Dissecting a scientific paper about evolutionary biology I

Scientific journal articles can be daunting with their technical jargon, footnotes, and statistics. However, understanding one is not an impossible task. Below, we've annotated basic parts of a scientific paper in evolutionary biology to give you an idea of how to extract the important information. Keep in mind that while different journals use different article formats, all of the major sections below are present in other scientific papers, but may occur in a different order or may be combined.

Article

Dunn, D. W., Segar, S. T., Ridley, J., Chan, R., Crozier, R. H., Yu, D. W., and Cook, J. M. (2008). A role for parasites in stabilizing the fig-pollinator mutualism. *PLoS Biology*. 6: e59

http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.0060059

Title

Check the title! It might seem obvious but it's easy to forget. The title of a scientific paper usually summarizes the most important point being made. It's worth looking up parts of the title that you don't understand.

Take a look at the title of this paper. A mutualism is a relationship that benefits both parties involved. So this paper is about how a parasite helps keep balance in the mutually beneficial relationship between figs and pollinators. What organism pollinates figs? What sort of a relationship do figs and their pollinators have? If the paper doesn't explain it soon, you'll need to look that up as well.

Authors

The order of authors usually reflects the distribution of the workload. The first author is the scientist that performed most of the work and orchestrated the completion of the manuscript. A footnote will usually indicate which author to contact regarding the paper. This is the person to contact if you want to examine the data supporting the paper, to request an interview about the research for a publication—or to find out about graduate work in his or her lab! Also be sure to check out the affiliations of the authors. Are they at universities, in industry, or in government? Sometimes this can give you an idea of why this research was done in the first place. These researchers are all from academia, and an Internet search reveals that James M. Cook is a professor who runs a lab focusing on parasitism and mutualism. No big surprises there.

Abstract

Spend some time on the abstract! The abstract summarizes the entire paper. Read the abstract first to get a thumbnail sketch of where the authors are going and whether or not the paper will be of interest to you. Like a chapter outline, the abstract gives you a general idea of what's coming and makes it easier for you to fill in the details later. Try translating the abstract line by line into plain English. This

might mean you have to look up some vocabulary words. Don't worry if you get hung up on a sentence. Just try to understand each one as best you can.

- "Mutualisms are interspecific interactions in which both players benefit." Translation: A mutualism is a relationship that benefits both species involved.
- "Explaining their maintenance is problematic, because cheaters should outcompete cooperative conspecifics, leading to mutualism instability." Translation: It's strange that mutualisms exist because it seems like one of the parties would evolve to "cheat" the system (to get a benefit but not provide one).
- "Monoecious figs (*Ficus*) are pollinated by host-specific wasps (Agaonidae), whose larvae gall ovules in their "fruits" (syconia)." Translation: Figs that have both male and female flowers on the same tree are pollinated by wasps that stay true to a single fig species. Each wasp offspring takes over an ovule inside the fig fruit and keeps that ovule from developing into a fig seed.
- "Female pollinating wasps oviposit directly into *Ficus* ovules from inside the receptive syconium." Translation: Female wasps have to get inside the fig fruit before they can lay their eggs in the fruit.
- "Across *Ficus* species, there is a widely documented segregation of pollinator galls in inner ovules and seeds in outer ovules." Translation: When we look at lots of different fig species, we see that pollinating wasps tend to lay their eggs near the center of the fruit and that the outside of the fruit is usually devoted to producing seeds for the benefit of the fig plant.
- "This pattern suggests that wasps avoid, or are prevented from ovipositing into, outer ovules, and this results in mutualism stability." Translation: It seems like wasps avoid using the outside of the fruit for their eggs (or are prevented from doing it)—and that this helps avoid the problem of cheaters mentioned above. (The reasoning here is probably not clear yet. That's ok. Hopefully, you'll be able to figure that out from the rest of the paper.)
- "However, the mechanisms preventing wasps from exploiting outer ovules remain unknown." Translation: Nobody knows why it is that wasps only use the inner part of the fruit for their eggs.
- "We report that in *Ficus rubiginosa*, offspring in outer ovules are vulnerable to attack by parasitic wasps that oviposit from outside the syconium." Translation: The new thing we've discovered is that in this particular fig species, wasp offspring living in the outside of the fruit might be attacked by *another* wasp species that lays its eggs into the wasp offspring from outside the fruit!
- "Parasitism risk decreases towards the centre of the syconium, where inner ovules provide enemy-free space for pollinator offspring." Translation: If a mutualistic/pollinating wasp lays its eggs near the center of the fruit, its offspring won't get parasitized by other wasps.
- "We suggest that the resulting gradient in offspring viability is likely to contribute to selection on pollinators to avoid outer ovules, and by forcing wasps to focus on a subset of ovules, reduces their galling rates." Translation: We hypothesize that the risk of having offspring parasitized in the outside of the fruits causes wasps to favor laying eggs near the center of the fruit, and so causes them not to co-opt so much of the fig fruit for their own reproduction.

• "This previously unidentified mechanism may therefore contribute to mutualism persistence independent of additional factors that invoke plant defences against pollinator oviposition, or physiological constraints on pollinators that prevent oviposition in all available ovules." Translation: Nobody has proposed this hypothesis before and we think it might be a factor that keeps cheaters from arising in the fig/wasp relationship.

When you read a paper on your own, you might not be able to translate every sentence of the abstract like that, but give it a try and just get as far as you can! It will help you understand the rest of the paper.

Introduction

The introduction is a short background section that explains the significance of this particular research within the context of what is already known. The introduction summarizes the authors' questions or hypotheses and the approach that they propose to address them. Unless you are familiar with the field already, don't skip reading the introduction! Odds are, you will need this information later. Try to restate the main point of each paragraph in plain English, but if a paragraph is just impossible to understand, press on ahead! Don't let one confusing paragraph stop you. You may be able to figure out what it means later or may not end up needing it at all.

In this introduction, the authors first explain the basic evolutionary conundrum of mutualisms: why doesn't one partner evolve to cheat the system? This gets at the idea of evolutionary stability—the ability of a particular trait or set of traits (in this case, the traits associated with the mutualism between figs and their pollinators) to be maintained for millions of years, despite all the random mutations that might be more advantageous for an individual and allow the organisms to evolve a different set of traits. The second paragraph explains the basic biology of figs' pollination by wasps—and why this mutualism might be evolutionarily unstable: "Trees need to produce both wasps and seeds for the mutualism to persist, but natural selection should favour wasps that exploit the maximum number of fig ovules in the short term, resulting in a conflict of interest between wasp and tree." The third paragraph explains more about fig fruits and wasp egg-laying, as well as hypotheses to explain these observations. Basically, fig fruit contain many ovules (which may end up producing a seed or may become the home for a wasp larva). Pollinating wasps preferentially lay their eggs in ovules near the center of the fruit—and several hypotheses have been proposed that might explain why they do this. This paragraph contains some vocabulary you may need to look up-but will be very helpful when you look at the Methods section later! The fourth paragraph addresses another hypothesis to explain the preferential egg-laying. This paragraph may be confusing and is not essential to understanding the main point of the paper. The fifth paragraph describes some specific hypotheses about why eggs laid near the center of the fig might ultimately do better—including the hypothesis that the authors want to explore: that "parasites are more likely to parasitize pollinator offspring in the outer layers of ovules," and that this is one of the selection pressures that favored the evolution of pollinating wasps that prefer to lay eggs near the center of the fig.

References

References are extensively cited in the introduction. These are papers that the authors use to frame their arguments and questions. The citations are literally a scientific scaffold for the authors to build upon. The format of citations depends on the journal, but usually in scientific literature, it is parenthetical and not footnoted. These references can be a handy way for you to learn about an area of research. If there is something in the introduction that you don't understand, you can look up the reference associated with that idea to find out more. For example, if you were confused by the paragraph about "optimal foraging" and really wanted to understand it, you could start by looking up reference 24, an article called "Oviposition strategies, host coercion and the stable exploitation of figs by their hosts."

Author Summary

This is not a regular feature of journal articles, but a unique feature of this journal. It is essentially a jargon-free take on the work, aimed at making the research accessible to the general public. It's very handy! Lucky you, if the paper you are reading has one of these!

Figures

Figures are an important part of the manuscript. They present key information graphically to helps readers visualize patterns in the data. Figures are referred to at the appropriate place in the text. When you see a reference to a figure, stop and spend some time studying the figure! Often, the figure will make it *much* easier to understand what the text is saying. The caption is important too. Captions often provide key information that isn't available anywhere else in the paper and must provide enough information so the figure can be interpreted without reference to the text. In other words, the figures can stand alone. If a scientist is already familiar with a particular area of research and wants to understand a journal article quickly, he or she may just read the abstract and study the figures to get the main points of the paper.

This particular figure is unusual because it does not display data. Instead, it is meant to help the reader understand the physical layout of a fig fruit and where wasps may lay eggs. Examining this figure will help you understand the third paragraph of the introduction. You can see that ovules closer to the center of the fig are more likely to contain pollinators and ovules closer to the outside of the fig are more likely to contain parasites or seeds. The figure also gives you a visual idea of what is meant by *style* and *pedicel*. This will be important later on because the authors will use "ovule length" (seed plus pedicel) as an indicator of distance from the fruit wall.

Results/Discussion/Conclusion

Results, Discussion, and Conclusions are often broken into separate sections depending on the journal. All three are combined in this journal, but we'll deal with each separately.

Results

The results section of a scientific paper is where the authors' analysis of the data is presented. Authors do not usually present the raw data. Instead, the results section summarizes the data using tables, graphs, figures, and statistics. This is the part of the paper to focus on if you want to know how reliable/reasonable the authors' later conclusions are. Typically, authors keep their reporting of the data and their interpretation of the data separate, so that the reader can better evaluate the interpretation. Results sections usually contain lots of statistics and figures. When you are first reading a challenging article, it may be helpful to ignore the statistics for the most part and pay more attention to the figures. You can look up more details of a statistical test if you need to. When you come across an unfamiliar statistic, remember that a *p*-value smaller than 0.05 indicates a significant result, but nothing about the size or importance of the effect.

The authors collected fig fruit from six different sites and studied where pollinating and parasitizing wasps were distributed in the fruit. The main result of this study is summed up by the third sentence here: "Inner ovules were significantly more likely to contain pollinators, and outer ovules, seeds or parasites." Be sure to check out Figure 2, which supports this result. What is the main trend that each graph shows? Pollinators were found in the ovules with the longest average length, and seeds and parasites were found in the ovules with the shortest average lengths. Males exit their ovules before females. Since those wasps were already gone, the authors don't know if the empty ovules belonged to pollinating wasps or to parasitizing wasps, but the authors report the length of those exited ovules anyway. At this point, you might want to start asking yourself, what do these data say about the authors' main hypothesis that parasites are more likely to parasitize pollinator offspring in the outer layers of ovules?

Figure 3 shows a different analysis of some of the same data. The larger symbols (i.e., the ones that form the two horizontal lines) show the length of ovules that either were parasitized (the top line) or were not parasitized (the bottom line). The curve shows the probability of an ovule being parasitized depending on its length. Make sure that you understand which points on this graph represent actual observations and which represent calculations based on observations. Do these data support the authors' main hypothesis?

The authors present a few other results statistically, but these are less important to the main point of this paper.

Discussion

Though not a distinct entity in this particular paper, the discussion is the place where authors explain their interpretation of their data and how it reflects on their hypothesis or informs their question. In this section, the authors also compare and contrast their findings with those of other researchers, discuss implications of the work, and highlight future directions for research. Some of these tasks may also or alternatively be done in a conclusion section. First, the authors examine what their data have to say about an alternative hypothesis that pollinating wasps don't use outer ovules because those ovules are "unbeatable" or somehow protected from pollinating wasps. They note that they found parasitic wasps in outer ovules—which implies that the outer ovule *used* to be occupied by a pollinating wasp, before it was the victim of parasitism. So clearly at least some outer ovules are accessible to pollinating wasps, arguing against that hypothesis.

Figure 4 presents the study's data in yet another way. The authors' hypothesis suggests that parasites prevent pollinators from laying eggs in outer ovules and that this allows the fig to produce seeds in those ovules—and Figure 4 supports this idea. It shows that, just as we'd expect based on the hypothesis, as we get closer and closer to the outside of the fruit, parasites replace pollinators as the dominant wasp—and furthermore, as we get closer and closer to the outside of the fruit, ovules are more and more likely to develop into fig seeds.

The authors then return to examining a different alternative hypothesis—that pollinators don't lay eggs in all the fig ovules because they run out of eggs. Some simple observations and calculations suggest that this is not the case.

Next, the authors acknowledge that their hypothesis (that parasitism matters for the fig/pollinator mutualism) might affect different species to different degrees—specifically, species with different sized fruits. To begin to address this issue, the authors examine two distantly related fig species with differently sized fruit, and find the usual pattern in both (as shown in Figure 5): parasites and seeds towards the outside of the fruit, pollinators towards the inside. At least for this tiny subset of all species, their hypothesis seems relevant—but of course, this is a topic for a future study.

Finally, the authors examine another alternative hypothesis—that egg-laying pollinators prefer inner ovules because eggs growing in those ovules will produce larger offspring. If that hypothesis were true, we'd expect pollinator offspring in inner ovules (i.e., longer ovules) to grow larger on average than pollinator offspring laid in ovules more towards the outside of the fruit; however, the authors found no relationship between offspring size and ovule length, which argues against this alternative hypothesis.

Conclusion

If this paper had a distinct conclusion, it would consist of paragraphs that reiterate the significance of this contribution to a larger question and highlight future directions for research. This is another place where unfamiliar ideas might be brought into play, so be prepared to look up a concept or check out an additional article to fully understand the conclusion.

Here, the authors remind us that their data support the hypothesis that parasites are more likely to prey on pollinator offspring in the outer ovules. This is significant because it suggests that this might select for pollinators who spare outer ovules in their egg-laying—which would contribute to the evolutionary stability of the mutualism. This is of

interest beyond the fig/wasp interaction, because it shows how a third player (here, a parasite) may contribute to the evolutionary stability of a mutualistic pair.

The authors also explain how their hypothesis might relate to another aspect of the fig/pollinator mutualism. This is a bit trickier to understand. First, recall the original mutualistic conundrum: natural selection should favor the evolution of cheaters, so how is it that this mutualism can be maintained through millions of years of evolution? Because pollinating wasps have much shorter generation times than fig trees, they can evolve more quickly than the trees can—and so we'd expect pollinating wasps to be the partner that evolves to cheat the other. However, the presence of parasitic wasps helps resolve this conundrum. They also have short generation times and evolve quickly—so could play a role in keeping the evolution of cheating in the pollinators in check.

The authors end by suggesting that their hypothesis may apply to other fig/wasp mutualisms, not just the species pair in this study, and that this would be an important area for future study. They also reference other cases in which a third player contributes to the evolutionary stability of a mutualistic pair. Check out references 4, 32, and 33 if you want more details on these other cases!

Tables

Tables, like figures, organize or summarize data. They must include informative captions and should be interpretable without returning to the body of the paper. When you see a table in a paper, try to determine what main messages it is intended to convey. The main point of this table can be found in the last column: levels of parasitism can be high, but vary substantially depending on location.

Materials and Methods

The methods section is often placed within the paper rather than at the end usually just after the Introduction. It provides details on sample, analytical tools, study design, equipment, potential sources of error, and methods of statistical analysis. Because this section is most relevant for readers conducting similar research (and is only skimmed by most readers), it may appear in smaller font. This section is necessary for other experts to evaluate the validity of a particular study, but it is often challenging to read and provides little insight for non-experts. You can usually get the information you need just by skimming and looking things up in the methods section if you have a question about how a particular result was obtained. For example, if you were reading this paper without a guide, you might have initially been confused by the idea of ovules exited by male wasps that was mentioned in the results section. You could skim the methods section for more details on that: "All syconia were early in the male flower phase with no exit holes made by male wasps. This was to ensure that female wasps had yet to emerge from their galls." Of course, if you wanted to conduct a related study of a fig species that lives near you, you would pore over the methods section to learn more about exactly how you could design your own study.

Supporting Information

A reference to supporting information is often provided at the end of the manuscript. This information will be useful to readers interested in details of the data or study design. The information may include items that cannot be printed (like video or audio), or items that don't fit within the page limits for the manuscript. Unless you are conducting research in a related area, you are unlikely to need to access the supporting information. If you are curious, take a peek at the supporting information for this article:

http://www.plosbiology.org/article/fetchSingleRepresentation.action?uri=info:doi/10.137 1/journal.pbio.0060059.sd001

Acknowledgments

Acknowledgments allow the authors to thank people, organizations, and funders that were instrumental to the work, but did not participate in writing or analyzing the data. In some journals, this section also provides a sketch of the work that each author did on the study in order to better allocate credit for the work. Funding sources are almost always acknowledged here—and might give you an idea of where to apply for funding for your own related work. The acknowledgments section may also include statement about competing interests or conflict of interest. Journals require authors to reveal whether or not they have a personal stake in the outcome the research being reported on (e.g., if an author is being paid as a consultant for a pharmaceutical company whose produces drugs that treat the disease being studied). Potential conflicts of interest must be revealed to readers so that they can judge whether this may have been allowed to influence the outcome of the study. Depending on the journal some of these items may be referenced in different places. For example, in the journal *Science*, acknowledgements are noted as an endnote in the list of references.

References

These are the published papers that the authors directly cite in their study. Formatting varies considerably from journal to journal. References are a valuable resource for finding additional information. You can often find at least the abstract of a reference online simply by typing the title of the paper into Google Scholar (http://scholar.google.com/). For example, using Google Scholar, you can find the full text for reference 32

(<u>http://www.webpages.uidaho.edu/~pellmyr/pubs/IJPS06CrabbPellmyr.pdf</u>) and find out how similar evolutionary dynamics may be at play in a mutualism between yucca plants and their moth pollinators.