Fishing for Genes:
How the Largest Gene Family in the Mammalian Genome was Found (and Why Idiosyncrasy in Exploration Matters)

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Abstract

In 1991, Linda Buck and Richard Axel identified the multigene family expressing odor receptors. Their discovery transformed research on olfaction overnight, and Buck and Axel were awarded the 2004 Nobel Prize in Physiology or Medicine. Behind this success lies another, less visible study about the methodological ingenuity of Buck. This hidden tale holds the key to answering a fundamental question in discovery analysis: What makes specific discovery tools fit their tasks? Why do some strategies turn out to be more fruitful than others? The fit of a method with an experimental system often establishes the success of a discovery. However, the underlying reasoning of discovery is hard to codify. These difficulties point toward an element of discovery analysis routinely sidelined as a mere biographical element in the philosophical analysis of science: the individual discoverer's role. I argue that the individual researcher is not a replaceable epistemic element in discovery analysis. This article draws on contemporary oral history, including interviews with Buck and other actors key to developments in late 1980s olfaction.

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1. Introduction

In 1991, Linda Buck, then a senior postdoc, and her PI Richard Axel identified the genes encoding the olfactory receptor family. Their discovery transformed research on the biology of smell. Olfaction had always occupied a mere niche in the grander world of science, only to become part of mainstream genetics and neuroscience overnight (Firestein, Greer, and Mombaerts 2014). "She devised this very clever scheme, and she got it." Axel recalled Buck arriving in his office. "When she showed me the results, I was silent for a while because the whole thing began to unfold in my head" (quoted in Barwich 2020a, b).

With this discovery's 30th anniversary, the hidden experimental history of Buck's work behind the scenes invites fresh attention. In the late 1980s, Buck was cloning her way through the nasal epithelium unsuccessfully for several years. She was not alone. Albeit a small field, several laboratories had entered a race to discover the olfactory receptors at the same time. None prevailed. The difficulties that long characterized the stagnation of research and Buck's final breakthrough are instructive, especially for science studies today. These difficulties point toward an element of discovery analysis routinely sidelined as a mere biographical element in the philosophical analysis of science: the individual discoverer's role.

The historical recency of this discovery offers a remarkable opportunity. Research for this paper benefitted from two chief sources. In addition to published documentation of historical events, this article draws on interviews of the author with historical actors key to this development, principally Linda Buck as the experimental protagonist in focus.[1]

This article focuses on Buck's experimental history to revisit philosophical thinking about the individual scientist as an epistemic element in the context of discovery. I begin by situating the importance of the olfactory receptor discovery in the broader context of science (section 2) before discussing the individual scientist's role in philosophical analysis of discovery (section 3). That is followed by a detailed analysis of Buck's discovery, including historical background, methodological challenges, and an analysis of her experimental solution (section 4). I end with a reflection on the lessons we can derive from such detailed examinations of

[1] These interviews were conducted between 2015 and 2018 as part of a larger project concerning a contemporary history of the science of smell; further details in Barwich 2020a.

individual discovery stories for broader philosophical discussions about scientific practice (sections 5 and 6).

2. A Nobel Nose

The scientific impact of the receptor discovery findings on olfaction is difficult to overstate. It is comparable to Hubel and Wiesel's revolutionary findings in the cat cortex and its influence on twentieth-century neuroscience on the visual system (Shepherd 2009). The receptor discovery was the starting signal for modern molecular research into the sense of smell. Buck and Axel (1991) did not merely discover the receptors responsible for odor recognition. Their discovery revealed that the olfactory receptors provide an exceptionally versatile molecular model for investigating structure-function relations in ligand-protein interactions (Firestein 2001; Barwich 2015a). The gene family encoding the olfactory receptors turned out to be the largest family, occupying up to 4% in most mammalian genomes (Zhang et al. 2007). That is notably more than the 'genetic space' allocated to the immune system.

This unexpected insight catapulted olfaction into mainstream science (complimentary analysis of the OR discovery with additional information and interviews about its impact on the field in Barwich 2020a, b). These receptors were the missing piece for evaluating any hypothesis about odor detection at the sensory periphery.

The identification of the olfactory receptors as members of a larger protein family, the superfamily of G-protein coupled receptors (GPCRs), also changed olfaction's standing as an experimental system in neurobiology. GPCRs are one of the most central entities in current biology (Barwich and Bschir 2017). GPCRs are molecular gateways involved in various critical cell-signaling processes. Up to 50% of drug-receptor studies target these proteins (Zhang and Xie 2012). Still, the general principles of molecular recognition in these cell membrane proteins are not entirely understood. GPCRs respond to an astonishing array of structurally diverse ligands such as neurotransmitters, hormones, peptides, proteins, airborne chemicals, and even photons (Snogerup-Linse 2012). A key feature of GPCRs is that, despite their functional diversity, this protein superfamily shares a significant amount of amino acid sequences preserved throughout

evolution. Their genetic characteristics pose questions about how the tremendous functional diversity in protein behavior evolved in light of such striking structural similarities.

Odor receptors (ORs) are of particular interest in this context. They offer a sub-system for modeling GPCR ligand binding because they constitute the largest and most diverse group of this protein superfamily (Firestein 2001; Barwich 2015b).

Buck and Axel received the 2004 Nobel Prize in Physiology or Medicine for their achievements (Buck 2005, Axel 2005). Historically, only a few scientific discoveries genuinely have had a comparable impact on defining an experimental system's outlook as discovering the odor receptor genes in research on olfaction (Barwich 2015a, b, 2018). That is visible also in publication metrics: "over the 30 years before 1991, 2,456 research articles used the keywords 'odor', 'odor receptor', 'olfactory receptor', 'odorant receptor' (American and British English spelling); within the 5 years after this landmark publication, there were an additional 4,110; and since 1991, the number stands at a remarkable 44,380 (as of February 8, 2020)" (Barwich 2020b, 749). Meanwhile, their original 1991 publication has become a modern science classic and was selected for a series of annotated research papers in *Cell*, celebrating fundamental breakthroughs in biology over the past 40 years (Firestein, Greer, and Mombaerts 2014). Further, *Cell* published an analysis of Buck's experimental account in its Commentary (Buck 2004) and BenchMark (Barwich 2020b) sections.

Buck's discovery must read like a classical success story to a modern audience. However, a critical part of Buck's discovery is why it looked far from being a success story for several years.

That part of the story begins a few years before the triumphant results and remains primarily disclosed in Linda Buck's memory and lab notes. It describes a long trail of failed experiments, cul-de-sacs, and discarded ideas. For three years, Buck was the only researcher in the Axel lab working on olfaction.[2] That is a very long time with minimal publishable results as a

[2] In the 1980s, Axel also collaborated with Steven Siegelbaum, another neuroscientist at Columbia, on the olfactory ion channels in catfish (Goulding et al. 1991). Nonetheless, this collaboration was not considered to address the olfactory system as such but was primarily intended to study the general nature of second messenger pathways (see Figure 1).

senior postdoc.[3] Retrospectively, Buck's fixation on finding these unknown genes would either spell out the end of her career or result in a Nobel Prize. What was the difficulty in finding the OR genes, and what established Buck's breakthrough? The answer involves an elegant and, at that time, unlikely use of an experimental tool.

How a discovery occurred can be equally as instructive to the development of an experimental system as the empirical data it provides. What makes specific discovery tools fit their tasks, and why some approaches are more fruitful than others, needs an answer that also includes the exploratory strategies of the individual scientists as characteristic of a specific discovery.

3. How *replaceable* is the individual scientist in discovery analysis?

A popular witticism among scientists involves the elusive logic of great discoveries. It seems like trying to find a black cat in a dark room. A tricky endeavor, "especially if there is no cat" (Firestein 2012, 65). The making of scientific discoveries seems a delicate art. How can you spot something novel while being sufficiently ignorant about its characteristics or even existence? Close engagement with individual scientists' exploration strategies becomes a valuable source for understanding this conundrum.

Philosophical interest in scientific discovery has attracted attention with a late twentieth century focus on scientific practice, which succeeded the debate on the logical structure of scientific theorizing. Their context-sensitive background presented discoveries as a subject for historians of science. Traditionally, philosophers have dealt with the more generalizable context of justification as the epistemic rationale underpinning the findings. They separated the unique context of scientific discovery from its logically tractable context of justification, with the latter reflecting the confirmatory basis of scientific hypotheses (Clarke 2017). Systematic interest in the epistemic foundations of scientific exploration thus has been sparse.

[3] That said, Buck published two articles prior to this discovery. Neither concerned olfaction but splicing in aplysia (in 1990 with Thomas Jessel) and selective expressions of a lactose-binding lectin gene (in 1987 with Richard Axel).

Often the rationally reconstructed and the historically real seem irreconcilable (Nickles 1980). The physiologist Root-Bernstein noted the disciplinary consequences of this epistemic separation as early as 1989:

> "A fundamental problem with philosophy of science is that many philosophers believe the context of discovery to be unknowable: How a scientific discovery occurs is a matter of unique historical, social, and psychological elements that cannot be explained logically or rationally. We can only determine whether the resulting hypothesis is correct or not." (1989, 473)

Contemporary studies in the philosophy, history, and sociology of science have remedied this neglect over the past decades. Prominent representatives of disciplinary transformation are Longino (1990), Barnes, Bloor, and Henry (1996), Rheinberger (1996), Kay (2000), Arabatzis (2006), Chang (2004), and others. This line of work has revealed how historical and societal parameters shape the outlook of research. For example, Klein and Lefèvre (2007) explicated the historical and social intersection between commerce and academia to understand the disciplinary rise of chemistry. Such contingent factors surrounding science shaped the character of the research community and scientific ideas. An excellent case for this is the heuristic influence of societal metaphors on scientific modeling. Such metaphors influence even physics. Consider research in the Soviet Union where the political analogy of collectivism entered physics and lead to views about the collective behavior of electrons, which were taken up, de-politicized, and mathematized by British physicists soon after (Kozhevnikov 2004). Examples are legion.

Overall, this kind of analysis also led to a reconsideration of scientific discovery in philosophical discourse, one that is inseparable from the context of justification as its historically and socially determined background (Schickore and Steinle 2006). Such analysis often highlights the variety of instances falling under the notion of scientific discovery. Philosophical interest here tends to center on communal achievements of discovery, often involving and attributable to independent groups of people with an analysis of how their work has come together in different ways (Clarke 2017). Yet, while the distinction between discovery and justification has received increased scrutiny and criticism, the consequences of such longstanding heritage of oversight remain to be undone.

What can we know about the reasoning that makes scientific discoveries possible? Moreover, what may such analysis reveal about the philosophical treatment of science? The road

to discovery appears strongly anecdotal and erratic for scientists and philosophers alike. There does not seem to be a general gameplan to derive proper guidelines or consistent rules for successful scientific discovery.

Focus on generalizable rules in the logic of discovery has overshadowed other elements that deserve notice. A central element is the idiosyncrasy that makes scientific discoveries so challenging to study in a general form: their individual context. In a series of essays, Root-Bernstein (1988, 1989) thus highlighted three elements, namely, the historical, the social, and the *individual*. Such focus on the individual discoverer is of greater interest for analysis in current science studies.

Is there more to an individual scientist's story than their biographical record and personal touch in the event of discovery? Can we subject the individual's reasoning to an epistemic treatment, perhaps to better understand exploratory strategies in action?

Scientific biographies pique the imagination, especially when explorers renegade against the ossified mainstream. Sometimes, such stories are misleading (Barwich 2018). At other times, these stories reveal something about the endeavor of science itself. They illustrate how much scientific reasoning is pluralistic also at its *personal* level—with individual viewpoints and disciplinary backgrounds, styles of reasoning, and strategizing.

A case in point is Barbara McClintock. Fox-Keller's *A Feeling for the Organism* (1983) shone a spotlight on 1983 Nobel Prize laureate Barbara McClintock, centering McClintock's personality in her intellectual journey solving a complex scientific problem. McClintock theorized about the general genetic principles driving plant evolution through her apperception of physical idiosyncrasies. She was looking for evidence for jumping genes by closely observing corn. Notably, Fox-Keller's analysis went beyond documentation of how McClintock's ideas were met by rejection based on sexist bias in the scientific community. In addition to positioning the individual scientist as representative of broader social structures in science, Fox-Keller's detailed analysis of McClintock also opened an epistemic viewpoint. She situated McClintock's particular reasoning style of trained perception in a broader epistemic context of methodological observation. Why and how does McClintock's particular strategy of exploration matter in the context of discovery?

Individual reasoning in discovery looks contingent, if not accidental, and lacks consistent comparison and evaluation criteria. Moreover, we think of scientific discoveries as showing us

something about the objectively real world, objective meaning intelligible independently of the individual who discovered it. Scientific achievements, such as the revelation of the DNA double helix, are bound to happen at some point in time (regardless of Watson and Crick's personalities or any other scientist involved – like Franklin, for that matter). It is harder to imagine that Beethoven's ninth symphony would exist if it were not for its particular creator. Besides, the reasons that guided a scientist to their finding may not bear directly on the things found. Many events resulted from sheer serendipity. Other ideas have obscure origins. Otto Loewi referenced a dream for his inspiration to conduct an ingenious experiment to test for the presence of neurotransmitters in synaptic transmission (Firestein 2015). Thus, the general attitude is that whatever circumstances have influenced scientists' reasoning, their results could have come about by any other means after all—could they not?

Root-Bernstein challenged this sentiment: "For one of the basic flaws in most logic-oriented accounts of discovering is a hidden assumption that anyone in the same position as the discoverer would have seen the same thing and drawn the same conclusions. (…) Thus, the discoverer is often left out of logical accounts of discovery, or is portrayed in such a way that any scientist could replace him" or her (1989, 478). However, why did *this* scientist 'see' something or did something at the time that others did not? What kinds of theorizing, strategies, interests, convictions, and attitudes converged that were mirrored in the experimental design? Root-Bernstein explicitly encouraged the pursuit of this viewpoint by asking: "Why not admit that discoveries derive from the ways in which particular scientists logically go about their work?" (1988, 29)

Scientists enact their thinking through their experimental design. To analyze the individual strategies that elevate some discovery strategies over others, facilitating a better fit between a discovery method and an experimental puzzle, a close-up look at the specific choices in using methods or discovery tools proves beneficial.

Here, Linda Buck and her search for the ORs take center stage. Several laboratories had been hunting for the OR genes without victory. A close examination of Buck's experimental design reveals why her particular strategy was a most unlikely choice at the time. However, it looks evident in hindsight.

While unconventional, her strategy was all but arbitrary.

4. Linda Buck and the Olfactory Receptors

The question driving Buck to embark on the search of the olfactory receptors was: "How could the olfactory system detect such an enormous diversity of chemicals?" (Buck 2004, 116) Today, the estimate is that the human olfactory system can discriminate about 1 trillion odor stimuli (Bushdid et al. 2014) with about 400 receptors in humans (even more in other animals, e.g., 1000 receptors in mice). In comparison, the largest known protein gene family before the olfactory receptors had been serotonin with a more moderate number of 12 members (today, their known number is 15).

The OR discovery split olfaction into two ontological stages: pre-receptor modeling centered on the chemical stimulus and post-receptor modeling aimed at the sensory system. Olfaction indeed changed so much in its modeling outlook that its research questions, evaluation of empirical data, and disciplinary objectives became notably incompatible (Barwich 2015b, 2018, 2020a). The ORs finally handed modern research on the molecular basis of smelling the keys to the brain. However, in the late 1980s, when Buck embarked on discovering these genes, olfaction was not a widely popular field. It did not promise to yield rewards in terms of awards, accolades, or funding (Barwich 2020a, 2020b). Thus, Buck's interest involved a significant risk with little expected value at the time, and her success did not come easy.

"When I first came into the field, olfaction was way off to the side," the Yale neuroscientist Gordon Shepherd remembered (quoted in Barwich 2020a,b). Shepherd was an exception in that he had highlighted the significance of smell for general studies of sensory processing early on. In the 1970s, Shepherd (Sharp, Kauer, and Shepherd 1975) looked at stimulus activation patterns in the olfactory bulb (a spherical neural structure in the brain's inferior frontal lobe). However, to model how the chemical stimulus was encoded into neural patterns required insight into the receptors and their binding repertoire (Shepherd 1991). Once discovered, the receptors would change the field. Shepherd's prognosis was right. Indeed, his expectations were exceeded.

While a niche interest at the time, concentrated efforts began targeting this family in the late 1980s and, with the advance of genetic tools, the discovery seemed close enough. Buck competed with a small number of other laboratories in her hunt for the OR genes. The molecular

biologist Randy Reed at Johns Hopkins recalled (quoted in Barwich 2020b): “By, whenever it was, in 1988, ’89, ’90, at least three labs, our lab, Parmentier’s lab in Belgium, and Richard’s lab with Linda, all were using, essentially, identical molecular cloning tricks. Clever little tricks.” (Others included Doron Lancet in Shepherd’s lab.)

The OR discovery seems like part of a genealogical series of studies on cell signaling and molecular detection from a broader perspective. The theoretical understanding of cell signaling mechanisms advanced in parallel with fundamental technological innovations. That is one way to tell the OR discovery story. However, it would miss a crucial part. Notably, all of the competing labs used the same experimental techniques and worked with the same theoretical assumptions about the ORs. Reed emphasized: “We all knew what those criteria [for olfactory receptors] were. It should be a family. They should be highly expressed in olfactory tissue. They should be relatively specific to olfactory tissue.” By the end of the 1980s, evidence suggested that the olfactory receptors may be part of a larger family of GPCRs.

Nothing happened, however, for several years. The standard methods did not yield any results. For a minor area like the olfactory research community, this could have been a death blow. “In my laboratory, I couldn't get anybody interested in the project,” Reed noted in personal communication.[4] Graduate students avoided the issue since no one seemed to get lucky, and the lack of results would spell a quick end to their aspirations. “We had essentially let the problem drop,” Reed described a growing frustration in the field.

“If you think about what happened if Linda just said: ‘I give up.’”

4.1 Historical Background: Evidence for a new GPCR family

The molecular machinery behind odor detection long remained a mystery (Barwich 2015, 2018, 2020a). The basic model of olfactory signal transduction[5] was established only a few years before the receptor gene discovery (Figure 1). Early electrophysiological work by Adrian (1953) and Gesteland, Lettvin, and Pitts (1965) started by measuring and individuating the responses of

[4] Randall Reed, personal communication, interview recorded via Skype, 04/26/2018.

[5] Signal transduction is a process in which external information (such as the chemical information from an extracellular airborne molecule in the environment) is transformed into electrical signals (by activating cell membranes through binding to appropriate receptors).

olfactory sensory nerves to a variety of odorants (i.e., the olfactory stimulus: volatile airborne molecules). Their work formed the foundation for understanding the olfactory mechanism as a signaling process based on changing membrane potential.

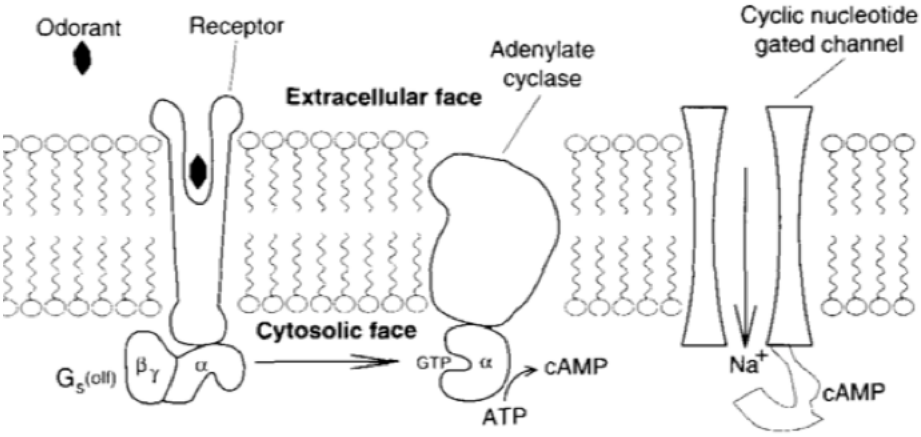

Figure 1 (Buck and Axel 1991, 176): *Olfactory signal transduction in the transmembrane domain of the cilia in the nasal epithelium. Odor recognition starts with the excitation of the olfactory sensory neurons, those cells in the nasal epithelium whose surface cilia are covered with transmembrane olfactory receptors. When an odorant binds to a suitable receptor (extracellular), the coupled G-protein subunit Golfα (intracellular) becomes activated and decoupled. In its inactive state Golfα binds GDP (Guanosine diphosphate), which gets converted into GTP (Guanosine triphosphate) when activated. The conversion into GTP stimulates adenylate cyclase, which results in the formation of cAMP (cyclic Adenosine monophosphate) from ATP (Adenosine triphosphate). This, in turn, causes a change in the membrane potential, i.e., leads to a difference of electric charge between the inner and outer membrane environment. This signal is carried to the so-called olfactory bulb (an area situated at the brain's frontal lobe) through the olfactory sensory neurons' axons.*

Two discoveries in the 1980s were vital in embedding research on smell into ongoing developments in molecular biology. These discoveries suggested that olfaction operated by a

second messenger pathway, just like any other signaling process (an assumption that was not necessarily common at the time).

First, cell-signaling mechanisms involving changes in membrane potentials suggested the presence of active adenylate cyclase, a regulatory enzyme. “It was reported about 1970 that there was a lot of cyclic AMP in the olfactory epithelium,” Buck said. Pace et al. (1985) experimentally established the fact of such reporting adenylate cyclase activity after measuring higher levels of cAMP (cyclic adenosine monophosphate, an important second messenger molecule in cell signaling responses) in olfactory stimulation with a series of odorants. In addition to this biochemical test, it was later confirmed physiologically (Firestein, Darrow, and Shepherd 1991).

Second, second messenger mechanisms with an active adenylate cyclase were known from other signaling processes mediated by a G-protein (the G stands for GDP-binding). Jones and Reed (1989) analyzed the messenger RNA in the olfactory epithelium, searching for such a protein’s amino acid sequences. They identified a hitherto undescribed G-protein subunit by comparing epithelium RNA with sequences of a known G-protein subunit (Gsα, coupled, for example, to ß-adrenergic receptors). This unit, Golfα, exhibited an extraordinary overall similarity of shared amino acid sequences with Gsα (88%), but it also had sequences that were only expressed in the olfactory sensory neurons. Golfα was linked further to the stimulation of cAMP.

The discovery of a G-protein subunit now provided an indicator for the presence of an olfactory GPCR. Reed highlighted the influence of these new molecular tools, their success in research on other GPCR signaling (in vision and adrenergic responses), and how their application in olfaction just felt naturally like the next step:

> “The ability to use biochemistry and pharmacology to get to receptors led to the cloning of those receptors and recognizing that the receptors for detecting adrenergic stimuli looked very much like and used similar systems as vision. I clearly remember this seminar from Jim Hurley, who was interested in vision. And [another seminar happened] shortly after I got to Hopkins that described the isolation and purification cloning of transducin. That’s the G-protein in vision. I remember walking out of that seminar saying: ‘That’s how olfaction works. It’s got to be the same!’”

These developments provided the backdrop against which Buck and Axel (1991) framed their modeling strategy. Everyone seemed to be playing by the same rules and with the same tools. Still, no one found these genes for years. What distinguished Buck's modeling strategy from that of her peers? Buck worked with the same tool as everyone else: the newly invented method of PCR. But Buck devised a new way to manipulate the material conditions of PCR to do something it had not been originally designed to do. She made the method fit the materials by tailoring its capacities to her theoretical model of the unknown ORs.

Scientific discoveries often hinge on the application of new tools and methodological ideas. In Buck's own words (quoted from Barwich 2020b), technology is not merely a way to test hypotheses derived from more or less well-defined data. Technology embodies an opportunity for exploration

> "that could allow you to 'see' things. [Seeing] then leads the questions. I think that often happens in science. You have a new technology that allows you to look at things, see things. Now you see things that you didn't imagine, and then you try to figure out what those things are that you're looking at. And that leads to new discoveries that were totally unanticipated."

4.2 Parts Unknown: Turning PCR into a Tool for Exploration

A critical key to discovery is knowing that whatever is found *is* something in particular. What if someone else had discovered the receptors? Unbeknownst to Buck and Axel, another lab (not working on olfaction) indeed had found a group of genes that looked like an unknown protein receptor gene family, potentially GPCRs. Later, when the ORs were identified, these genes turned out to be part of the olfactory receptor family (Parmentier et al. 1992). The Parmentier group had also been looking for the ORs as part of the GPCR family because it was a lucrative topic "to find new G-protein coupled receptors," Reed explained, especially "new therapeutically important G-protein coupled receptors." Parmentier's sequences were found in the testes. Thus, these sequences were considered to be of potential interest to contraception studies. However, not much attention was paid to their specific nature and family membership at this time. They did not look like *olfactory* GPCRs.

Moreover, the nature of GPCRs—including their size and amino acid sequences—remained undecided. GPCRs were a markedly new entity in the field of biochemistry (Barwich and Bschir 2017). “There was only one GPCR known at the time,” Buck confirmed. “That was Opsin [in vision]. Maybe the beta-adrenergic receptor was published then, but if so, that was only two receptors. (…) So there weren't many [GPCRs] known at the time.” Since knowledge of GPCRs was underdetermined, it was also unsettled by what features the ORs would be identifiable as GPCRs.

Besides, the ORs may not have been GPCRs. Experimental reports were discordant at the time, suggesting a few other alternatives: “odorants were also reported to directly open ion channels in olfactory cilia, suggesting that, like many neurotransmitter receptors, odorant receptors might be ligand-gated ion channels (…).” Alternatively, “odorants were reported to depolarize other cell types and to even alter the membrane potential of artificial liposomes”; thus, “for Buck (2004, 116), “it was not at all clear what kind of proteins the odorant receptors were or, for that matter, whether they even existed.”

“Nothing was expected,” Buck emphasized in personal communication. She thus explored several methods, screening and cloning away, to no avail (Buck 2004, 116):

> “I first tried an unconventional approach in which I replica screened an olfactory cDNA library with large amounts of 32p-labeled genomic DNA or brain cDNA. [… Then,] I also tried a cDNA subtraction approach to identify genes selectively expressed in olfactory sensory neurons and, in addition, tried to develop a way of cloning genes that were related but not identical. These efforts yielded some genes that appeared to be specifically expressed in olfactory sensory neurons, but none belonged to a family, so I set them aside.”

The breakthrough arrived with the new genetic tool of Polymerase Chain Reaction (PCR). Today, the invention of PCR by Kary Mullis, 1993 Nobel laureate in Chemistry, and the discoveries that followed its achievement, are praised as "highly original and significant, virtually dividing biology into the two epochs before. P.C.R. and after P.C.R. (Wade 1998)." Yet PCR was reasonably new when Buck started searching for the receptors (Saiki et al. 1985, Saiki et al. 1988).

Buck immediately sensed the possibilities of this tool:

> “When the PCR papers came out, I was thrilled. Because I thought that PCR would open up the door to many things. I mean, it would just be a miracle. (...) It would open the way

to doing many different things. (...) Spectacular. Just think of the microscope. What the first microscope allowed people to do; they could look, they could see things. To me, it is all about being able to see things!"

PCR is a method based on the natural process of DNA replication (Figure 2). DNA replication involves an enzyme (polymerase) duplicating DNA strands that are targeted by primer pairs. Primers are short sequences of nucleotides that bind to specific genome sequences in a complementary fashion. This procedure can be replicated exponentially through repeated reaction cycles, producing vast amounts of specific gene strands. The obvious advantage in the invention of this method is that it solved the scarcity of genetic material (for the history of PCR, see Rabinow 2011).

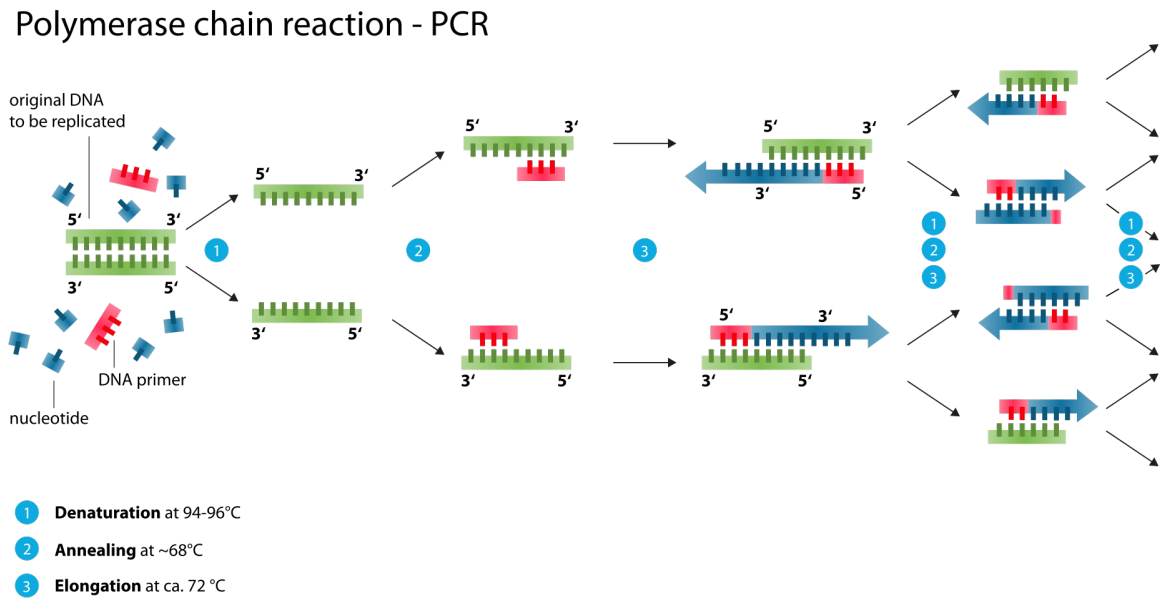

Figure 2 (image from Enzoklop 2014): *Basic principle of PCR. Two primers (short sequences of nucleotides; red) are designed to bind to specific genome regions that one wants to amplify. When primers do not bind to genome regions, they will not amplify them.*

Buck was not the only one using PCR to search for the olfactory receptors. The GPCR sequences in the testicles mentioned above were also found just this way. So what made the critical difference?

PCR did not seem the most suitable tool for *new* genetic discoveries. Based on a copy and paste mechanism, it was designed to be an experimental method that amplifies known materials, not to find unknown ones. To amplify particular genome regions, one already had to be familiar with their characteristic nucleotide sequences. *The* precondition of mapping PCR primers is that those parts of the genome sequences one wants to amplify are already established. The tricky part of doing PCR is the primer design, targeting these specific genome domains. Buck first followed the standard procedure: testing the few known primer pairs for GPCRs. These pairs *should* have yielded at least some olfactory receptor genes if they belong to this larger GPCR family and share specific amino acid sequences. However, they did not.

Buck's tests only yielded a known dopamine receptor (Buck 2004, S117-8). The absence of identifiable ORs meant one of two things: ORs may not be GPCRs. Alternatively, ORs may present an entirely new family of GPCRs with sequences not yet linked to the known GPCRs. But how to catch such unknown sequences?

Here, Buck tried something hitherto unconceived. She used the material restrictions of PCR as a targeted searchlight by using two modifications of PCR in tandem: degenerate primers and reverse transcriptase PCR. Recognizing the brilliance of this strategy requires some background on the necessary technical details.

Primers in PCR are called degenerate when some positions of their sequences have more than one possible base: "for example, in the primer GG(CG)A(CTG)A the third position is C or G and the fifth is C, T or G" (Linhart and Shamir 2002). The degeneracy of a primer describes the number of its unique sequence combinations (6 in the example cited). Degenerate primers, therefore, are less specific and allow for amplifications of related yet heterogeneous genetic sequences. The design of primer degeneracy fundamentally shapes the success of the application: the degeneracy of a primer can easily be too high, therefore lacking domain specificity that results in the amplification of unrelated sequences, or it can be too low, thereby requiring a lot of sequencing and manual labor (Koelle 1996). Primer design in PCR was a notoriously laborious and challenging task. Even five years after Buck's breakthrough, the use of degenerate primers was called delicate: "The identification of novel members of gene families by PCR using degenerate primers has been considered more of an art than a science, so much that the method books I've come across have been too timid to discuss the considerations that go into the design of this experiment, much less give a protocol for its execution." (Koelle 1996)

Meanwhile, degenerate primers in PCR were used successfully to find new members of an already known GPCR family. However, the crucial difference here was that part of the sequences was already established (Libert et al. 1989). That was not the case for Buck's target after she realized that the olfactory receptors potentially constituted an entirely *new family*, which meant no sequences were established and, thus, no template for degenerate primers was at hand.

Buck's idea to use degenerate primers to find an entirely new family with no known sequences may have sounded like a punt than systematic experimental planning to some of her peers. Understanding how gene sequences related to a protein family is essential to the design of primers. The concrete sequences were unknown, and it was also unclear just how big the olfactory family might be and how diverse its members are. After the failure of using the published GPCR primer pair, it was not certain whether olfactory receptors were GPCRs for sure. Suppose they were, how then did the olfactory sequences relate to already known members of GPCRs which, as mentioned, were young entities in the inventory of molecular biological research at the time?[6]

In a way, degenerate primers in PCR appeared as a most unsuitable and unlikely discovery tool to pick. It became evident only later: "[t]he simplicity of recognizing how the application would work is like hiding in plain sight" (Firestein, Greer, and Mombaerts 2014, 177). In the end, the success of Buck's program hinged on an unorthodox yet strategic combination of two variations of PCR: Buck's design of degenerate primers and her choice of using RNA instead of DNA.

4.3 Merging the Method with the Materials

Buck's experimental plan comprised three major steps:

[6] The existence of cell surface receptors as a molecular gateway in cell signaling was doubted deep into the 1970s. Crucial for their wider acceptance was the pioneering work of Robert Lefkowitz on the adrenalin receptors in the mid- and late 1980s (Barwich and Bschir 2017). By sequencing ß-adrenergic receptors Lefkowitz showed that these proteins were part of a much larger family of cell surface receptors, together with rhodopsin and the nicotine receptors (Lefkowitz 2013). Lefkowitz received the Nobel Prize in Chemistry for his work on GPCRs belatedly in 2012.

1. Primer design to amplify sequences.
2. Reverse transcriptase PCR (RT-PCR) to identify specific sequences.
3. Northern blot to confirm whether these sequences were tissue specific.

Step 1: Fishing for genes (primer design)

Buck's degenerate primer design was, in a sense, both theory-driven and exploratory. She later reported how her previous interest in gene diversification mechanisms in the immune system informed her primer design strategy. The immune system responds to a wide array of structurally diverse pathogens. The olfactory system likewise was known to respond to a wide variety of structurally highly diverse volatiles. Here, Buck's primer design constituted an experimental expression of her theorizing about genetic recombination in the olfactory system:

> "My background was in immunology and I had also been trying to develop a method to identify rearranged genes in the mammalian nervous system, the idea being that such genes might provide insight into its cellular and connectional diversity. I was intrigued by the possibility that gene rearrangement or gene conversion might be involved in the generation of a varied set of odorant receptors or regulate their expression, as with antigen receptors in the immune system. (…) At that point, I decided to conduct an exhaustive search for GPCRs in the olfactory epithelium by using a number of different degenerate primers in a *combinatorial fashion*. (…) The idea was that *different parts of an olfactory receptor GPCR might be related to different non-olfactory GPCRs*." (Buck 2004, S117-8, emphasis added)

Buck considered that the ORs might not all share a set of sequences with all other GPCRs (as expected more generally). Some ORs, she assumed, may share some of these sequences with some other GPCRs (while other ORs share other sequences with other GPCRs). A Wittgensteinian mosaic of genetic resemblance, not similarity sets.

Buck designed eleven degenerate pairs in a combinatorial pattern using parts of the known GPCRs sequences to capture potentially related non-GPCR patterns. "For the degenerate primers, I collected all those sequences of the known ones [GPCRs], which was a very limited number, and aligned them by hand. And then design degenerate primers that give you combinations, which have the capability of amplifying up *any* of those GPCRs." Moreover, she

went the extra mile. "When it came to the GPCRs and the general primers, I thought, 'Okay. There are different GPCRs known… maybe they're GPCRs, but maybe they are some other kind of [other] receptor, maybe the nuclear type receptors.' So I actually designed the general primers not only for GPCRs but also for the nuclear receptor family."

Just how would Buck know whether she caught the right genes?

Step 2: Identifying the catch (using RNA instead of DNA)

Buck tested the primers with reverse transcriptase PCR (RT-PCR) on tissues isolated from the rat olfactory epithelium. That constituted the second ingenious twist in Buck's experimental set-up. RT-PCR is another variant of PCR. Instead of DNA, it traces RNA expression. RNA sequencing allows for tracking down tissue-specific expression characteristics in protein coding. ORs should be highly expressed in olfactory epithelial cells, which would shine a spotlight on ORs instead of other members of the GPCR superfamily.

RT-PCR gave Buck 64 matching cDNA bands with GPCR sequences. Which one contained the OR sequences? ORs were assumed to exhibit a high degree of variation based on their binding capacity to structurally highly diverse ligands. It stood to reason that the olfactory receptor family might consist of a heterogeneous group of genes. Thus, Buck was looking for a band with multiple genes.[7]

To find such a multigene family, Buck cut all 64 cDNA bands into fragments with restriction enzymes. Here, using epithelial RNA revealed itself as a keystroke of genius in Buck's design: PCR applications with genomic DNA only yielded gene families in equimolar amounts. With RNA, however, Buck could compare the molecular weight of different bands. The trick was to find a band where the fragments' molecular weight was larger than the uncut band (Malnic et al., 2010), revealing a multigene family. Bands containing only one gene would show fragments where their molecular weight matched the original band's weight. However, bands containing multiple genes would show fragments with a molecular weight higher than the weight than the original band (Figure 3).

[7] The term "band" refers to the products of electrophoresis. Here, you load the genetic material into a gel and run a current through the gel— "running a gel" —to see the materials separating and "wandering down" based on their molar weight. The separated generic fragments are called "bands."

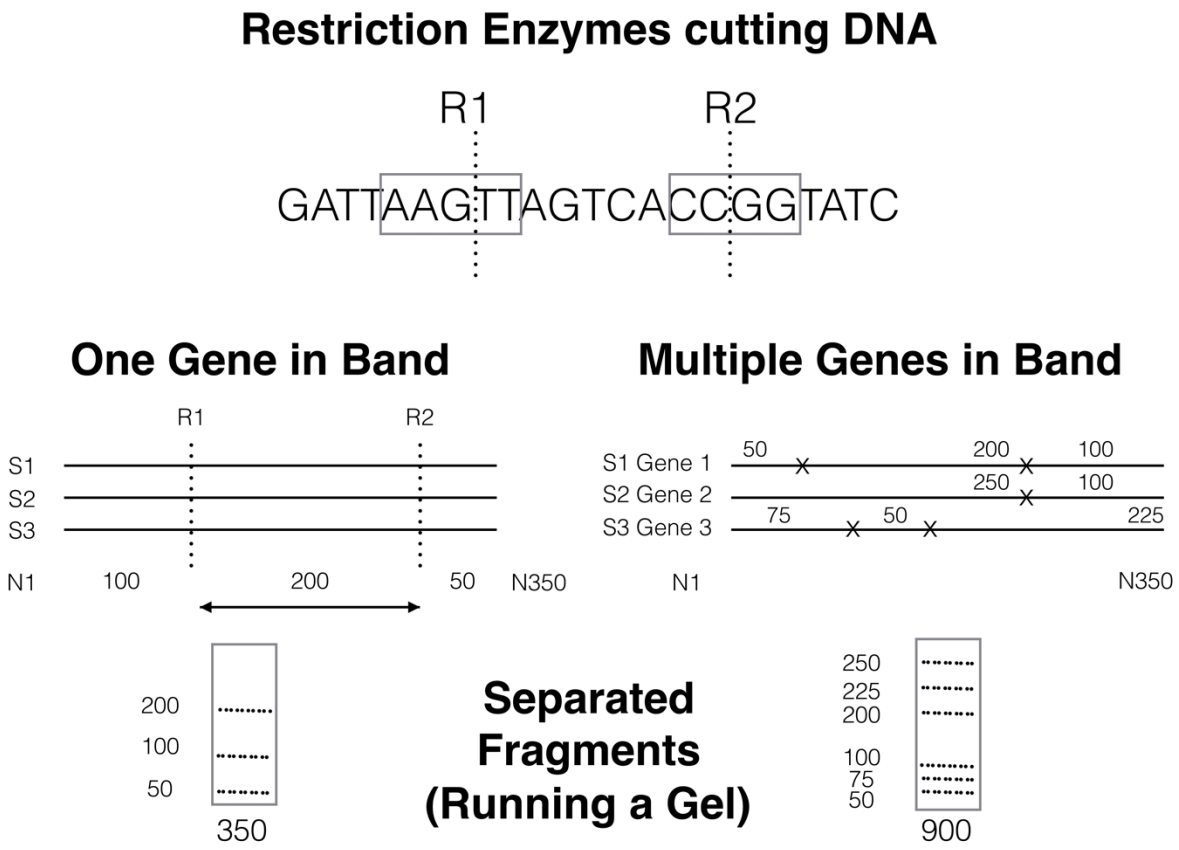

Figure 3 (image adapted from Barwich 2020b): *Restriction enzymes target and cut specific sequence regions. Top: Schema of two restriction enzymes (R1, R2) targeting particular nucleotide sequences and cutting amplified DNA bands into fragments. R1 (targeting the sequence AAGTT, cutting between AAG and TT) and R2 (targeting the sequence CCGG, cutting between CC and GG). Middle left: representation of band containing one gene (R1 and R2 cutting amplified nucleotide strands, S1-S3, at the same locations, resulting in equal-sized fragments adding up to the same size as the original band). Middle right: representation of band containing multiple genes (R1 and R2 cutting amplified nucleotide strands, S1-S3, at different nucleotide locations, resulting in fragments of different sizes). Bottom left: after running a gel, separated fragments add up to the size of the entire band, here: 50+100+200=350 (containing only one gene). Bottom right: after running a gel, separated fragments add up to more than the*

original band size, here: 50+75+100+200+225+250=900 (containing more than one but multiple genes).

Buck found that one band matched this set-up. Moreover, it stood out: lane 13 (Figure 4) “almost shouts the finding” (Firestein, Greer, and Mombaerts 2014, 177).

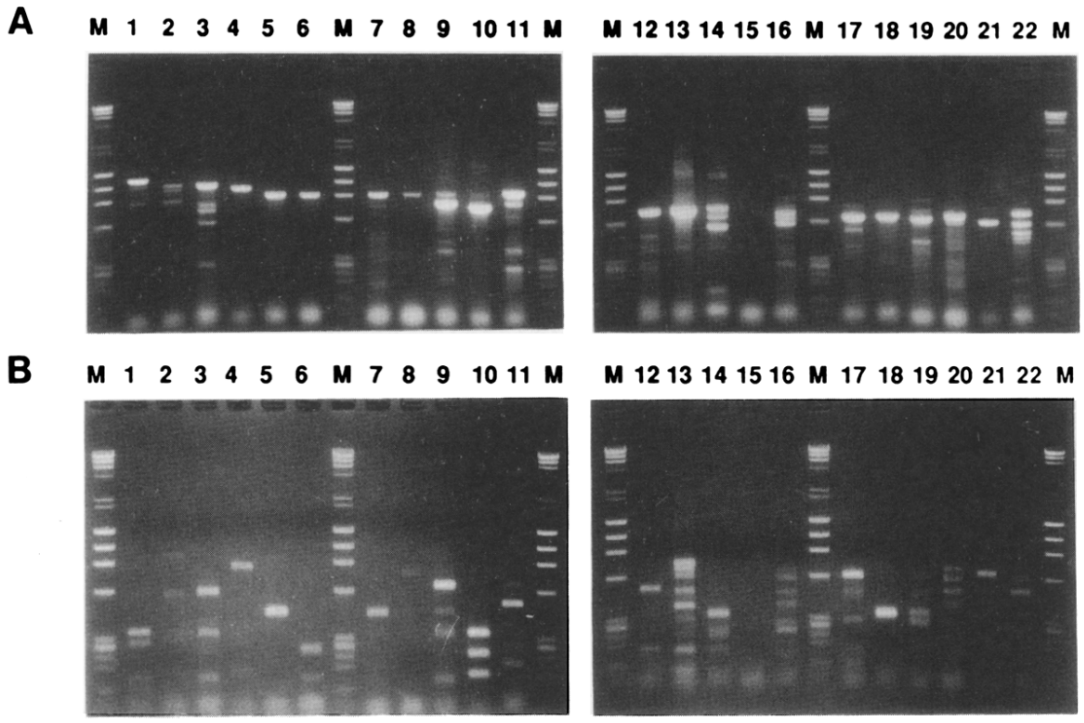

Figure 4 (image from Buck and Axel 1991, 177): *The lanes (1-22) represent fragments of amplified cDNA bands from the rat’s epithelium tissue. Lanes labeled "M" are marker lanes, meaning they provide references of known genes for comparison. There is a significant difference in lane 13 (B) because the molecular weight of lane 13 is much greater than the original cDNA band size, suggesting multiple genes being present. Compare the congregated bulk of fragments on top of lane 13, for example, to lanes 7, 12, or 21.*

Exceeding all expectations, the member size of this novel multigene family turned out to be huge. Buck and Axel discovered a GPCR family with over 500 genes (in mice), with one gene coding for one receptor type. (Today, the number of known olfactory receptor genes in mice

exceeds 1000.) To put this in perspective, before their discovery, estimations for the number of olfactory receptors was around 30-50.

Step 3: Double-checking the results (Northern Blot)

In a third and concluding step, Buck tested her new GPCR sequences in lane 13 for a family relation. She tested whether the same primer pairs can amplify these large fragments. She cloned the band and sequenced all its fragments to find that they exhibited significant similarities with the few known GPCR sequences despite a great diversity of these structures. Making sure these GPCRs are essentially olfactory (instead of some other) GPCR, Buck conducted a Northern blot (studying gene expression by isolating RNA) to compare the expression of the genetic material extrapolated from the epithelium with the genetic material of other tissues (e.g., brain, retina, liver), where those sequences were not detected.

Technically, the story of the receptor discovery did not end here. A less-known fact is that conclusive proof for these genes' functional identity as *olfactory* GPCR was obtained nearly a decade later by Stuart Firestein and his student Haiqing Zhao at Columbia. Zhao et al. (1998) tested several odorants on an isolated odor receptor, the rat OR-I7 (details in Barwich 2020a, Ch. 2).

Still, in 1991, the nature of these new genes left hardly any doubt about their identity.

4.4 The Impact of the Discovery

The results spread like wildfire in the olfactory community. Reed remembered: “Linda probably immediately knew it’s what she was looking for, and as soon as I read that paper, or I heard what the criteria were, it was clear that was it.”

The OR discovery was formative of the field, and its timing mattered. Reed stressed: “The greatest danger I thought to the field was that we would have gone another decade without finding receptors and people gave up. Right?” Ultimately, the OR genes opened the field of olfaction to mainstream science and funding. Shepherd responded: “That you had the biggest family in the genome really made it very attractive. We went from being kind of just a smaller

field struggling to maintain ourselves just in terms of funding to a field in which we are now a part of the mainstream. So that was very important."

Olfaction has advanced significantly as a rising model system for molecular biology and neuroscience (Barwich 2020a). With the discovery of the receptors, it now was possible to trace the olfactory signal further to understand its implementation in higher brain processing, exploring the organizational principles of odor activation in the olfactory bulb (at the frontal lobe) and the olfactory cortex.

To date, these developments are still in active progression (Kurian et al. 2021).

5. Why Idiosyncrasy in Exploration matters

The story of Linda Buck and the OR discovery highlights two things. On the one hand, it shows the importance of support for exploration-driven research in science. On the other hand, it illustrates that the individual scientist is not a replaceable element in epistemic evaluations of exploratory reasoning in scientific discovery. These two considerations can be tied together, as both carry similar implications for the impact of science studies on the language of science in funding and education contexts.

Could anyone else have done what Buck did? Traditional ideas about scientific discovery, focusing on the impact of technology in the justification of results, suggest that the discoverer constitutes mainly a biographical or narrative element, not an *epistemic* factor in philosophical analysis. Contemporaries in the philosophy of science have foregrounded various epistemic factors fostering discoveries in terms of broader communal efforts (e.g., Clarke 2017) in light of previous work on conceptual and social changes in science (Fleck 1935; Kuhn 1962). Integrating the individual researchers with their idiosyncratic pathways that led to their discoveries into philosophical analysis about knowledge production in science in this context adds something crucial for understanding the reality of scientific practice. To be sure, focus on the individual does not imply to conclude that *only* one individual could have produced a specific discovery or recognized the findings as what they are. Rather, it highlights the historical fact that discoveries cannot be understood solely as communal achievements, which simplify scientific reality (and turn it into an image it is not).

Feyerabend also pointed this out in *Against Method* (2010[1975], 3): "Now it is, of course, possible to simplify the medium in which a scientist works by simplifying its main actors." Feyerabend talked about the danger of eliminating the pluralism of ideas and approaches that each person embodies from their education. Still, a similar concern about pluralism applies to the philosophical analysis of a scientist's work and experimental reasoning.

Caution against the simplification of the medium in which scientists work also involves their individual epistemic space as experimenters, shaped by a variety of factors. Scientists show significant perspective variations based on their general training in parallel with their personal interests and background. Therefore, reintegrating the individual scientist in philosophical discovery narratives can serve as a valuable (and irreplaceable) philosophical tool that helps to probe the scope of alternative reasoning and observation required in exploring and teaching to examine scientific puzzles.

In support of this claim, I explicated how exploratory thinking in discovery analysis can be specified by analyzing choices in experimental design that were not accounted for by the general historical and social context. The question was how to make something theoretically conceived visible with an experimental set-up and further identify its appearance (which may diverge from assumption)—in Buck's case, a genetically diverse family with a large membership. Buck's 'feeling for the method' in her use of PCR indeed reminds Fox-Keller's (1983) portrayal of McClintock. Buck had an intuitive grasp of the material conditions of PCR and its potential to reorganize the materials in its application.

This soon was evident to me in my analysis of Buck's experimental work. However, I also was faced with the limits of current conceptual tools to analyze exploration research, even the tools of recent science studies. Part of my lessons drawn from the present analysis thus concern a conceptually underdetermined philosophical framework for discovery studies.

The discoverer might be detachable from the justification of discovery results in a general epistemic sense. Yet she is not replaceable for understanding the epistemic uncertainties, the scope of options, and the design of testable alternatives in exploration-driven research. These latter factors are vital to analyzing and defining exploratory research and discovery contexts, including their epistemological foundation.

Easier said than done, as analysis of exploratory-driven research suffers from an absence of precise terminology and methodological standards. "Exploratory experimentation" emerged as

a term in recent philosophical and historical studies of science with one chief and understudied caveat: "Initially designed to debunk philosophical ideas about theory testing, the concept 'exploratory experimentation' quickly exposed the poverty of our conceptual tools for the analysis of experimental practice" (Schickore 2016, 20).

Such remaining conceptual poverty for analyzing exploratory research in science studies matters beyond philosophical disputes about the nature of science. Our conceptual tools in the meta-analysis of science, or the lack thereof, also shape evaluations of experimental practices in official scientific contexts, such as education and funding policies. A look at the guidelines of big funding agencies, including the United States' National Institute of Health (NIH) and National Science Foundation (NSF), show a robust normative tilt toward hypothesis-driven research as best practice (Madsen 2007). By contrast, exploration-driven research is regarded as merely preliminary and insufficient in its methodological rigor and epistemic standing, as, Elliot, and Burian (2009) also observed:

> "A number of scientists and philosophers have argued that the best science is hypothesis driven and that science's pivotal activity is to test hypotheses. From this perspective, descriptive, exploratory, and inductive methodologies, although sometimes necessary, are fundamentally preparatory."

Exploratory methodologies are more than merely preparatory, however. They carry the potential of being revisionary for the development of a field, including its experimental outlook. Buck's experimental history demonstrates that curiosity-driven research is not arbitrary or accidental. Strategic exploration is not devoid of testable parameters and references to general theories and established models against which its success is measured.

6. Outlook: Toward Cognitive Theories of Observation in Scientific Practice

Exploration essentially builds on the notion of *observation* and *trained intuition*. Scientific intuition involves "informal patterns of expectation born of experience" (Meyers 1995, 757). Such patterns constitute a form of 'tacit knowledge' in Polanyi's (2015 [1958]) sense. Scientific training distinctively shapes observational abilities, focus, and inferential procedure (Daston 2008). Naturally, such tacit knowledge blends with researchers' individual backgrounds and

other skills, including their reasoning strategies. For example, Buck's theorizing embraced a blend of several ingredients: personal, educational, and epistemological. On a personal level, Buck later linked her style of scientific reasoning and interest in mechanisms of recombination puzzles to her upbringing in her Nobel biography:

> "My mother was a homemaker who was exceptionally kind and witty and loved word puzzles. My father was an electrical engineer who, at home, spent much of his time inventing things and building them in our basement. It may be that my parents' interest in puzzles and inventions planted the seeds for my future affinity for science, but I never imagined as a child that I would someday be a scientist" (Buck 2004b).

Traditional philosophers of science may distrust the introduction of psychologisms. However, we have sufficient reason to integrate epistemic-psychological elements into philosophical analysis of scientific practice. Skilled observation is not theory-neutral and strongly shaped by epistemic context. This context includes an individual's background with their specific research training and trajectory, cross-disciplinary pollinations, and even non-scientific activities such as art.

> "Observation is theory directed. We all know this, yet we fail to take the crucial step of realizing that every individual has a different set of theories in their head and a different personality. They will therefore apply what they know and perceive what they see in different ways" (Root-Bernstein 1989, 478).

Philosophical work needs an adequate *psychologically informed theory of observation* to counter the conceptual poverty of exploratory practice in science. Weaving this thought into the conclusions of my discovery analysis serves as an invitation for further philosophical work. To be sure, the idea to adopt cognitive theories for philosophical analysis of scientific developments is not entirely new. It was previously undertaken, for instance, by Nersessian (1992, 2002), in her work using cognitive science to understand concept development and mode-based reasoning in science. It was also advanced by Thagard (2014), who argued for the inclusion of current cognitive theories in philosophical arguments on theorizing in science. Lastly, Paul Churchland (1995, 2013) proposed a contextual theory of meaning based on modern ideas of connectionism in cognitive science—and how such perceptual theory also applies to scientific reasoning. Observation in science, Churchland argues, must be understood via the acquisition of perceptual and cognitive prototypes, including the underlying (re-)learning mechanisms.

Such cognitive theories of scientific practice have made great progress in targeting general cognitive strategies in science. But I think such theories now also promise to provide new grounds for revisiting the basis of reasoning that also underlies the still underexplored factor of *individual variation* in science. Focus on scientists' idiosyncratic reasoning strategies, the story of Linda Buck has shown, is critical to understand the specific impact of exploratory reasoning behind some discoveries that may not be visible from a broader perspective on communal developments in a field.

So this article must end where future work in philosophy must pick up.

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