Shaped by History

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EXCERPT

Systematics, the study of the evolutionary history of life on Earth, is arguably a foundational subdiscipline in the biological sciences. But as the scientific process has matured, systematists have been forced to grapple with an important question: is what we do really science? In this lecture, I address that question by providing a brief history of the field, beginning with Linnaeus' system of nomenclature, continuing to Darwin's contributions of evolutionary thinking to the field, and concluding with Hennig's legacy of advocating for objective methods for hypothesis testing. This history of the field reveals the distinctive challenges systematists face in attempting to reconstruct events of the past, a task not unfamiliar to many humanities scholars. I then use my own work elucidating the evolutionary history and refining the taxonomy of *Eragrostis*, a large genus of grasses, to illustrate how historical and scientific approaches work together to yield insights into how biological processes shaped natural history.

Shaped by History

Amanda L. Ingram
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  For most of the people attending this lecture, explaining how your special field might be viewed through the lens of the humanities would be a piece of cake. But for me, it's not so easy: I'm a scientist, after all, and a botanist to boot. If I'm completely honest, human-focused questions, which are really at the heart of the humanities, wouldn't rank terribly high in the list of things I find intellectually compelling. But as I thought about how I might approach Cheryl's assignment, I began with some careful examination of the questions that *do* interest me and the approaches I take to answer them. This led naturally to a rather uncomfortable subject that crops up periodically in my special field of systematics: is what systematists do *actually* science? So, today I'm going to explore that question, and in doing so, we'll see how my research might relate to the humanities.

  Now, I imagine you have taken a science class at some point, and as a consequence, you probably know how science is done: by using "the" scientific method. In case those memories are dim, let me remind you of the fundamentals. A scientist begins by making some sort of observation about the world, and uses those observations to generate a hypothesis. A hypothesis is a specific sort of question that, ideally, is falsifiable, or possible to disprove. A scientist then designs an experiment to test that hypothesis. The experiment unfailingly involves controls, or unmanipulated conditions. There is also at least one experimental variable that *is* manipulated in the other conditions. Data are collected and analyzed, and then one evaluates the hypothesis. Was it refuted? Interesting! Was it not refuted? Also interesting! Regardless, the outcome will lead to more questions—maybe something like, "Why is this so?"—more observations, more hypotheses, and so on. If you have enough curiosity (and time and money), it's a wonderfully self-sustaining process that allows you to probe ever deeper into the inner workings of our world.

  Let's work through an example together. Imagine that you're scrolling through Twitter

one day and come across, as one does, The Keeling Curve. This is a daily report of the concentration of carbon dioxide (CO_2) in our atmosphere as measured at a Hawaiian research station established by Charles David Keeling in 1958 (Keeling et al. 2001). The interesting observation here is that atmospheric CO_2 concentrations have been rising dramatically over the past half century.

A scientist might consider that observation and wonder how rising CO_2 levels will affect photosynthesis. As I hope you know, plants take CO_2 from the air and use it to make sugars. One might predict that if more CO_2 is available to plants, then the plants might be able to make more sugar. So, on the basis of an interesting observation and some prior knowledge, a scientist might formulate a hypothesis: rising CO_2 should increase photosynthetic rate in plants.

Next the scientist would design an experiment. This would undoubtedly involve including conditions with "normal", or control, CO_2 levels and elevated CO_2 levels. If one wanted to design a more elaborate experiment, one might also use a couple of different kinds of plants to see if they respond uniformly. Maybe you'd choose one of the plants I work on (e.g., *Eragrostis tef*) and then another grain crop (e.g., wheat). You'd grow both species at both CO_2 levels and measure how much photosynthesis they do, fully expecting to find that both do better at higher CO_2 levels. You'd collect your data and analyze them, and you know what you would probably find? That wheat does in fact do far better at higher CO_2 levels (e.g., Blandino, et al. 2020), but tef doesn't really improve (e.g., Sage and Kubien 2003). So, your prediction is partially supported, but how do we explain the species-specific differences? You would go back to what is known about the plants (e.g., while they're both grasses, they actually use different photosynthetic pathways—could that explain this difference in response?), design a new experiment, and continue gaining a deeper understanding about how the world works. It's a marvelously effective and efficient method.

Now, I am a card-carrying scientist, with all of the appropriate degrees and job titles. But the thing is, in my main research activities I don't *actually* use this method, or at least not all of it. So, what are the questions that interest me, and how do I go about finding answers to them? Am I really a scientist?

As I've mentioned, my scientific subfield is called Systematics. It's a relatively young discipline, one that wasn't possible to imagine until we had a good handle on how evolution works. But it has much deeper roots in an older field called Taxonomy, which is focused on

assigning names to the organisms around us and building classifications to organize the planet's vast biodiversity.

I would argue, as many have done before me (e.g., Yoon 2009), that Taxonomy is an instinctive activity for humans. For one thing, humans like to talk, and it's hard to talk about things if you don't have a name for them. As an example, we could refer to some of the trees in the Fuller Arboretum as "large trees with simple leaves that have rounded lobes and that make oval-shaped acorns that are about ¾" long and have a warty cap and that are reasonably palatable if you know how to prepare them". But it's far clearer to refer to them as white oaks, or better yet, *Quercus alba*. All of those facts about the trees are, in a way, embedded in the name, at least to those who have taken the trouble to learn about them. So, practicing taxonomy by naming things provides shortcuts to facilitate accurate and efficient communication.

But names alone aren't always enough to help us cope with the vast and complex world we live in, so humans also have an inherent tendency to develop classification systems to organize this information (Yoon 2009). To stick with our tree example, you might notice that there are actually several kinds of trees out in the arboretum that make acorns. Some of them may have unlobed leaves or pointy lobes, and the acorns might be different shapes, but they are undeniably more similar to each other than they are to any of the other fruits that you see in Indiana. So, we would group these together as oaks, or members of the genus *Quercus*, and, if you're really sophisticated, use adjectives like *alba* or *rubra* (or if you prefer, white or red) to differentiate among them. But even if you can't distinguish the various kinds of oaks, just knowing that they *are* oaks tells you a great deal: they're trees, their wood is probably useful, and they make fruits that are acorns. So, classifications and taxonomy work together to facilitate communication, organize the vast biodiversity surrounding us, and even make predictions about the species' traits.

Classification and taxonomy have been happening for as long as humans have existed (Yoon 2009), and possibly before. But naming and classifying really came into their own as rigorous pursuits with the father of modern taxonomy, Carl Linnæus. Linnæus established many of the conventions that we continue to use today in naming organisms in his influential publications, including *Species Plantarum*, which was published in 1753. Linnæus is credited with formalizing the Latinized binomial, as you can see in a page from the text (Fig. 1), and his classification was based on floral characters, as you can see in the header of this page. As an

aside, this classification system was seen by some as quite scandalous. Linnæus called it the "sexual system" because it's based largely on reproductive characters, and he had the temerity to call hermaphrodite flowers "perfect" and unisexual flowers "imperfect". This did not sit well with some—one critic called the system "loathsome harlotry" (Gribbin and Gribbin 2008)—but it turns out to be effective in its ease of application, and many of the categories that Linnæus identified are still in use today. (But to be fair, Linnæus also described a group that included, among other things, some grasses, some sedges, and maple trees. Even the least botanically-inclined among you might conclude that this is a far less intuitive group than, say, *Quercus*.)
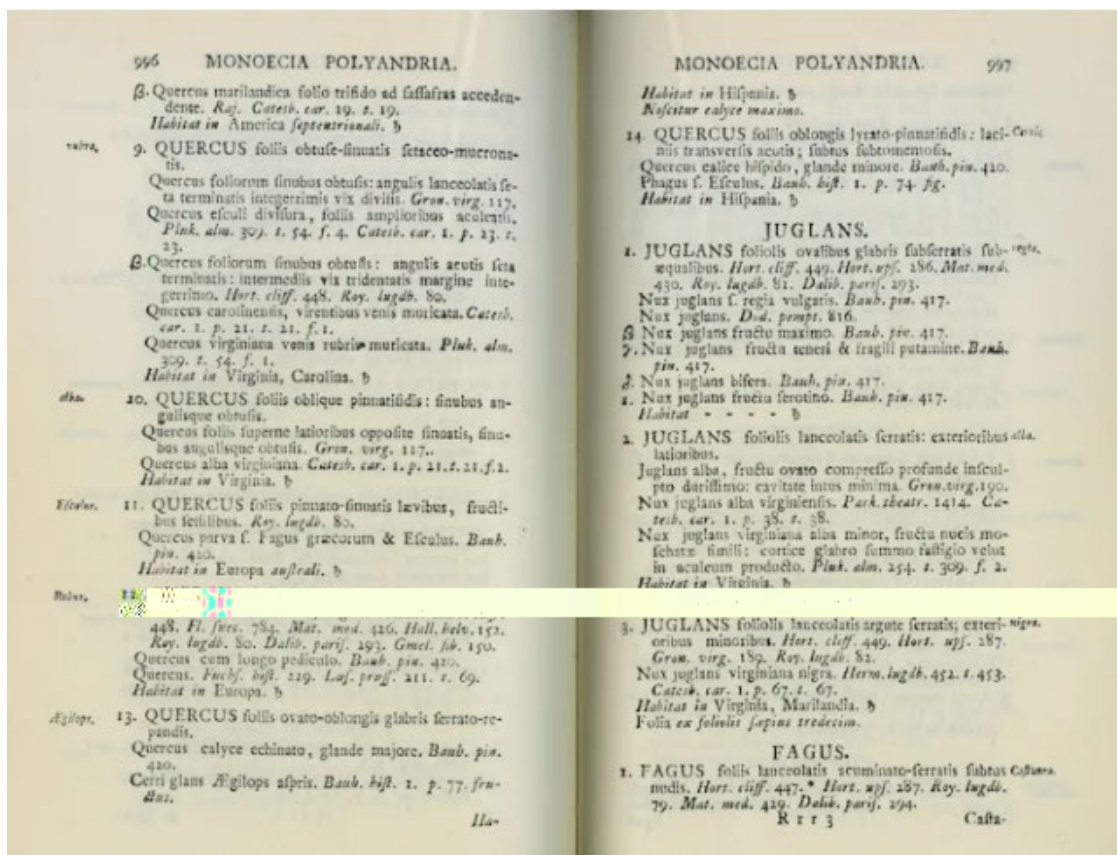
Fig. 1. Pages from *Species Plantarum*. The left page includes descriptions of several species of *Quercus* (oaks); the specific epithets are in the margins.

Linnæus' system arrived at an opportune moment, at least viewed from the perspectives of a Euro-centric assessment of scientific progress. Around the time that Linnæus developed his taxonomic conventions and classification system, European naturalists were increasingly travelling the world and "discovering" species not yet known to Western science. Linnæus'

system allowed names to be applied and enabled rapid categorization of, and thus communication about, the incredible biodiversity found throughout the world.

Before I move on, I want to take a moment to acknowledge some of the troubling ways scientists in my field typically frame our exploration of the natural world. Let's start by returning to our friend the white oak. Its full name is actually *Quercus alba* L. That "L." at the end is called the authority, and it's essentially a citation of the person who named the species, giving that individual credit for discovering it. "L." is our abbreviation for Linnæus, and many species names bear his authority. But did Linnæus actually discover white oaks? Of course not! For one thing, this species is restricted to North America, and Linnæus never visited this continent. He sent plenty of his students to explore North America (many of whom suffered tremendously or even died on these expeditions; Gribbin and Gribbin 2008), but even they were not the first humans to know these plants. Indigenous peoples already knew and had named this species. But because an enterprising European guy had access to vast collections of plants from all over the world and access to publishing to disseminate his work, he continues to receive the credit for "discovering" and naming thousands of species. This is just the tip of the iceberg of the complex and often disturbing history of colonialism in my field.

But back to taxonomy and classification: does what I've described sound like science? There is certainly a great deal of observation that goes into putting names on things and putting them into groups. You need to understand the species' physical characteristics and how they compare to other known species to figure out if they're a different species and with whom you might group them, so you could argue there's data analysis. But are there hypotheses? Not really. And are there manipulative experiments? No.

But I hope you're all thinking, surely this practice has changed since 1753? And, of course you would be right. In 1859 Charles Darwin published a figure (and a lengthy explanation of it, in *The Origin of Species*) that eventually changed everything for taxonomy and classification (Fig. 2). This unassuming collection of intersecting lines elegantly illustrated Darwin's radical idea that all species that live and have lived on our planet could trace their origins to a single common ancestor and that the biodiversity we see today arose as a consequence of billions of years of selection on naturally-existing variants (as well as some other evolutionary processes that came to be understood later).
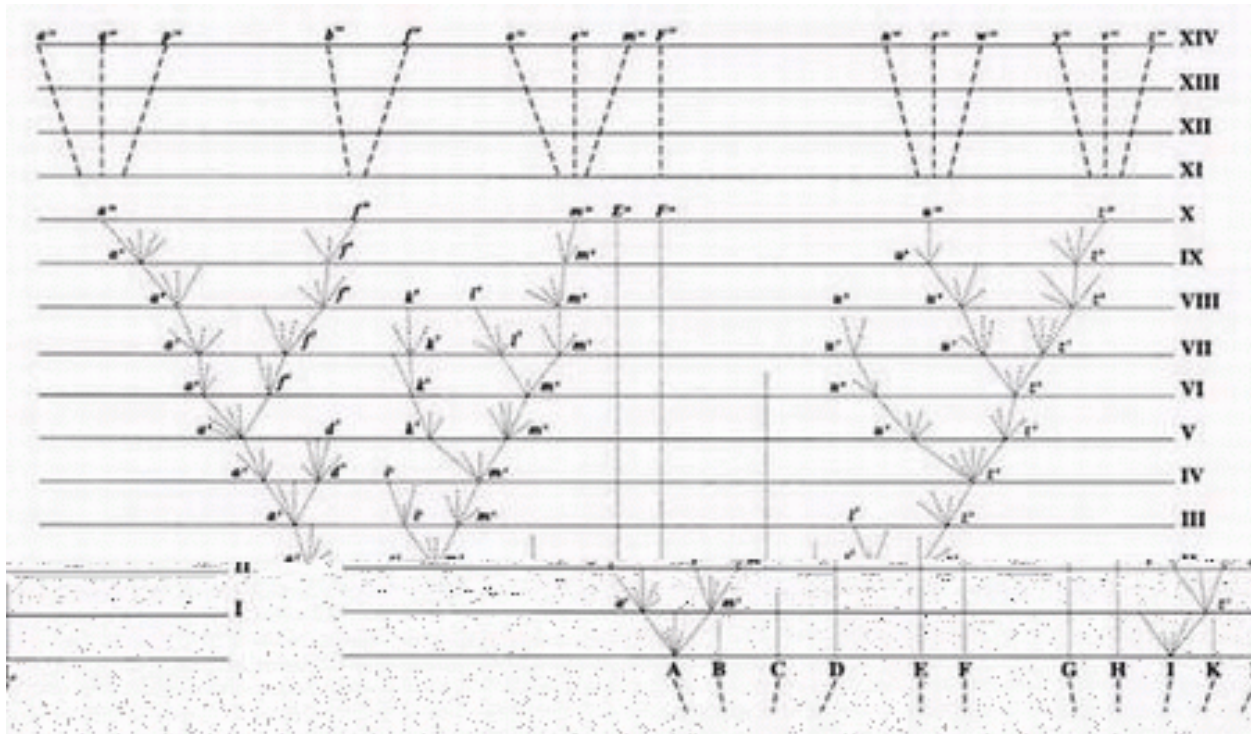
Fig. 2. An illustration from *On the Origin of Species*.

So how did I get all of that from this diagram? First, Darwin intended for the lines (or branches, as I will call them) to represent lineages. The places where these branches intersect, the nodes, represent common ancestors of two or more lineages. The top of the diagram is the present; the bottom is the past. Branches that peter out before the present represent extinct lineages, which may (or may not) be represented in the fossil record. Darwin envisioned the ends of these branches as representing individual species in multiple genera, so we might imagine that the five leftmost lineages are oaks, while the next three are chestnuts. There are only a handful of species represented here, but in theory we could extrapolate out to all known species, connect the lineages, and trace them back to the ancestor of all living species.

After setting out his theory for how evolution by natural selection works, which is generally the part of this treatise that gets all the attention, Darwin went on to outline what I find even more interesting: the implications of his theory and this diagram for classification. By the 1850s there was increasing appetite for classifications to *mean* something and to be more than a convenient way to organize biodiversity (Yoon 2009). In other words, the naturalists of the time were growing dissatisfied with so-called artificial classifications like Linnæus' sexual system,

which, after all, produced that group that lumped together maples and some grasses. Instead they sought to produce what they called *natural* systems of classification. Some naturalists at the time felt that natural systems should reveal the plan of the Creator; Darwin (1859) rejected this idea and instead returned to an idea put forth by Linnæus, "…namely, that the characters do not make the genus, but that the genus gives the characters." Darwin went on to say, "that community of descent—the one known cause of close similarity in organic beings—is the bond, which though observed by various degrees of modification, is partially revealed to us by our classifications." In other words, if we get it right, our classifications should reveal something about the relatedness of taxa, and this is because groups share features because they share a common ancestry. For example, we put species in the genus *Quercus* because they make acorns. The *genus* gave us that character because the genus *Quercus* is a natural entity, not a human construct. Oaks make acorns because their common ancestor made acorns, and the character was passed down, through natural processes, to our present-day species. A natural classification might then allow us to predict that all oaks share a more recent common ancestor than any oak and a chestnut, and we can identify those most recent common ancestors of groups of species on the diagram.

This was a tremendous insight. Now classifications can be viewed as hypotheses of properties of nature. Rather than representing groups of species that are simply more similar to each other than they are to other equivalent groups, as is the case with Linnæus' artificial system of classification, taxa now can be viewed as hypotheses of common descent. "Natural" taxa came to be thought of as groups that could be traced back to a single common ancestor. So, when Darwin's ideas are realized, classifications move beyond being a simple filing system and instead represent the underlying biological processes that generated the diversity of life.

So, are taxonomy and classification science now? We still have the observation piece, and now I've introduced the idea that classifications represent hypotheses explaining biological phenomena. But there are still no experiments. And I haven't really explained how Darwin came up with this branching diagram. In other words, what were his data, and how did he analyze them?

The truth is, this diagram you're looking at is really a thought experiment based on Darwin's extensive observations of organisms and his hypothesis about how species originated and diversified. The letters don't represent actual taxa, there were no data, and no algorithm was used to construct the tree. But over the ensuing decades, taxonomists began building diagrams

representing evolutionary histories that did incorporate real taxa and real data, among them a figure (Fig. 3) produced by Charles Bessey in the early 20th century (Bessey 1915).
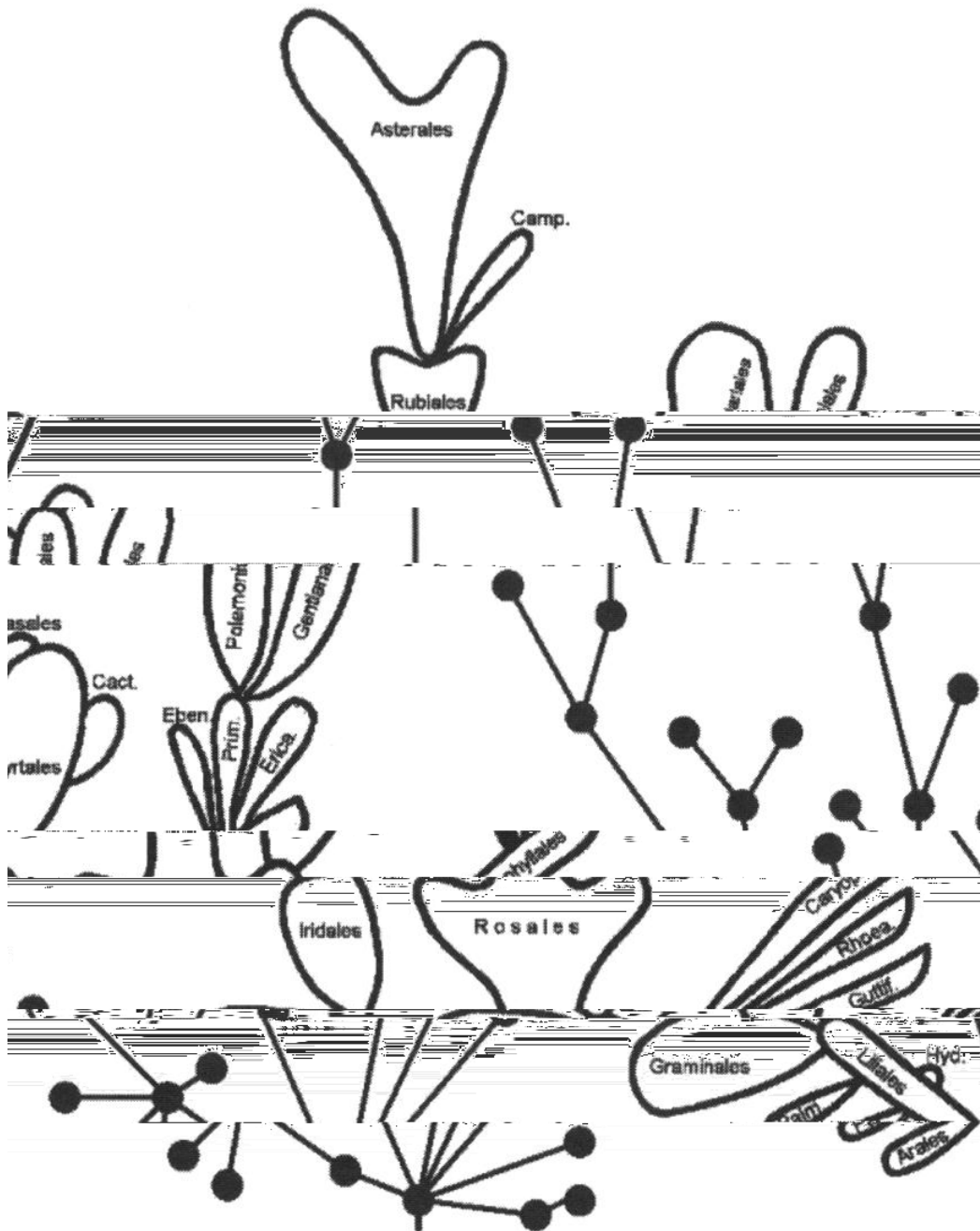

Fig. 3. Bessey's "cactus", an early phylogeny of the flowering plants.

Known fondly as Bessey's Cactus, this diagram is a culmination of Bessey's encyclopedic knowledge of the flowering plants and his attempts to arrange taxonomic Orders by

their "advancement". Each "pad" represents an Order; the groups at the bottom are characterized by what Bessey thought of as more "primitive" characters, while those toward the top are more "advanced". Even though this diagram depicts real data assembled from real taxa, there are two serious issues with it (and with many other similar classifications developed during the late 19th and early 20th centuries.) First, Bessey's focus on distinguishing primitive and advanced groups was wildly misguided: a clear implication of Darwin's theory was that all living species descended from a common ancestor. All living species, therefore, ultimately belong to the same ancient lineage, and we all possess characters that are the result of billions of years of evolution. None of us is really more advanced than any other. But this has been a challenging idea for people to accept, and vestiges of this mindset linger in my field even today—we humans are accustomed to thinking of ourselves as special and somehow better than all other living species, and we often frame evolutionary trajectories accordingly.

The second problem with early evolutionary trees like Bessey's was that the method for arranging taxa was quite subjective. As these hypotheses of evolutionary relationships proliferated, systematists became concerned with how to evaluate the merits of competing hypotheses. Was there a repeatable method underlying the generation of these diagrams? There wasn't, so how do we evaluate ideas based on subjective approaches? When philosopher Karl Popper introduced the idea that hypotheses should be falsifiable, systematists began to wonder if their hypotheses met this mark. (They did not.) Interest grew in developing more objective methods for producing and testing falsifiable hypotheses of evolutionary relationships, which might in turn form the basis of classification schemes. These methods needed to have a firm philosophical grounding, and they needed to be applicable to the wide array of data that a systematist might want to incorporate. Enter Willi Hennig.

Willi Hennig was a German entomologist who published his most influential work, *Phylogenetic Systematics*, in 1950. The text was translated to English in 1966 and quickly became a lightning rod for controversy and debate, sometimes friendly but often vitriolic, that has continued into this century. One of Hennig's key contributions was to set the stage for developing an objective, repeatable method for constructing these trees based on something more meaningful than perceptions of overall similarity. Since we can't directly observe what happened in the past, he (and other like-minded systematists who followed) advocated for relying on the principle of parsimony, or Occam's razor: the simplest explanation is the preferred explanation.

In this case, that meant building a tree that required the fewest evolutionary changes to represent what we know about organisms and how their traits have changed over time. This enabled the characters, inherent and natural features of organisms, and a simple optimality criterion, minimizing evolutionary steps, to work together to produce trees on which we could base or evaluate classifications.

Hennig also refined our understanding of what natural groups should be. In addition to all members of a taxon tracing their evolutionary history back to a single common ancestor, Hennig felt strongly that a natural group should include *all* descendants of that common ancestor. This may sound uncontroversial, but it called into question the validity of many familiar taxa, a concept that can be illustrated with the flowering plants. Many of you have probably learned that there are two kinds of flowering plants: monocots and dicots. The distinction comes from how many cotyledons, or embryonic leaves, they have. One of the things we've learned in the last few decades is that this distinction may be obvious and useful, but it doesn't accurately define natural groups. Evolutionary trees of the flowering plants show us that monocots all share a common ancestor, and no dicots are derived from that ancestor. Monocots are a natural group according to Hennig's criteria. However, if you trace back to the common ancestor of all dicots, it turns out that some of the descendants of that ancestor have one cotyledon: dicots do *not* meet Hennig's criteria for natural groups. The names "monocot" and "dicot" aren't equivalent—the former describes a complete lineage, whereas the latter is incomplete and therefore unnatural.

Together, these ideas led to rigorous methods for tree construction that were gradually adopted by the community, as well as a more nuanced understanding of how to define natural groups that may form the basis of classifications (Hull 1988). This stronger conceptual framework, paired with technological advances in computing and DNA sequencing, has enabled systematists to generate and analyze data sets that include large numbers of taxa and an enormous number of characters. The systematics community continues to argue about exactly which data and which methods of analysis are best, but the one thing we can probably agree on is that we've come a long way from diagrams like Bessey's that are based on opinion. Now we have the ability to produce trees, which we have come to call *phylogenies*, that are far more accurate, with methods that are far more objective. These phylogenies have enabled some giant leaps forward in our understanding of how life has evolved. For example, some groups that I suspect will sound familiar to you—invertebrates, fish, reptiles—are now known to be unnatural

groups that fail to include *all* descendants of their most recent common ancestor. Vertebrates arose from within the lineage that includes all of the animals without backbones. Tetrapods arose out of the lineage that includes all the fishes. Birds are reptiles. This has reframed the way we think about the history of these groups.

Phylogenies can provide insights beyond assessments of the hypotheses represented by our classifications, too. We can put dates on the nodes, either by working backward based on the rate at which DNA typically evolves or by calibrating with fossils. This can help us understand what the world was like when the ancestors of living groups existed. Where were the continents? What was the climate like? This in turn can help us understand how taxa have dispersed all over the world and how characters have evolved. Phylogenies can also be used to understand more recent questions of evolutionary origins. Curious about where SARS CoV2 came from? Sample the sequences of coronaviruses in a bunch of wild animals, build a phylogeny, and determine which host animal has coronaviruses sharing a most recent common ancestor with our current plague.

So, let's get back to my question: is what contemporary systematists do science? We make observations about living creatures, we develop hypotheses about how they might be related, and we collect large quantities of data. We analyze those data with mathematically complex algorithms to generate phylogenies, which allow us to evaluate our hypotheses and ask more questions. Yet we never do any manipulative experiments! And this is because what we're fundamentally trying to do is to understand the *history* of our organisms. What did extinct ancestors of the species we now know look like? How did these species diverge? How have they spread around the world? How did new characteristics evolve? All of these are questions about the past, whether quite recent or unfathomably ancient, so I would argue that we are, at heart, historians in lab coats. We're trying to make sense of the outcomes of *nature's* experiments rather than experiments of our own design. Unlike humanities scholars who study history, most systematists have little interest in humans and instead spend our lives occupied with the obscure, underappreciated organisms of the world, but in many ways our activities mirror those of historians. We rely on archives of information (e.g., DNA sequences preserved in our genomes) to draw inferences about what happened in the past. As is true for historians, our data sets are often incomplete—in the case of the evolutionary process, many lineages go extinct and leave no trace of their existence, just as many conversations, letters, or other artifacts that might shed light

on human history never make their way to an archive. And our datasets may even be misleading thanks to the strong forces of nature shaping the species that have survived, mirroring the way peoples' biases and selectivity affect the information they leave behind. But we do our best to use these wildly incomplete data to form an understanding of our past. Sure, we apply scientific laboratory techniques and mathematically complex analytical methods to collect and make sense of our data, and we generate and test hypotheses, so, begging Stephen Colbert's forgiveness for this coinage, you might say our work has some "scienciness" to it, but at its heart, one could make the case that systematics is a historical exercise.

So I think this is where we can see how my scholarly work relates to the humanities. In Cheryl Hughes' 2001 LaFollette Lecture, titled "Integrity", she wrote, "Humanistic studies give us insight into ourselves, our values and ideals, and our development over time." I submit that systematics gives us insights into *nature*, and what *nature* values, and the development of biodiversity over time.

So what aspects of nature interest me, and how do I go about understanding what nature values? First and foremost, I am a *plant* systematist. I could give another entire lecture on why I'm so fascinated with plants, but for today's purposes, let's just say that I love that plants aren't bound by many of the rules that seem to limit the ways animals exist in this world. Two rule-defying attributes that are particularly interesting to me are hybridization and polyploidy. Plants are notoriously promiscuous, happily mating with other species on a regular basis, producing offspring that are often quite viable and even fertile (Rieseberg and Carney 1998). This is often associated with polyploidy, or having more than two sets of chromosomes. Polyploidy turns out to be extraordinarily common in plants—somewhere between 30-80% of extant plant species are some sort of polyploid (Otto and Whitton 2000). We don't fully understand why plants don't mind having extra chromosomes or why they're able to mate so freely, but we do know that all of that extra genetic material put together in new combinations is terrific fodder for evolution, and understanding the historical patterns of these processes is a first step toward understanding how and why polyploidy and hybridization influence plant evolution.

I'm also motivated to better understand the histories of human-plant interactions, so these complementary interests in economically important plants and polyploidy are what brought me to the group I've spent most of my career working on: the genus *Eragrostis*, commonly known as the lovegrasses. The most well-known economically important species in the genus is

Eragrostis tef, commonly known as tef, a cereal crop grown in Ethiopia and used to make breads like injera. Tef seems like a wildly improbable crop: its grains are tiny, and it's prone to flopping over or dropping its fruits at inopportune times. But it thrives in the challenging conditions in Ethiopia, tolerating heavy soils, unreliable rains, and wild elevational variability, making it an essential crop for food security in this low-income nation. Several other *Eragrostis* species provide forage for livestock, and others are nasty weeds important for their negative economic impacts. But most of the approximately 400 species of *Eragrostis* are little wild grasses about which relatively little is known. They can be short-lived annuals, completing their life cycle in a couple months, or long-lived perennials. If you look closely enough, you'll find that they exhibit striking morphological diversity, especially in their floral structures. They grow all over the world and on a variety of soils, but they're most diverse in the tropics and subtropics and in disturbed areas or sites where they might experience some water stress. This is likely due to the fact that they use a modified form of photosynthesis called C_4 photosynthesis, which enables them to conserve water and continue cranking out sugars to build their bodies and fill their fruits (because yes, grains are fruits) even when water is limiting and light levels are high.

I started working on *Eragrostis* largely because I was curious to know what wild species gave rise to tef. Knowing this could facilitate improvement of the domesticated species because wild relatives often harbor desirable traits that can be bred into the domesticate by hybridization and selective breeding. Comparisons with wild relatives also provide key insights into how human interactions shaped the history of crop plants. I found tef's closest wild relative easily enough (Ingram and Doyle 2003), but on examination of the phylogenies that addressed that question, it became obvious that some more fundamental work on classification was necessary (Ingram and Doyle 2004). In the phylogeny in Fig. 4, one can see that most of the species named *Eragrostis* can trace their origins to a single common ancestor, but others fall outside this lineage. There are also a handful of species, referred to as segregates, who have at times been placed in *Eragrostis* and at others have been moved to their own genera. Some segregates are in the main *Eragrostis* lineage, but others fall outside it. This phylogeny also reveals that not all descendants of this common ancestor are named *Eragrostis*. As a consequence, one of the major questions that has occupied me in the research lab is how *Eragrostis* should be circumscribed. The work I'll describe here has been focused on improving my sampling of key *Eragrostis* species, sequencing some genes, and building phylogenies to understand the group's history and
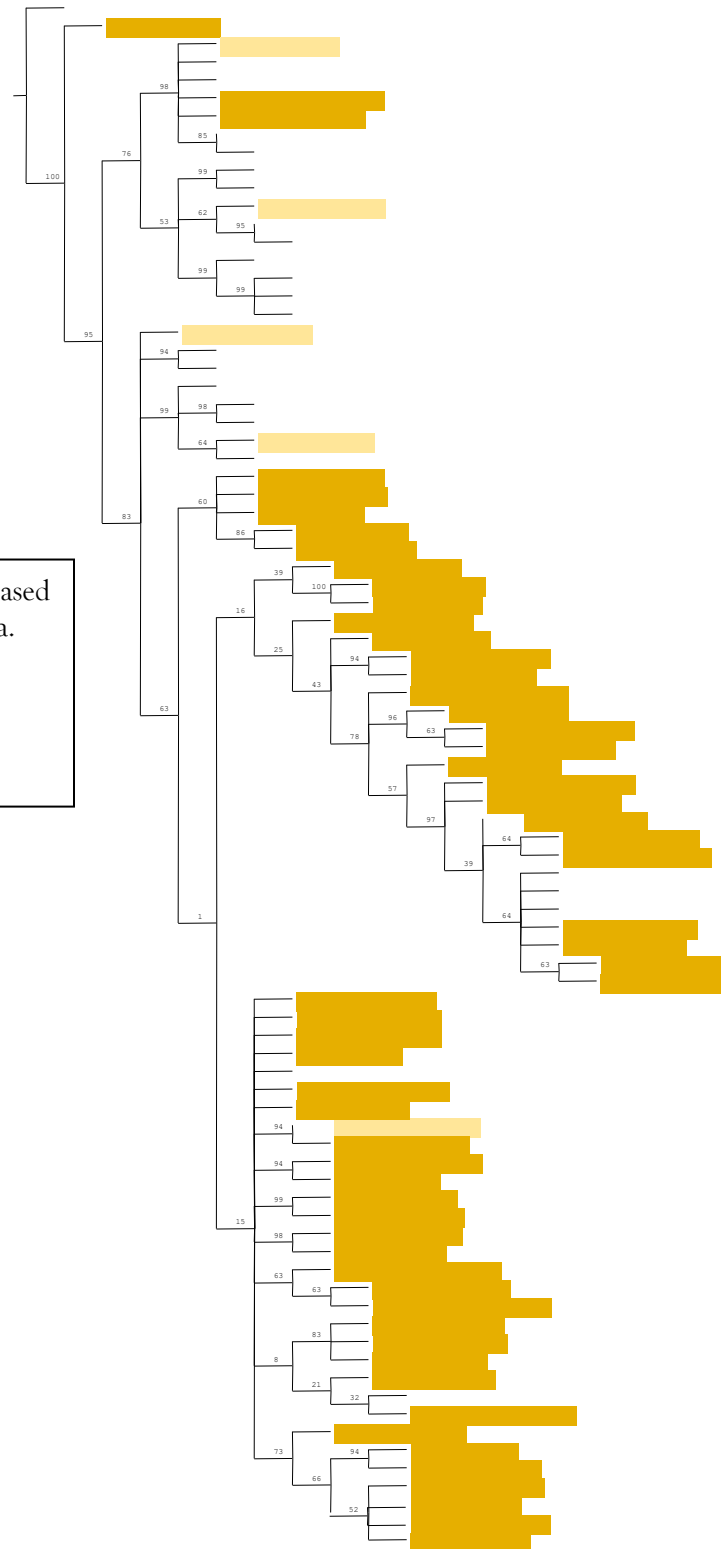
to test the hypotheses represented by these names.

Fig. 4. A phylogeny of *Eragrostis* based on *trn*L-F and *rps16* sequence data. Species highlighted in orange are *Eragrostis*. Species highlighted in yellow are *Eragrostis* segregates.

Before I reveal some of the things my collaborators and I have learned, it's important to know how past botanists have decided to place species in *Eragrostis*. The first official mention of *Eragrostis* was actually in Linnæus' *Species Plantarum*, but he applied the name as a specific epithet, not as a generic name. In 1812, just twenty short years before some Presbyterians kneeled in the snow and founded our college, a French botanist named Palisot de Beauvois correctly realized that the species to which Linnæus applied this name did not actually belong in the genus *Poa* and instead elevated the name to the rank of genus. His description of *Eragrostis* lists several characters by which one might recognize a grass that belongs in this genus, including two characters related to the organization of flowers. First, in grasses, flowers are organized in structures called spikelets. In *Eragrostis*, spikelets contain many flowers. Additionally, those spikelets are arranged in an inflorescence type called a panicle, a structure with multiple levels of branching. A few other characters have come to be recognized as important in placing species in *Eragrostis* in subsequent years, including having three veins on a leafy structure in the spikelet called the lemma. If we view this through the lens of modern systematics, we effectively have hypotheses of characters present in the common ancestor of all extant species of *Eragrostis*. In other words, this name represents a history, and my job is to deepen our understanding of that history. Let's look at a few examples to illustrate how these plants' appearances seem to have misled us about their history and what my collaborators and I have done about it.

The first trickster we'll talk about is a species I encountered when on a collecting trip through the southwestern US and Mexico with some colleagues from the California Botanical Garden. One of them, Hester Bell, was working on a grass called *Distichlis spicata*, so we made lots of stops in salt flats, or playas, to collect them. On one such day, we happened to grab something that superficially looked like *Distichlis* but on closer inspection turned out to be a species named *Eragrostis obtusiflora*. It was a surprise (to me) to find it there: *Eragrostis* species aren't common in soils that are quite this alkaline, and its non-reproductive parts were a bit unusual—it had leaf apices that can puncture skin, a creeping habit, and, as we discovered later in the lab, atypical leaf anatomy and microhairs. Despite that, it had all the usual reproductive features that cause botanists to put species in *Eragrostis*. We were excited to see what the DNA told us, and sure enough, the sequences revealed that the reproductive features were misleading us: *E. obtusiflora* is in fact quite distantly related from all the other *Eragrostis* species. So, we

gave it a new generic name, *Kalinia* (derived from the Arabic for alkaline), so that our classification better reflected the evolutionary history (Bell et al. 2013).

A different sort of story can be told about some grasses in the genus *Ectrosia*, which I first met in Australia. I was keen to see these plants in person because some previous phylogenetic analyses of the subfamily to which *Eragrostis* belongs had suggested that *Ectrosia* was closely related to *Eragrostis*, but the results were quite preliminary because neither genus had been sampled very thoroughly (Columbus et al. 2007). *Ectrosia* differs from *Eragrostis* in several ways, largely related to the reproductive structures. At a glance, they look almost nothing alike, but the differences are actually quite simple: *Ectrosia* doesn't have as many flowers in each spikelet as *Eragrostis*, the flowers are more spaced out in *Ectrosia*, and the lemmas in *Ectrosia* spikelets have long awns, or projections. The last feature is quite striking and makes *Ectrosia* species quite distinctive. But again, the DNA sequences help us understand how much weight to give those simple morphological changes: the phylogenetic analysis reveals that *Ectrosia* is nested within *Eragrostis* and seems to have evolved in Australia (Fig. 4). They're really just gussied-up *Eragrostis* species, and the awns are probably a simple adaptation to help the seeds disperse. The species have been renamed.

Now let's examine one final case: that of *Eragrostis walteri*. If a grass that is narrowly distributed in Namibia and that has no economic importance can be famous, *Eragrostis walteri* would qualify. This is a species that was originally described by Pilger in 1941. He placed it in *Eragrostis* because the multi-flowered spikelets look like those of other *Eragrostis* species. The plants also have paniculate inflorescences. A phylogenetic analysis of morphological data in the mid-1990s confirmed this placement (van den Borre and Watson 1994). But in 1984, R.P. Ellis made the shocking discovery that *E. walteri* deviates in one striking way from other *Eragrostis* species: it has leaf anatomy typical of grasses that use "normal", or C_3, photosynthesis.

Most people probably need some context to understand why this was so shocking. First, let's talk briefly about how these two forms of photosynthesis differ. C_3, or "normal", photosynthesis occurs within a single cell. CO_2 diffuses into the cell, is bound to the most abundant enzyme on the planet (Rubisco), and is converted to sugars. This works well for many species, but there's a slight hitch: Rubisco is a terrible enzyme. It can't tell the difference between O_2 and CO_2, so sometimes O_2 binds to it instead of CO_2. This is inefficient, and it also generates a byproduct that has to be destroyed by the cell. Most plants carry on despite this

inefficiency, but the problem becomes worse when plants live in certain types of marginal habitats (hot, water-limited), and this inefficiency becomes untenable (Sage and Kubien 2003).

Happily, a number of solutions to deal with Rubisco's shortcomings have evolved. One that has evolved dozens of times in the land plants is C_4 photosynthesis (Sage and Kubien 2003). Plants that use this pathway basically don't give Rubisco the opportunity to bind with O_2 by artificially increasing the CO_2 concentration where Rubisco is present. They do this by altering a few things about their anatomy. First, Rubisco is present only in certain leaf cells, the bundle sheath cells. These are interior cells that are buffered from atmospheric gases by the surrounding mesophyll cells. CO_2 diffuses into these mesophyll cells and is bound to a three-carbon molecule, producing a four-carbon (or C_4) molecule. That four-carbon molecule is then shuttled to the cells housing Rubisco, where the CO_2 is released so that it can bind to Rubisco and be used to make sugars. This is an energetically expensive process, but it allows plants to thrive in places where they wouldn't otherwise. It also requires a huge number of changes: veins have to be closely spaced so that no mesophyll cells are very far from the cells with Rubisco. Additionally, a bunch of proteins have to be localized in a very specific way, and many of them are in much higher abundance than they are in a C_3 plant. But as I said, this suite of characters has evolved repeatedly in the land plants, including at least 20 times in the grass family (GPWG II 2012).

But what's really interesting about this is that phylogenies tell us that more or less all of these evolutionary transitions in photosynthetic pathway appear to have been in one direction: C_3 photosynthesis is the ancestral condition, and C_4 photosynthesis is the derived condition (GPWG II 2012). Reversions from C_4 to C_3 photosynthesis were thought to be impossible, which, if you think about it, is fascinating. So much *does* seem possible given the vast timescales on which evolutionary change occurs, so it's a bold claim to suggest that a change is irreversible. But then came this revelation that *E. walteri* has anatomy typical of C_3 species! All other *Eragrostis* species are C_4, and in fact, all of the species in the subfamily to which *Eragrostis* belongs are C_4 (GPWG II 2012), so if *Eragrostis* is a natural group, then *E. walteri* must have reverted to C_3 photosynthesis from C_4. In other words, *E. walteri* appeared to have escaped the constraints of its history.

Hopefully now you can understand why *E. walteri* was famous, at least in certain circles. Well, some colleagues and I decided to test the hypothesis that this species' name represented, so we got our hands on some material. First, we needed to confirm that *E. walteri* does, in fact, use

C₃ photosynthesis by conducting analyses of isotopic composition. It does. Then we sequenced some DNA to confirm that its morphology wasn't tricking us and that it actually was a descendent of the most recent common ancestor of all *Eragrostis* species. When we got the sequences back, it was immediately obvious that the morphology had, in fact, tricked Pilger and all of the subsequent agrostologists who studied this species. Its sequence was highly divergent from other *Eragrostis* species, and a phylogenetic analysis of a broad sample of grasses revealed that it is actually related to a large group of C$_3$ grasses (Ingram et al. 2011).

So, what did we learn? There are certain evolutionary paths that still look extremely difficult, and certain histories may be impossible to escape. Historical constraints, set by nature's values, are real. But it also illustrates how important it is to look carefully and to try not to dismiss data that are inconsistent with your initial hypothesis. When I finally had a chance to meet *E. walteri* in person, it actually made no sense to me that Pilger had put it in *Eragrostis* in the first place. First, it grows *in* a stream! *Eragrostis* hardly ever does that, especially not in a mucky stream like this species does. Second, the lemmas actually have five veins, not three as we see in the rest of the genus. The lemmas also have pointy ends, a character not typical of the genus, though not impossible given what we've learned from *Ectrosia*. There were clues to its actual history all along, but then, hindsight is 20/20.

While I've elaborated on several examples where initial hypotheses of relationships, as represented by taxonomy, were inaccurate, I should point out that systematists have actually done pretty well overall. Most of the *Eragrostis* species I've sampled do fall into a single natural group (Fig. 4), and with additional sampling and more data, I'll be able to make some inferences about the group's history: where the genus originated, what the most recent common ancestor may have looked like, how polyploidy has influenced diversification in the group, and so on. These phylogenies can have practical value, too. For example, one of the challenges with growing tef is that its grains sometimes fall off before they can be harvested. But fruit dehiscence, as this is called, varies quite a bit in the genus, so some colleagues and I are using the historical framework that phylogenies provide to understand how this trait has evolved, what genes are involved, and how we might imagine and effect a future in which tef is a more efficient crop.

I hope you've learned today that systematics is a field whose roots were in organizing and naming life, but the field has evolved to begin probing the historical patterns that our

taxonomies can reflect. We infer these patterns by looking at a snapshot of time, and by doing so, we can see what worked, what nature valued, how history shaped life, and what constraints biology put on evolution. And this historical perspective allows us to consider how our own actions may shape the *future* of our planet's biodiversity. We know, for example, that atmospheric CO_2 concentrations have fluctuated in the past, but they've never risen at this pace (Lüthi et al. 2008). Systematists, with our sense of the extreme age of our planet and the vast expanses of time required for lineages to adapt to changing environments, understand that we're not giving other species the time they need to adjust to the new normal. Our understanding of the organisms we devote our careers to and their pasts can also help us predict who will win and who will lose in a new nature. Those C_4 grasses that are so well-adapted to drought and heat, conditions we know we're likely to see more of in the future? They evolved at a time when atmospheric CO_2 had *dropped* dramatically (Sage and Kubien 2003)—they'll lose one of their most important competitive advantages in a future where the habitats that can support them will almost certainly become more abundant. And as the phylogenies have told us, it is exceedingly unlikely that they'll revert to a form of photosynthesis that *can* take advantage of the new conditions. Who will take these grasses' place? Or will any species be able to take their place? What will happen to Ethiopians who rely on a C_4 crop for food security? So, this is why systematists spend our time exploring the consequences of nature's experiments. By understanding what shaped our past, and what nature valued, we can prepare for our future.

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