7

The Challenge

Scientia (Latin): knowledge.

In the challenge, you describe the specific knowledge you hope to gain. This starts with the question that drove you to do the research. You did the work to discover the answer. From the question, we sometimes formulate a hypothesis and we usually state specific objectives, which describe the information we will present. Some authors only pose the question, whereas others do all three, offering a question, framing it into a hypothesis, and then describing specific research goals. Each approach has its place, but the question is the core of it all. If you don't have a question, you are not doing good science. If readers can't tell what it is, you are not writing good science.

7.1. QUESTIONS VERSUS HYPOTHESES

There are people who argue that without a hypothesis, it isn't science. That view grows from a strict focus on Popper's argument that a theory is only scientific if it can be falsified.[1] But Vaghjiani and Ravishankara (example 6.1) had no hypothesis about the rate constant for the reaction between methane and hydroxyl radicals; they did have a question, "what is the rate constant?" Was their work not science?

1. K. Popper, *The Logic of Scientific Discovery* (1934; Routledge Classics, 1959).

Different fields of science have different traditions about questions versus hypotheses. One community I work in submits proposals to the National Science Foundation Biology Directorate and demands hypotheses. Another submits to Geosciences and is slightly baffled by biologists' obsession with them. Framing a hypothesis can be a powerful tool for organizing your thoughts and structuring your research, but a hypothesis merely takes your question and makes into a falsifiable prediction. The question, defining the knowledge gap, is still the key.

We often use hypotheses to test whether a relationship exists—to develop a theory. We don't necessarily frame one to evaluate the nature of a relationship. An evolutionary biologist might ask if flower color controls whether pollinators visit a particular plant—they might frame the hypothesis: "hummingbirds prefer red flowers." That is testable and falsifiable. But Vaghjiani and Ravishankara knew that methane reacts with hydroxyl radicals and that the reaction has a rate constant, they just didn't know what it is. What would they have hypothesized?

Interestingly, even biologists who routinely frame hypotheses for proposals might still ask a question in the challenge of a paper. I can't give any global advice as to whether to pose a question or to transform it into a formal hypothesis; you need to know the culture of your field. Just remember that the question comes first and must be clear.

7.2. QUESTIONS VERSUS OBJECTIVES

Despite the importance of the question, many authors define their challenge by stating "Our objectives were" rather than by saying "Our question was." That is, they focus on the information they will collect, rather than the knowledge they hope to gain. They assume that the question is obvious from all they have said in the introduction and they don't need to state it explicitly. They are almost always wrong.

Focusing on objectives instead of questions is weak science and weak storytelling. If you leave the question unstated and implicit, and jump straight to specific data-collection goals, the reader has to figure out what your question was and whether you even had one. You leave it to them to figure out how the work will advance knowledge. That violates principle 1—it is the author's job to make the reader's job easy.

Focusing on objectives also doesn't engage SUCCES. It doesn't create unexpectedness or curiosity—at least not the curiosity you want. A reader will wonder about your objectives. Why did you do this work? What was the purpose? What was the underlying question these tasks address? Is it possible that you were just aping experiments published by others without a real question of your own? Those are not questions about your science but about you and your motivation, and there is an underlying criticism embedded—why are you wasting my time with this?

You have a question that drove your work. Make it clear. Then you can tell us how you answer it.

7.3. WHAT COMES AFTER STATING THE QUESTION?

After posing the question, a good challenge briefly lays out the research approach. This is where you tell us about specific objectives and the information you will generate. If you tested whether a pollutant is carcinogenic, did you use mechanistic toxicology or epidemiology? If you were identifying bacteria in nature, did you grow them in culture or sequence DNA extracted from environmental samples? If you are measuring the rate constant for a reaction, did you do it in gas phase or aqueous solution? Here, stating objectives can be useful in providing a map that helps readers assess the rest of the paper. Were your methods appropriate for the question? Did you learn what you hoped to? Where are the remaining gaps?

Some papers also provide a brief overview of the Conclusions, usually starting with language like "In this paper we show . . ." After a full Introduction, the authors telegraph their Conclusions. This telegraphed OCAR works well for an impatient audience but one that still wants to see how arguments develop. It is more front-loaded than normal OCAR, but not as much as ABDCE or LDR. This approach is common in the biomedical literature, but is not unknown in other fields. I suspect it evolved to help readers screen papers quickly—knowing the proposed conclusions makes them easier to evaluate as you read.

7.4. GOOD CHALLENGES

In papers for specialist journals, a good challenge almost always condenses conceptually to "to learn X, we did Y." That is, they present the question and lay out an approach to answering it, as illustrated in example 7.1. This paper explored how living in a complex environment may enhance brain development.

Example 7.1

Our goal in this study was twofold. First, we tested whether an animal's physical environment would affect hippocampal attributes. Specifically, we tested whether food-caching mountain chickadees (*Poecile gambeli*) housed in captivity differed in hippocampal volume, hippocampal neuron number and neuronal density as compared with fully developed wild-caught conspecifics. We predicted that captivity, with reduced environmental complexity and restricted memory-based experiences (compared with memory-based experiences afforded in the natural environment), would reduce hippocampal volume, neuron number and, potentially, neuron density.[2]

2. L. D. LaDage, T. C. Roth, R. A. Fox, and V. V. Pravosudov, "Effects of Captivity and Memory-Based Experiences on the Hippocampus in Mountain Chickadees," *Behavioral Neuroscience* 123 (2009): 284–91.

Why is this a good challenge? It is long and technical, without linguistic flourish. The authors, however, do several things well. First, they remind us of the overall issue—fundamentally a question about controls on brain development, a topic of wide interest and import, even though the specific question is narrow. In fact, the specific question about captive birds and their hippocampuses, by itself, might seem more a candidate for a Golden Fleece award for pointless Ivory Tower science than for the Faculty of 1000 website of interesting and important papers, which is where I found it. The authors did an excellent job of connecting their specific question to the larger problem. They go further, though—even after posing a tight question, they frame a hypothesis that defines their measurements and the data that would falsify or support it. As you read the rest of the paper, you know what the authors think and what they did; you can easily follow along as they assess their results and develop their Conclusions. Nicely done.

Example 7.2 is from a paper looking at the mechanisms of light perception and visual responses in marine invertebrates. What chemicals create the signal? Some work suggests that diacylglycerol (DAG) has a role in signaling. But the authors argue that DAG can't completely explain observed responses and so propose an alternative signal molecule: phosphatidylinositol bisphosphate (PIP_2), which breaks down to DAG.

Example 7.2

Despite the tantalizing evidence for DAG and/or its downstream products in visual transduction and the synergistic role of calcium, in no instance has application of such chemical stimuli fully reproduced the remarkable size and speed of the photocurrent. This may imply that yet another signal may be missing from the proposed schemes. In other systems PIP_2 has been shown to possess signaling functions of its own, independent from those of its hydrolysis products. . . . These observations prompted the conjecture that in microvillar photoreceptors PIP_2 may help keep the channels closed and its hydrolysis could promote their opening. In the present report, we examined the consequences of manipulating PIP_2 on membrane currents and light responsiveness in is qolated photoreceptors from *Pecten* and *Lima*.[3]

The authors clearly lay out the problem—DAG can't explain existing observations. They hypothesize a new mechanism that involves PIP_2 and briefly describe the experiments they did to test this hypothesis— "to learn X, we did Y." Even though this was in a specialist journal, the authors use strong words to engage curiosity and attention, notably *tantalizing* and *remarkable*.

As an example of a telegraphed OCAR structure, consider example 7.3. This paper explores how the transcriptional machinery at tRNA genes may interfere

3. M. Del Pilar Gomez and E. Nasi, "A Direct Signaling Role for Phosphatidylinositol 4,5-Bisphosphate (PIP2) in the Visual Excitation Process of Microvillar Receptors," *Journal of Biological Chemistry* 280 (2005): 16784–89.

with DNA replication in a way that can promote chromosome breakage. They examined how certain proteins regulate tRNA gene transcription.

Example 7.3:
Although Rrm3 and Tof1 might collaborate to set the rate of fork progression through tRNA genes, there is no evidence that this rate is subject to physiological regulation by mechanisms that determine the balance of activity between Rrm3 and Tof1. Furthermore, tRNA gene regulation is not known to be tied to fluctuations in the rate of DNA replication other than by mechanisms that generally tune the proliferation rate to nutrient availability and overall cellular fitness for growth and division. However, there is evidence that the appearance of double strand breaks (DSBs) in DNA can trigger repression of the tRNA genes. Specifically, tRNA gene transcription is actively repressed in cells treated with UV light or the alkylating agent methane methylsulfonate (MMS). The secondary DNA lesions generated in UV-irradiated and MMS-treated cells include DSBs. This fact, as well as the observation that tRNA gene repression requires a protein kinase (CK2) previously implicated in adaptation to chromosome breakage, led us to hypothesize that the canonical DNA-damage response (DDR) checkpoint pathway controls tRNA gene transcription. The aim of the present study was to test this hypothesis. In pursuing this aim, we discovered an unexpected system of regulation in which checkpoint proteins specialized for signaling replication stress repress tRNA gene transcription during normal proliferation. These proteins also convey repressive signals to tRNA genes in cells exposed to genotoxins that cause replication interference. These data provide the first evidence that the fork-pausing activity of tRNA genes is regulated by the checkpoint system that has evolved to control replication.[4]

The authors synthesize existing knowledge to pose a clear hypothesis; they could have stopped by saying that their aim was to test that hypothesis. Had they done so, this still would have been a fine challenge. But then they highlight that in testing the hypothesis they discovered a new regulation system, in which the mechanisms that control the rate of DNA replication also regulate tRNA transcription. This both prepared you for the story to come and raised the curiosity factor to intensify the challenge.

These examples all came from papers in specialist journals written using OCAR story structure and IMRaD sections; they all used the "to learn X, we did Y" form. When a paper uses a different structure, the challenge is often condensed to focus more intensely on the question. Example 7.4, from the field of physical chemistry, illustrates this in an LDR-structured paper; it is a report in *Science* that doesn't use subheads to break up the sections. The authors ended the paper's lead with a

4. V. C. Nguyen, B. W. Clelland, D. J. Hockman, S. L. Kujat-Choy, H. E. Mewhort, and M. C. Schultz, "Replication Stress Checkpoint Signaling Controls tRNA Gene Transcription," *Nature Structural and Molecular Biology* 17 (2010): 976–81.

strongly worded question to ensure that you didn't miss the challenge and to effectively engage the broad audience of this journal.

Example 7.4:
However, three decades of work in the gas phase have explored how the specifics of the forces between atoms involved in isolated chemical reactions determine the final energy partitioning as the reaction moves from the transition state. Is knowledge of these specifics completely immaterial to reaction dynamics in solution?[5]

These authors define the knowledge gap and ask an interesting question that confronts the reader—is the three decades of work on gases "completely immaterial" to solution-phase reactions? This taps into SUCCES: it draws on simple by asking a clean and straightforward question, and it draws on unexpected and emotion by using the highly charged phrase "completely immaterial" to challenge decades of high-quality science.

7.5. BAD CHALLENGES

If the challenge is unclear, readers will be left adrift. If they don't know where the paper is going, how will they know whether they got there? A challenge is ineffective if it doesn't concretely state the question or hypothesis or if gives the reader the wrong impression as to what it is.

The most common type of unclear challenge is where authors focus on the information, rather than the knowledge they are trying to acquire; they leave off the "to learn X . . ." and just say "we did Y." They focus on the objectives, rather than the question. I think many authors fall into this trap because they know the material so well that the question seems obvious. No reader, however, knows as much about your work as you do, and your thinking is rarely completely apparent. The challenge is too important to leave it to assumption, hope, or chance. You must make the question clear. If you fail to do this, your papers will lack power, and your proposals will likely lack funding.

So let us evaluate some weak challenges and discuss how to improve them. The first example explores why the immune system sometimes breaks down.

Example 7.5
Some T-cells may be anergic—that is, unable to proliferate after being restimulated with an antigen. Some anergic T-cells are unable to link to the T-cell–antigen presenting cell (APC) interface. Here we examined the structural

5. A. C. Moskun, A. E. Jailaubekov, S. E. Bradforth, G. H. Tao, and R. M. Stratt, "Rotational Coherence and a Sudden Breakdown in Linear Response Seen in Room-Temperature Liquids," *Science* 311 (2008): 1907–11.

characteristics of anergic mouse T-cells and we tested their functional response to being rechallenged with antigen-loaded APCs.

Here the authors tell us which data they will collect, but they don't specify the knowledge gap. What is the question? Presumably these researchers are trying to figure out what makes a T-cell anergic, and from that to understand why immune systems break down, with the ultimate goal of finding ways to prevent the breakdown. But that thought process is opaque—the implicit question is too deeply buried. This would have been much stronger if they had clarified the "to do X" part of the challenge, perhaps like this:

"To determine what causes mouse T-cells to be anergic, we evaluated the structural characteristics of T-cells and how they responded to being rechallenged with antigen-loaded APCs."

That simple addition would have clarified the question and made it stronger.

Example 7.6 is from environmental science and is about how herbivores structure plant communities.

Example. 7.6
We evaluated the possibility that hares influence the structure of shrublands by acting as keystone herbivores that maintain gaps between the shrubs and so influence the competitive interactions of plants recruiting into those gaps.

In this one, the question itself is unclear. What do hares do that influences shrubland structure and competitive interactions? What does the investigator hope to learn?

There are several implied hypotheses within this challenge: (a) hares control plant community structure, (b) they maintain gaps between shrubs, and (c) they influence competitive interactions within the gaps. Those hypotheses should be explicit and concrete. This should also describe the experiment that will test those hypotheses. Consider this as an alternative:

"We hypothesized that hares control the structure of shrublands by foraging on shrub seedlings in the gaps between mature plants. If true, hares act as keystone herbivores by maintaining these gaps, in which grasses can outcompete shrub seedlings. We tested this hypothesis by following hare movement to determine where they eat and by analyzing their feces to determine what they eat."

This states the hypothesis and suggests its larger implications—hares are keystone species that have a disproportionate impact on ecosystem structure and function. It also briefly identified the experimental approach used to test the hypothesis.

Example 7.7 is also from environmental science, specifically grassland ecology and global change biology. The authors pose two goals, both of which are clear. The problem is their sequence.

Example 7.7
The study had two goals. First, we aimed to constrain our estimates of grassland plant production by comparing measurements based on two techniques: maximum biomass at the end of the season and periodic measurements of photosynthesis. Second, we examined the response of grass growth to a combination of elevated CO_2 and increased temperatures, conditions that are expected to occur with climate warming.

Why is this challenge weak? The authors presented the goals in an order that is more chronological than intellectual. First they validated their methods, and then they assessed what the data meant (i.e. We did X to learn Y). But we expect the most important objective to come first, defining a study's overall thrust. Following objectives should elaborate and refine that main goal. Starting with the data-collection goal gives readers the impression that the work is primarily comparing approaches for measuring plant growth—narrowly useful, but not broadly engaging.

The interesting question is posed in the second objective: how will plants respond to climate change? This is the *To learn Y* part of the challenge. Because it comes second, it seems subordinate to the methods comparison. The language reinforces that hierarchy; the first goal is stated using strong verbs: the authors will "constrain" and "compare" measurements. The second, in contrast, uses weak language: they will "examine" a response. To fix this problem, we need to switch the order of the objectives and highlight the core question:

"The primary goal of this study was to evaluate how grass growth responds to a combination of elevated CO_2 and increased temperatures, conditions that are expected to occur with climate warming. To validate our plant growth measures, we used two approaches to estimate plant production: maximum biomass at the end of the season and periodic measurements of photosynthesis."

Now this seems like a more interesting paper—the real question is clear and is unmistakably about a topic that is broadly interesting and relevant.

A good challenge must define not only the data you collect but the knowledge you hope to gain. If you read something and can't find a clear statement of the question or hypothesis, or if that question itself is unclear, the challenge will be weak and will weaken the entire story. With a proposal, that weakening is likely to be fatal. Remember that the critical part of the challenge is not "we did Y" but "to learn X."

EXERCISES

7.1. Analyze published papers

Evaluate the challenge of each paper. Does it clearly frame the question? Could you write it better? If so, how?

7.2. Write a short article

In your short article, did you clearly pose the question? If not, rewrite the piece to do so.

8

Action

You are not just presenting your results, you are telling a story.

In OCAR, action makes up the main body of the story and includes everything between the challenge and the resolution. In a paper, this includes the Materials and Methods, the Results, and most of the Discussion. In a proposal, it is the description of what you intend to do. Because so much goes in these sections, you could write an entire book on it (and many have). Particularly when it comes to how to present data (tables, figures, etc.), there is a wealth of information. Because this is a book about *writing*, I focus on how to integrate these sections into the overall story and how to use story structure to present them most effectively.

In writing the action, the critical message is to remember the last S in SUCCES—*story*. You are not just presenting your results, you are telling a story. You are, of course, free to write papers that simply present experiments and data; but journals are equally free to reject them. It's not that readers aren't interested in your techniques and results—we are. We want to know what you did and what you found. That is the concrete core of the science, and we must be able to evaluate it to assess the validity of your conclusions. But without embedding the action within the larger story, the paper easily becomes aimless, incoherent, and dull. What is the point of all that work? What do these results mean? Do they answer your question? Do they support your hypotheses and conclusions?