endoderm). Often called a “belly piece.” This embryo will not continue development past early stages.

Ventralization

Any manipulation of an embryo that results in an overabundance of ventral structures. Ventralization leads to a ventralized embryo.

Spemann-Mangold organizer

The region of a vertebrate embryo that will differentiate into the notochord. The notochord itself has organizer activity – it induces ectoderm above it to become the neural tube. Ectoderm farther from it becomes skin. This organizer is located at the edge of the blastopore in vertebrates and, more specifically, at the dorsal-lip of the blastopore in frogs.

Cellular differentiation

The developmental process of a cell becoming mature. This involves narrowing down its fate as well as specializing its functions.

Radial symmetry

An organism that has three or more fold symmetry perpendicular to a central axis. For example, anemones, jellies, and many flowers are classically considered to be radially symmetric. However, note that recent work has found that cnidarians, like anemones and jellies, may also exhibit bilateral symmetry.

Vegetal/animal poles

Before an embryo has clear axes that correlate to its juvenile or adult form, it may still exhibit asymmetry. One asymmetry common in animal embryos is vegetal vs. animal. The vegetal pole is rich in yolk while the animal pole is rich in cytoplasm.

Cytoskeletal microtubules

The cytoskeleton is the scaffolding within a cell that gives it structure and also forms the “highways” in a cell that other proteins and vesicles can travel on, instead of relying on diffusion. One major element of this are microtubules, which are long filaments made up of tubulin. Microtubules also form the mitotic/meiotic spindle and propelling structures like cilia and flagella.

Cortical determinants

Proteins, nucleic acids, and small molecules in a cell that help to regulate gene expression. These are often asymmetrically distributed in egg cells and early embryos. They will help to determine future axes and cell types of an organism.

Transplantation

Moving part of one organism to another organism. The two organisms can be the same species or different species

Genetic manipulation

Experimentally modifying the genetics of an organism. This is a broad term. Usually it refers to genetic modification (aka gene editing) via genetic engineering. Genetic modification makes a permanent and heritable change to the DNA of an organism or cell within an organism.

Cleavage

Morphogens

Diffusible molecules (often proteins) that affect cellular, tissue, or organismal morphology. They have different effects at different concentrations.

Morphogenic gradient

The concentration gradient of a morphogen.

Actin Filaments

Polymers of actin subunits, also called microfilaments. These are important components of the cytoskeleton and are especially important in cell-shape changes and cell movement.

Cleavage

Early stage of embryogenesis characterized by rapid cell division and low rates of transcription and translation.

Blastula

A cleavage-stage embryo. They often appear fairly undifferentiated (a ball of cells).

Germ layers

A “layer” of cells formed in the early embryos through the process of gastrulation (see below). They are some of the first tissues specified in a developing animal embryo. Nearly all animals share the same three germ layers – ectoderm, mesoderm, and endoderm.

Gastrulation

The process of cells and tissues moving relative to each other to produce the three germ layers of an embryo and a primitive body-plan.

Autonomous specification

Cells or tissues whose fate is specified based on inherited factors (proteins and RNAs)

Conditional specification

Cells or tissues whose fate is specified based on interactions with the environment (other cells and morphogens)

Epiboly

Cell movements characterized by the thinning out of a sheet of cells to cover other cells.

Involution

A type of cell movement. Involuting cells in a sheet undergo shape changes to bend underneath the sheet of cells to form a second sheet. This has an effect similar to folding a piece of paper in half.

Intercalation

Cells moving between each other to form a thinner sheet.

Invagination

Cell movements characterized by the formation of a lined pit in a sheet of cells.

Delamination

Cells in a sheet losing their affinity for the surrounding cells and leaving the sheet.

Ingression

Delaminating cells moving into a cavity in the organism.

Fate-map

A map of an embryo showing the future tissues/organs cells will eventually differentiate into.

Notochord

In vertebrates, this is an embryonic structure that orients the anterior/posterior axis of the developing tissues. It is a flexible rod of mesoderm just under the future spinal-cord and brain. It signals to overlying ectoderm

Homologous

Genes or morphological characters from different species that are inherited from a common ancestor.

Synapomorphy

A gene or morphological character that defines a taxonomic group. For example, a vertebral column in vertebrates. A synapomorphy is a homologous character that is not found in species outside the group.

Genetic Toolkit

Bicoid

A protein unique to one group of flies that specifies anterior structures. It is a “maternal effect” protein in that the egg makes Bicoid mRNA before fertilization.

Osteogenic cell

A bone stem-cell. They divide to produce osteoblasts.

Osteoblast

A non-dividing bone cell which secretes the mineral matrix that will become the hard part of bone.

Phylum

A monophyletic group of organisms that share a body-plan. We belong to Phylum Chordata along with all the other vertebrates and organisms with a notochord. Other famous phyla include Mollusca and Arthropoda.

Signal transduction pathway

A cascade of chemical interactions involving proteins that carries a signal from one part of a cell to another.

Ligand

A molecule that binds to another molecule. In signal transduction pathways, ligands are usually the signal coming from outside of the cell that bind to receptors on the cell membrane.

Receptor

Can receive and transmit a signal from a ligand. In signal transduction pathways, receptors are usually cell-membrane proteins that change conformation in response to ligand binding.

Gene regulatory network

The set of genes and genetic interactions leading to a particular cellular, tissue, or organismal outcome.

Downstream effects

In a pathway, these are events that happen after a reference event. For example, triggering a pathway has a downstream effect of turning on its effector genes.

Wiring diagram

A graphical representation of the genetic interactions of a gene regulatory network.

Fate restriction

As a cell matures, its cell-type becomes more and more restricted. For example, an ectodermal cell can be restricted to a neural fate. This neural cell can now be restricted to a (say) glial cell fate. A neural cell cannot become a skin cell, though a generic ectodermal cell can. A glial cell cannot become a neuron, though a generic neural cell can.

Progenitor cells

A cell that gives rise to specific cell-types.

Neutrophils

A type of white blood cell that is part of our innate immune system.

Switches

Any molecule (or structure) with at least two conformations that have different effects on a system. In developmental genetics, switches are usually proteins that form different protein/protein partnerships depending on their conformation. Their conformation depends on other elements in the environment, for example a ligand. Switches in developmental genetics often determine a fate-decision by a cell.

Paracrine signaling

Chemically based (often protein) cell-cell communication where the two cells are nearby.

Juxtacrine signaling

Chemically based (often protein) cell-cell communication where the two cells must be touching.

Autocrine signaling

Chemically based (often protein) cell communication where a cell secretes a signal that it receives itself.

Kernels

Higher-level gene regulatory modules that make initial broad cell-fate decisions.

Batteries

Lower-level gene regulatory modules and outputs that differentiate cells

Plug-ins

Small gene regulatory modules that can be used in a variety of gene regulatory networks.

Patterning

Any type of development that results in differentiation across a tissue, organ, or organism.

Specification

Cells or tissues making cell-fate decisions.

Micromeres

Small blastomeres that arise from unequal cleavages in early development.

Cis-regulatory element

A piece of DNA that acts as a switch to help turn a gene on or off.

Regionalization

Regionalization

The process of splitting a developing organism into different parts, often along an already defined axis. For example, differentiating frontal brain from midbrain, hindbrain, and spinal cord.

Segmentation cascade

In *Drosophila* and some other insects, the anteroposterior axis develops simultaneously. This occurs through stepwise regionalization of the embryo. First, broad regions (head vs. tail) are determined, these determinations cause differential gene expression of downstream components of the segmentation cascade leading to further divisions of the axis into smaller regions.

Maternal effect genes

mRNAs and/or proteins can be made by the mother and deposited in the egg before fertilization. These are maternal effect genes since the sequences of the mother's alleles define the gene function, at least at an early stage of development. One maternal effect gene is Bicoid.

Gap genes

Early-acting genes in the segmentation cascade that divide the *Drosophila* embryo into broad regions. Loss of function mutations in gap genes often cause missing segments in the larva.

Pair-rule genes

Later-acting genes in the segmentation cascade that divide the *Drosophila* embryo into regions of two-segment periodicity. These will be divided in half by the segment polarity genes, which are expressed in every segment.

Serial Homology

Organs or appendages that are built in several locations along a developing body. These can differ from each other somewhat (for example arms and legs) but share common structures and a common developmental program.

Locomotion

Movement, or the ability to move around.

Apical ectodermal ridge

A ridge of cells at the distal end of a growing tetrapod limb (arm or leg). These cells send signals to the cells under them to keep growing. They can also change their signal over time to help specify the proximal/distal axis of the limb.

Genetic Complexity

Positive selection

In genetics, this refers to new genetic variants (mutants) being selected for at a locus. Over time, this increases the genetic variation across species, at least at that locus.

Purifying selection

In genetics, this refers to new genetic variants (mutants) being selected against at a locus. This maintains the original sequence over evolutionary periods of time.

Organismal fitness

The fitness of an organism, typically measured by the number of offspring it produces. Because this is highly dependent on environmental variables, this is actually measured across a population. To see if a particular allele affects fitness in a species, we can group individuals with the same genotype at a locus and compare average fitness between genotypes.

Pleiotropy

A gene or mutation that affects multiple aspects of an organisms phenotype. A pleiotropic constraint occurs when two different functions of the gene would be optimized with different alleles.

Gene variants

Different sequences of the same gene in a species, usually called “alleles.” Usually these are very very similar in sequence with only small changes between the variants.

Synonymous changes

Genetic changes in a protein coding region that do not cause a change in protein sequence.

Pairwise comparisons

Comparing two things at a time. In the context of this chapter we can compare two genetic sequences to each other at a time. So, for example, we might say humans and mice have 3 nucleotide differences in an exon, humans and maize have 10 differences, and mice and maize have 11 differences.

Ectopic expression

A new location or time of expression of a gene. That is, a gene is turned on in an “unusual” place or developmental time.

Point mutations

A single nucleotide change in a strand of DNA (note that this will result in the complementary change in the second strand of DNA).

Indels

An insertion or deletion of bases from a genome.

Duplications

In a genome, this is a class of mutation that results in an existing sequence of DNA being duplicated elsewhere in the genome. Duplicated regions will often be found quite near their originals but they can also be found farther away, even on another chromosome.

Genomic complexity

The number of “parts” in a genome. For this class we are concerned with coding portions of genes and their regulatory regions. Complexity would increase if more exons (coding regions) or more regulatory regions were added to the genome.

Organismal complexity

The number of “parts” in an organisms phenotype. This can refer to anatomical parts, physiological processes, behavior, and/or the “extended phenotype” (the way an organism changes its environment to promote its own fitness).

SPRE

Signaling Pathway Response Element, a regulatory region of a gene that allows the gene to switch on or off in response to the activation of a Signaling Pathway. This switch may also require the presence of local activators (tissue dependent transcription factors).

Exonic mutations

Mutations in exons, the coding regions of genes.

Functional domain

A protein domain with a specific function. This may include a catalytic site, a portion that binds to DNA or other proteins, etc.

Repressor domain

A protein domain involved in repressing a biological function, often by binding to another protein and preventing it from acting.

Nonsense mutation

A mutation that converts a nucleotide codon that codes for an amino acid (protein monomer) into one that codes for a stop codon. This causes a premature end to translation and a protein missing its C-terminal end.

Horizontal transfer

The uptake of foreign DNA into the genome of a host. Animals typically get exogenous DNA only from retroviruses, but plants, fungi, and bacteria are more able to incorporate DNA from the environment into their genome.

Transposon insertion

A transposon, the remnants of a retroviral infection, is a piece of DNA that can “jump” into different portions of the genome, sometimes taking surrounding DNA with it.

Pseudogene

A genetic sequence resembling a protein-coding gene that does not actually code for a functional product. These may be the remnants of a genetic duplication/transposition or may be once-functional genes that accumulated mutations over time.

Enhancer element

A piece of regulatory DNA that may contain many individual protein-binding sites. An enhancer element typically drives gene expression under specific circumstances (for example, in the limb mesoderm during limb development).

Novelty

Functional innovation

Mutations in an evolutionary lineage that change existing phenotypic characters so that they perform a new function. A functional innovation either increases fitness for the organisms in their ancestral ecological niche or allows them to exploit a new ecological niche.

Novelty

A new feature in an evolutionary lineage. Sometimes we restrict these to new features that are not build from existing features (functional novelty).

Realizer genes

Genes downstream of patterning genes that change cell and tissue characteristics to build a morphological character.

CRISPR

A genetic manipulation technique that directly edits the genome of an organism. It allows researchers to target a specific genetic locus.

Elytra

A beetle's hard wing covers. Singular: elytron

Gin trap

Segmentally iterated spiky defense structures on beetle pupae that disappear during metamorphosis. These are found only in abdominal segments and are thought to be serial homologs of parts of the wing.

Evolvability

Population-level evolution

Evolution within a population or a species. This level of evolution introduces new genetic variation (alleles) into a population and acts on these alleles to change their frequency.

Macroevolution

Evolution above the population level. That is, the origination of new species and evolutionary patterns seen at higher taxonomic levels.

Molecular genetics

All aspects of genetics that occur at the molecular level – for example mutations and gene expression.

Modularity

Modularity refers to discrete units being used to build up a larger structure and/or process.

Robustness

A process or structure that is resistant to change.

Adaptability

A process or structure that is able to change in response to external factors.

Weak regulatory linkage

Processes or structures that are linked by a common regulatory event that triggers their formation.

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