



Appendix

A Quick Guide to Finding and Comprehending Research Articles in Developmental Biology

Research for Research

If you ask a scientist why they get up in the morning to do this work (or go to bed in the morning as some experiments may require), a common response is for the thrill of discovering new knowledge. But what knowledge is considered new? In order to determine the next best questions to investigate, scientists must first understand what the currently accepted ideas in the field may be—an understanding most certainly requiring the analysis of the field's scientific literature.

Throughout *Developmental Biology*, we encourage and provide numerous opportunities for you to move from the content and ideas in the textbook to the cutting-edge research found in the scientific literature. This appendix is designed to offer a brief but constructive guide both to finding relevant articles in the field of developmental biology and to understanding how information is organized and presented in a research paper. These are necessary skills for every scientist—skills that will provide you the keys to unlocking our current understanding of how development works!

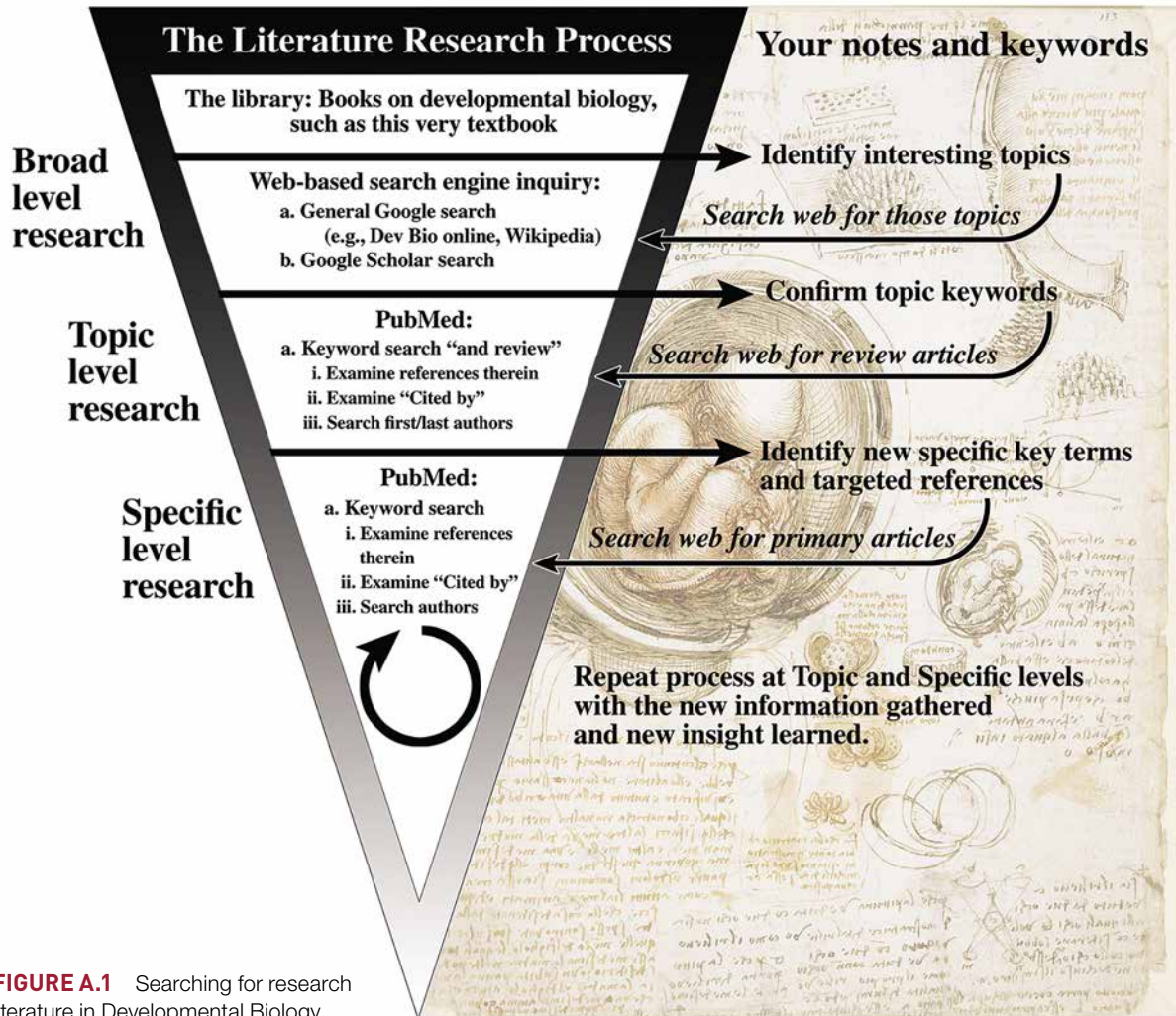
Finding the research

There is no better place to start your search for knowledge in developmental biology than this textbook. We hope that *Developmental Biology* provides you with a single source for a foundational understanding of the key concepts and overall state of thinking in the field of developmental biology. However, the field is enormous, and it would be a serious misconception to think that this textbook alone could cover it all. Additionally, in today's world of ubiquitous access to information, it is ever more important to understand how to navigate through this information strategically. Therefore, it will be important for you to learn how to search through many different sources in ways that help you find the most relevant information as efficiently as possible. The goal is not only to review the state of the field, but also to identify what the most logical next questions in the field may be. Only then can you have confidence that your coverage of the material is complete.

We present here a suggested process for conducting research in the scientific literature (**FIGURE A.1**). When beginning this process, it is wise to first gain a broad exposure to the different ideas surrounding your interests. You read that right: “your interests.” There are few activities that allow you to truly follow your own path of curiosity, and researching the scientific literature is one that is under your control—so enjoy the process and own it. The more prepared you are for this process, the more you will enjoy it. Consider establishing a literal or virtual notebook designated to serve as a recorded history of your journey. It is particularly helpful to establish some guiding questions that can be used to evaluate the utility of a given source. For example:

- How does the limb form?

Whether you are motivated by an assignment in your course or by some surprising result from an experiment, start by broadly acquainting yourself with your topic of interest (see Figure A.1, Broad level research). In the example above, you would



first need to review the basic anatomy and concepts governing limb development. As mentioned earlier, this textbook would be a great place to start. Are you interested in figuring out why our arms are different from our legs? Start with reading the chapter on limb development. Visit a library for additional books on the broad topic. During your exploration of these resources, note the aspects of this topic that pique your curiosity, in order to identify refined subtopics within your broad interest. For example:

- What controls cell identities along the proximal to distal axis that may underlie the differences between forelimbs and hindlimbs?

During this process, also identify keywords related to your subtopic(s). For example:

- Proximal-to-distal axis determination, forelimb, hindlimb, chick, mouse, and hox genes

Once you are prepared with some foundational understanding of your topic and a starting bank of keywords, you can conduct some general web-based searches. It is always advisable to meet with your institution's librarian and become acquainted with the databases that may be available to you. As resources will differ between institutions, we will keep this guide focused on more generally available databases. The first and most obvious resource is the web. Using your favorite search engine (e.g., Google), enter some of your keywords to see what kinds of websites are discovered. Consider

taking advantage of the different types of media filters, which can often help identify great sources sometimes overlooked by unfiltered searches. For instance, an image-based search might uncover a perfect schematic model of your topic that is linked to the original article or to a website with highly relevant content. Most certainly you may find the online resources of this textbook (which you should take advantage of), as well as websites like Wikipedia. It is prudent to view general websites, including community-curated sites like Wikipedia, as relatively superficial and potentially error-prone sources. However, the goal at this stage of your literature investigation is to continue to build a record of pertinent topics that could increase your comprehension of the subject and guide your research toward more specific subtopics. Another way to filter your search is to use the “Google Scholar” search function offered by Google. Although this usually isn’t the best way to explore the primary literature in developmental biology, it can prove useful for uncovering pertinent articles that are sometimes missed by other search methods.

At this point, if you have kept appropriate notes of your findings, you may find that while the amount of information is still manageable it is steadily increasing. How should you organize this information? One way is to create a visual representation of the accumulating ideas. For example:

- Draw out a large “concept map” of your topic: *Limb development* → *proximodistal (sources)* → *hox genes (sources)* → *epigenetic regulation (sources)*.
- Consider using software like Google Slides, PowerPoint or Adobe Photoshop to build your concept map, and include some pictures of the sources or relevant data so you can visually organize and experiment with how all these ideas could fit together.
- Continue to refine your subtopics, filtering questions, and keywords: *How do wrists and hands form more anteriorly, while ankles and feet develop in the posterior part of our bodies? In other words, what are the different regulatory mechanisms of hox gene expression that govern the differences between distal structures of the forelimb and hindlimb?*

Navigating the PubMed database

PubMed is the primary database we recommend for searching for research articles in developmental biology (**FIGURE A.2A**; <https://www.ncbi.nlm.nih.gov/pubmed>). PubMed is a database for all life science publications, and developmental biology is well represented there. Going to PubMed with your framed ideas and keywords already in hand will make it easier for you to make strategic queries and to refine the list of articles you might want to look at more closely. As you march deeper into the literature research process, we recommend first collecting related “review” articles on your topic (see Figure A.1, Topic level research). A review article is a synopsis of a particular topic within a field. These articles are usually written in relatively accessible language, and they provide an overarching summary of a given subtopic—the perfect next-step literature for you to engage with as you continue to build your concept map and your knowledge. To continue with our example, it would be unlikely for there to be a review article covering all of limb development; instead, there would be reviews of more specific subtopics. For instance, in May 2019, a search for “*limb development and review*” yielded 4863 articles! That is too many, which is why your collected keywords are so important. A search for “*limb development and hox and review*” yielded a much more manageable number of 69 articles—all reviews.

There are some real advantages to starting off your reading of the primary literature with a few review articles. A review article frames the problem within the broader scientific field, and also often identifies some of the most immediate questions that researchers should contemplate trying to answer. Most importantly, statements made in reviews are backed up with citations to supporting papers in the primary literature. These citations are fantastic clues to help you find important papers you should contemplate reading yourself. With those references noted, return to PubMed and search

(A)

Make sure "PubMed" is selected. Type in keywords here.

Change format between Summary (as shown) and Abstract (for full description).

Change search filter: Most Recent and Best Match are the most common choices.

Click on the article you want. (B)

Search results items: 14

1. [Similarities and differences in the regulation of HoxD genes during chick and mouse limb development.](#)
Yakushiji-Kaminatsui N, Lopez-Delisle L, Bolt CC, Andrey G, Beccari L, Duboule D. *PLoS Biol.* 2018 Nov 26;16(11):e3000004. doi: 10.1371/journal.pbio.3000004. eCollection 2018 Nov. PMID: 30475793 Free PMC Article
2. [A role for HOX13 proteins in the regulatory switch between TADs at the HoxD locus.](#)
Beccari L, Yakushiji-Kaminatsui N, Woltering JM, Necsulea A, Lonfat N, Rodríguez-Carballo E, Mascré B, Yamamoto S, Kuroiwa A, Duboule D. *Genes Dev.* 2016 May 15;30(10):1172-86. doi: 10.1101/gad.281055.116. Epub 2016 May 19. PMID: 27198226 Free PMC Article
3. [Convergent evolution of complex regulatory landscapes and pleiotropy at Hox loci.](#)
Lonfat N, Montavon T, Darbellay F, Gitto S, Duboule D. *Science.* 2014 Nov 21;346(6212):1004-6. doi: 10.1126/science.1257493. PMID: 25414315 Free Article

(B)

Full text links

Save items

Other articles that cite your selected article will be listed here.

(C)

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PLOS COMPUTATIONAL BIOLOGY

FIGURE A.2 Navigating PubMed. (A) The PubMed homepage provides an opportunity to search this database with keywords. Several dropdown menus enable you to refine your search criteria and display your results in different formats. (B) Selecting an article to further explore provides both the abstract and potential mechanism to acquire a pdf of the article. Additionally, this page

lists suggest similar articles and potentially those articles that have cited your chosen article. (C) Clicking the "Full text" links will bring you to a page where the pdf can be downloaded. If an article is not freely available, then you may have to look for the specific article of interest through an institution's library page to take advantage of potential subscriptions.

for them directly. In PubMed you can also click on the authors' names to find other papers published by those same investigators (finding similar papers is best achieved by clicking on the "corresponding author"). Moreover, while on a PubMed page for a specific article, you can also see a list of "similar articles" or even other articles that have cited the article you're considering. These are all great ways to find additional references of relevance (**FIGURE A.2B**). The new information gained from the review articles should help you to further refine your ideas and to generate new keywords that you can use for more targeted searches of PubMed.

Getting a PDF for an article

Next, you need to obtain the electronic full text or PDF versions of the articles you want to read. Not all articles are freely accessible. If you are affiliated with a university or the like, then your institution may have subscriptions to certain journals, in which case access to the PDF versions can be granted through your library's resources. Fortunately, some journals are open access, and all "PubMed Central" articles are free to download. On the PubMed page for your selected article (see Figure A.2B), you can access the full text by clicking "Full text links" at the upper right. This will bring you to the journal's website and the article's access point (**FIGURE A.2C**). The article access pages of different journals all look different, but there will be a clickable link to download the PDF somewhere on this page.

Defining the Anatomy of a Research Paper

Once you have found an article of interest, the next step is to extract and analyze the findings of the study. What were the authors' research questions and hypotheses? What approaches did the authors use to test their hypotheses, and are their conclusions actually supported by their stated findings? These are only a few of the many questions you might consider when reading a research paper. Here's another question: Do you have to read the entire article? It depends on what you are hoping to gain from the study. Maybe you are an experienced scientist merely seeking the methodology for a given technique that you plan to use in a totally different context, in which case reviewing the methods and some of the results might suffice. Alternatively, you might be a new student of developmental biology and could really benefit from reading the article all the way through, from beginning to end. Or you might be a student doing research to write a predoctoral grant and have more than 100 papers you need to digest while meeting a short deadline. How do you properly vet this volume of literature to have confidence your grant aims are justified? Whatever the situation may be, research papers generally have a consistent organization, so all readers can have confidence that specific types of information will be provided in specific locations of the paper. Once you understand this organization, you can efficiently read a study and extract the specific information you seek.

A typical research article is divided into the following sections: Title, Authors, Abstract and/or Summary, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, and References. To illustrate what these different parts contain, we have selected an open-access article that is free for us to annotate and that relates to our sample topic—the role of hox genes during limb development. We thank Dr. Denis Duboule (the "corresponding author"), who granted us permission to use this article for this purpose.

Author contributions

The list of authors behind the study is important for you to understand should correspondence be necessary (**FIGURE A.3**). Unless otherwise indicated, each author in the list contributed to the study in different amounts and/or ways. Usually, the first author in the list played the largest role in both the experimentation and the writing, while each successive author played significant but lesser roles to help the study come to completion. The one exception to this rule is the last author, who is usually the head of the laboratory and consequently would have played a role in most if not all aspects

RESEARCH ARTICLE

Similarities and differences in the regulation of *HoxD* genes during chick and mouse limb development

Title

First author (often the person who directly contributed the most to both experiments and writing)

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Last author (often the principle investigator and lab head for the study, this person is involved in most aspects of the research and consequently is usually the “**corresponding author**”)

Author summary

The shapes of limbs vary greatly among tetrapod species, even between the forelimbs and hindlimbs of the same animal. *Hox* genes regulate the proper growth and patterning of tetrapod limbs. In order to evaluate whether variations in the complex regulation of a cluster of *Hox* genes—the *Hoxd* genes—during limb development contribute to the differences in limb shape, we compared their transcriptional control during limb bud development in the forelimbs and hindlimbs of mouse and chicken embryos. We found that the regulatory mechanism underlying *Hoxd* gene expression is highly conserved, but some clear differences exist. For instance, we observed a variation in the topologically associating domain (TAD; a self-interacting genomic region) boundary interval between the mouse and the chick, as well as differences in the activity of a conserved enhancer element situated within the telomeric regulatory domain. In contrast to the mouse, the chicken enhancer has a stronger activity in the forelimb buds than in the hindlimb buds, which is correlated with the striking differences in the mRNA levels of the genes. We conclude that differences in both the timing and duration of TAD activities and in the width of their boundary may parallel the important decrease in *Hoxd* gene transcription in chick hindlimb buds versus forelimb buds. These differences may also account for the slightly distinct regulatory strategies implemented by mammals and birds at this locus.

Key

Background

Significance of the problem (may or may not include key questions or hypothesis to be addressed)

Approach/methods

Results

Conclusions

New revealed concepts (may or may not include new questions)

FIGURE A.3 The anatomy of a research paper: Authors and Abstract.

of the work, from its inception and funding to the experimental design and writing. The last author is also most often the “corresponding author” (as seen in our example paper by the Duboule lab). Feel free to reach out to the corresponding author should you have questions about a paper you are studying.

Title and Abstract

The first impression a reader has of a study comes right away, while searching through articles in PubMed, in the form of the paper’s Title and Abstract. The title often tries to capture the essence of the major findings, while also providing the cellular or molecular mechanistic context and the species used in the analysis (see Figure A.3). Giving the title a critical read before and after you read through the full article can help you better see these different elements. The title of our example paper touches all the bases:

- “Similarities and differences in the regulation of *HoxD* genes during chick and mouse limb development.” (The main result is red; the cellular context is orange; and the species are blue.)

The Abstract or Summary is a densely packed synopsis of the purpose, approach, findings and conclusions of the study (see Figure A.3, lower half). Knowing how to unpack an abstract into these discrete parts is an invaluable skill, which enables you to quickly assess the value of a given article in relation to your objectives. Some papers have a true abstract, while some others, like our example from *PLoS Biology*, may have both an abstract and an author’s summary. Regardless, these summaries usually provide some background context that sets up the significance of the study’s question and/or hypothesis. They also state the experimental approach taken to answer the question and/or test the hypothesis. The most significant findings are concisely presented and tied to the authors’ conclusions. Lastly, abstracts usually end with the implications the new findings might have on the broader scientific field. As you read over various abstracts, we encourage you to try to visually code these different types of information as we have exemplified here (see Figure A.3, color-coded breakdown).

The Introduction

The purpose of the Introduction section is to provide the minimum amount of background necessary to support the reader’s ability to 1) understand the rationale for why the study was conducted, 2) appreciate the logic of the experimental design that was employed to address the study’s questions, and 3) interpret the study’s results and recognize how they impact the current thinking in the field (FIGURE A.4). Importantly,

FIGURE A.4 The anatomy of a research paper: The Introduction. See Figure A.3 for key to color-coding.

Introduction

The Introduction sets up the problem and provides contextual background to better understand the study.

Tetrapod limbs are organized into three parts bearing skeletal elements—the stylopodium (humerus/femur), the zeugopodium (radius/fibula, ulna/tibia), and including the acropod (phalanges, metacarpals/metatarsals) and the tarsals [1]. Limbs can display large variations in their morphologies species or within the same species—as a result of their adaptation to different functions and ecological niches. For example, frogs display particular shapes of carpal and tarsal elements, with an elongated proximal tarsal whenever detectable [2], whereas geckos’ forelimb skeletal elements resemble those of their hindlimbs [3]. Another example of this morphological flexibility are the forelimbs of bats, which have digits early on similar to those of other mammals but that subsequently elongate to make flight possible [4].

References

1. Zeller R, Lopez-Rios J, Zuniga A. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nature reviews Genetics*. 2009; 10: 845–58. <https://doi.org/10.1038/nrg2681> PMID: 19920852

Linked citations and references can often help you track down relevant background articles fast.

The last paragraph(s) of the Introduction usually provide an additional summary of the entire investigation.

The structural categories of background, methods, results, and conclusions are often rearticulated at the end of the Introduction.

In this context, birds are a fascinating taxon, as they evolved forelimbs (wings) and hindlimbs may rely upon variations of the bimodal gene regulation mechanism described at the *HoxD* locus. Thus far, this mechanism has been analyzed only during the development of forelimb buds. Therefore, it remains unclear how much regulatory variation, if any, may be scored between fore- and hindlimbs of the same species or between different ones.

To tackle this issue, we used a comparative regulatory approach involving chick and mouse embryonic fore- and hindlimbs, mostly for two reasons. First, chicken embryos, unlike mice, display striking differences between the morphologies of their adult forelimbs and hindlimbs (Fig 1A and 1B, left). Second, it was reported that *Hoxd* gene expression domains during chick fore- and hindlimb buds’ development showed important deviations when compared to their mouse counterparts [23,31]. These features suggested that the bimodal regulatory system at work at the mouse *HoxD* locus may be operating slightly differently during the development of the avian appendicular skeletons.

Here, we combine the analyses of transcriptome, 3D genome conformation, histone modification, and mouse genetics to show that this bimodal regulatory mechanism is highly conserved in birds. However, in chicken hindlimb buds, the duration of T-DOM regulation is importantly shortened, which accounts for the concurrent reduction in *Hoxd* gene expression in the zeugopod. By using mutant mouse embryos lacking a large part of T-DOM, we also uncovered regulatory differences between fore- and hindlimbs. Therefore, although the general principles of these regulatory mechanisms are similar either amongst tetrapod species or within the same species between the fore- and hindlimbs, slight differences are scored, which may partly contribute to the observed morphological differences.

for your literature research, a good introduction is also a treasure trove of relevant references, from reviews to supportive primary literature. These references are all great candidates for further reading, depending on how directly they relate to your chosen research interests. Most introductions start by *introducing* the problem being addressed and provide general background to establish the necessary context. An introduction then becomes more specific, to the point of ending with another brief summary of the study's objectives, methods, results, and conclusions (see Figure A.4, color-coded text). From the Abstract and this last section of the Introduction, the reader should be able to get the “take-home” points of the study—at least from the authors' perspective. It is important to note that the research presented in these articles is meant to be critically analyzed and validated by the scientific community, which does so by asking challenging questions and repeating the work. Results are never absolutely true: they are limited by experimental uncertainty (their statistical significance), and they may be valid only under the same conditions used in the study.

The Results

The Results and the Materials and Methods sections are undoubtedly the most detailed and technical parts of a research paper. They are purposefully written in direct statements, with little to no prose dedicated to background or interpretation. The Results section is supposed to provide objective and concise statements of the findings; however, this section may also include some minimal, transitional articulation of the question to be addressed and the approach taken, and some brief conclusions. Because of the lack of explanatory language in the Results, you may need to do some additional reading to fully understand how the experiments were conducted.

The Results section of our sample paper is conveniently broken up into subsections, which help to demonstrate the intellectual flow from one finding to the next (FIGURE A.5). Data are usually presented in tables and in figures that are accompanied by descriptive figure legends. Callouts for the pertinent figures are provided in the text. A well-designed figure has orienting labels that identify its different parts, which is further supported by the figure's legend.

Some figures are not included in the main body of the article but rather are provided as “supporting information” or “supplemental materials.” Supporting information is only accessible from the journal's website (see Figure A.5, right). Video recordings, for instance, are usually called out as online-only supplemental material.

The Discussion

The Discussion section may begin with a brief reiteration of the major findings and conclusions of the study, but more often it is broken up into topical subsections (FIGURE A.6). The purpose of the Discussion is to 1) justify the authors' interpretations of the results, 2) relate the findings to the broader context of the field, 3) address similarities and differences with other, comparable studies, 4) discuss the broader implications of the work to the field and beyond, and 5) offer what may be some of the next logical questions. Often the Discussion also includes a summary illustration or schematic that proposes a “model” of how the authors interpret their findings (see Figure A.5, bottom). Knowing that these types of perspectives are communicated within the Discussion section can help readers find them quickly.

Materials and Methods

The Materials and Methods section is similar to the Results section, in that it describes as directly and concisely as possible the experimental design and methodologies performed (FIGURE A.7). Fortunately, it is convention to break up this section into as many discrete subsections as reasonable, so that readers can quickly locate pertinent information. Importantly, if a method was described in a previously published paper, then that paper will be cited, and the authors will describe only their own alterations to the protocol. Therefore, you may need to read additional papers solely for information about a protocol of interest. Although the Materials and Methods section is meant to enable other laboratories to repeat a given experiment, in practice space limitations

Results The Results section is often broken up into subsections based on the questions of the study and provides direct statements of the findings.

Transcription of *Hoxd* genes in mouse and chick limb buds

We first used whole-mount in situ hybridization (WISH) to compare the expression patterns of *Hoxd* genes in mouse fore- and hindlimbs at embryonic day (E)12.5 (Fig 1A) with those observed in chick at either Hamburger–Hamilton stage (HH)28 (equivalent to E12.25–E12.5, see also S1 Fig (Fig 1B)) or HH30 (equivalent to E13–E13.5, see also S1 Fig). In mouse fore- and hindlimbs, the amounts of *Hoxd13* and *Hoxd12* mRNAs were high in the prospective

Review the data presented in figures in the article. Figures are often designed in panels with labels on the top and left sides of the columns and rows, respectively.

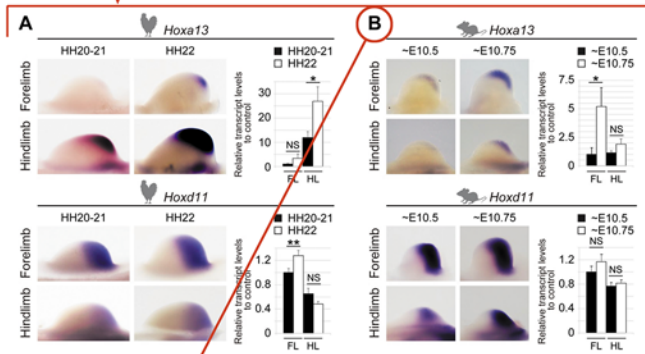


Fig 6. *Hoxa13* expression in chicken limb buds. (A) Expression patterns of *Hoxa13* and *Hoxd11* and mRNA steady-state levels in chick FL and HL buds from HH20 to HH22. A stronger expression of *Hoxa13* is observed in chick HL bud when compared to FL bud (top). mRNA level of *Hoxd11* increases in FL bud as development proceeds yet seems to decrease in HL bud (bottom). Expression levels are normalized to *Gapdh* and shown as fold change relative to FL bud at HH20–21. Error bars indicate standard deviation of three biological replicates. NS, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$, Welch two-sample t test. (B) Expression of *Hoxa13* and *Hoxd11* in mouse FL and HL buds from E10.5 to E10.75. mRNA levels of both genes in FL and HL buds increase as development proceeds. Expression levels are normalized to *Gapdh* and shown as fold change relative to FL buds at E10.5. Error bars indicate standard deviation of two or four biological replicates. * $p < 0.05$; NS, $p > 0.05$, Welch two-sample t test. For both A and B, individual numerical values of RT-qPCR are given in S1 Table. E, embryonic day; FL, forelimb; HH, Hamburger–Hamilton stage; HL, hindlimb; RT-qPCR, quantitative reverse transcription PCR.

Supporting information

S1 Fig. (Related to Fig 1) *Hoxa* gene expression in mouse and chick limb buds. (A, B) Comparison of developmental stages between mouse and chick limb buds. (C, D) Whole-mount in situ hybridization analysis of E12.5 mouse and HH28 chick FL and HL buds with expression

Access to supporting materials requires returning to the journal's website.

- Navigate to the “Supporting Information.”
- Navigate to the supporting figure of interest, which you may be able to download.

The figure legend contains concise descriptions of the presented data. Note the orienting lettering (A, B).

FIGURE A.5 The anatomy of a research paper: The Results.

It is increasingly common for the last figure in a paper to serve as a representative model of the paper's findings.

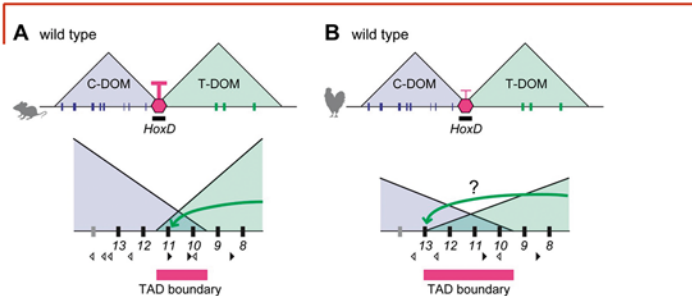


Fig 8. Model of TAD boundaries at the mouse and chicken *HoxD* cluster. (A, B) TAD boundaries at the *HoxD* locus in mouse (A) and chick (B) limb buds. (A) In the mouse, the boundary is dynamic and moves along a few genes within a window determined by a series of CTCF sites. Accordingly, T-DOM enhancers interact with promoters up to *Hoxd11* (green arrow in A). (B) In chick, the boundary appears slightly displaced toward the *Hoxd13* locus. This latter situation may enable T-DOM enhancers to interact with *Hoxd13* more efficiently in chick than in murine limb bud cells (green arrow in B). Black and white arrowheads indicate the orientation of CTCF motifs. C-DOM, centromeric regulatory domain; CTCF, CCCTC-binding factor; TAD, topologically associating domain; T-DOM, telomeric regulatory domain.

in the journal often preclude the inclusion of some important methodological details. Fortunately, there is always a corresponding author, who should be able to provide more detailed protocols.

Reading the article and exceptions to the rule

The consistent organization of information in a research article makes it easy to read the parts in the order that best meets the reader's needs. Each article serves different purposes for different readers, and a reader's level of expertise can also influence how much of an article they need to read. After reading the Abstract, a highly experienced scientist might jump directly to the figures and figure legends, then perhaps to the Discussion to see how the authors are interpreting the results. Only after this might the

FIGURE A.6 The anatomy of a research paper: The Discussion.

The Discussion section provides an articulation of the study’s conclusions and attempts to frame these findings in the context of currently accepted ideas.

Discussion

Often broken up into subtopics.

Conservation of the bimodal regulation in birds

Although the expression of *Hox* genes belonging to the *HoxA*, *HoxC*, and *HoxD* clusters during limb development are globally comparable between mammals and birds, clear differences are nevertheless apparent. For instance, *Hoxd* gene transcription is reduced in the proximal part of the developing hindlimb buds in birds, i.e., in a cellular domain in which their function is absolutely required for proper mouse hindlimb development [55,57]. Also, although *Hoxd12* is expressed in the mouse limb buds like *Hoxd13* (i.e., mostly under the control of C-DOM), its expression in the proximal avian forelimb buds resembles that of *Hoxd11*, suggesting it is controlled by T-DOM. The impact of these differences in *Hox* gene expression on the variations of limb morphologies is difficult to assess, particularly in the absence of experimental genetics in birds. Unlike in developing spines, in which a clear correspondence was established between *Hox* transcript domains and differences in vertebral formula in birds and mammals [58], such a direct relationship is more difficult to propose in the case of limbs for which many other genetic components are involved on top of *Hox* genes.

~~Because these expression specificities depend on the implementation of global regulations~~

scientist return to read the Results section, and, if they need more context, they might skim the Introduction and Materials and Methods. As your own skills and experience grow, so will your comfort level when you analyze research articles.

There are some extreme exceptions to the format described in this Appendix. For instance, articles published in the journals *Nature* and *Science* (the two highest impact factor journals) often merge the Introduction, Results, and Discussion into one continuous “Letter” or “Report.” Nevertheless, all the usual elements are represented within these formats—you just have to tease them apart.

FIGURE A.7 The anatomy of a research paper: The Materials and Methods.

The Materials and Methods section provides extremely concise descriptions of the most critical and unique aspects of the experiments conducted.

Materials and methods

Ethics statement

All experiments involving animals were performed in agreement with the Swiss law on animal protection (LPA), under license no. GE 81/14 (to D. D.), after evaluation by the ad hoc comité consultatif de l’expérimentation animale du Canton de Genève.

Animal experimentation

Chick embryos from a White Leghorn strain were incubated at 37.5°C and staged according to [50].

In situ hybridization and colorations

WISH was performed as described previously [65]. For *lacZ* staining, embryos were fixed in 1x PBS (pH 7.39–7.41), 2 mM MgCl₂, 4% PFA/PBS, 0.2% glutaraldehyde, and 5 mM EDTA for 20 min at room temperature and washed 3 times for 20 min in 1x PBS, 2 mM MgCl₂, 0.2% NP40, and 0.01% sodium deoxycholate. Samples were stained in 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 0.5 mg/ml X-gal at room temperature overnight fol-

This section is broken up into many subsections describing the methods and analyses performed, so it is easy to quickly find relevant information.

Note: These are not comprehensive descriptions of the protocols followed by the researchers. This section serves to provide the minimum information needed to replicate the experiments.