# ADJUSTING TO THE WORLD:
# HOW PLANTS SURVIVE AND PROSPER

A Resurrection of the 1986 Ph.D. Thesis of Dr. Stanley Rice
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This document is a translation of my 1986 Ph.D. dissertation from scientific jargonese into plain English. As such it is an attempt to rescue this work from near oblivion. It is nearly impossible to find my thesis through search engines; I can't even find it. When I completed it in 1987, theses were available only as copies from the universities in which they were piled up. Some old theses have been uploaded to the Web, but if mine is one of them, I am unaware of it. I wrote it at a time when main-frame computer terminals were just becoming available for word processing—sometime between Volkswriter and WordPerfect. Drafts of the work were printed on green folded computer paper. I took it with me to my first job in 1987 on big computer tapes that used software even then becoming obsolete. Published papers came from chapters 1 and 2 (of four) of the thesis, and the manuscripts were printed on a daisy-wheel printer using a program known as PFS:Write. Somehow amidst this technological confusion, electronic files were lost. I drew all the figures lovingly by hand, using drafting pens and rub-off Zipatone letters. There were no computer-based image files when I started; Cricket Graph became available a little after I finished.

And since I now have to rewrite the whole thesis into a word processor, why not translate it? My ability to write in a clear and interesting fashion has increased dramatically since I wrote the nearly impenetrable prose of my thesis. I never was very interested in writing for scientists. My passion, even as I worked on my thesis, was to share my understanding of the world with students and with citizens in general. The titles of my four books give you an idea of what I want to say:

- Encyclopedia of Evolution
- Green Planet: How Plants Keep the Earth Alive
- Life of Earth: Portrait of a Beautiful, Middle-Aged, Stressed-Out World
- Encyclopedia of Biodiversity

The thesis began, "The hypothesis is tested…" This pretty much sets the tone for the whole thing. Passive voice is used. Emotion is muted. As I reread my thesis for the first time in nearly thirty years, I found the alternate use of coffee and beer to be helpful.

The thesis, as do all theses, contained a nearly exhaustive literature review, as things stood in 1987. I here make no attempt to update this review, nor do I cite the literature that I cited in the thesis. As for the latter, nobody wants to read an almost thirty-year-old literature review; as for the former, it is impossible. Back in 1987, I went to the library to look at every journal I could. The University of Illinois biology library even had such obscure journals as the *South African Journal of Botany* (obscure to us, not to the South Africans). Today, you can use online

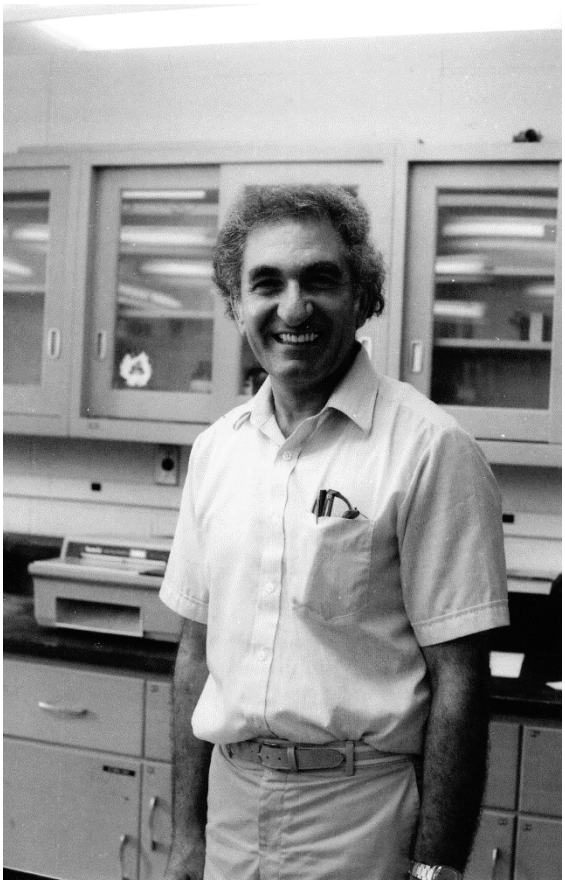
scholarly search engines. Or, easier yet, just use Google Scholar. Accordingly, my reference lists are actually "For Further Reading" for those who are interested in historical works.

Another thing that I can do this time around, which I could not do in my original thesis, is to use photos. Theses were (and perhaps still are) starkly dispassionate; photographs were not permitted unless they conveyed essential information. But this time I can include photographs, including color photos of the experiments and field sites.

This also explains something about what I have always been interested in: the Big Picture. In retrospect, I might have been better off studying some small self-contained system rather than trying to understand the whole world. And as a matter of fact, the only portions of this thesis that were ever published were the limited, focused chapters in which I developed ways of quantifying what I called phenotypic flexibility using just one (amazing) species of plant.

The original title was "Environmental Variability and Phenotypic Flexibility in Plants." Obviously I could not deliver on that title, but I learned a lot by taking a few steps into this topic. Here is the idea: The way plants survive and prosper (I did not study reproduction, the basis of evolutionary success) is by adjusting to the variability in their habitats. So far, I have only stated the obvious. But I meant something more: I meant that plants that live in highly variable habitats are able to adjust to that variability *more* than plants that live in less variable habitats. This was a testable prediction, though I used only a total of twelve species of plants from a total of three habitat types. In doing this research, as will be explained in the chapters themselves, I had to deal with immense difficulties of external and construct validity, even though at the time I had only a vague idea about what these were. This topic also relates to ecological succession (e.g., a farm going back into forest), as I believed that environmental variability declined as succession proceeded.

I did this thesis work at the University of Illinois at Urbana-Champaign under the oversight of the late Fakhri A. Bazzaz, who completed his career at Harvard. And while to most thesis advisors, my topic would have been considered intolerably vague, it actually fit right into Fakhri's approach to science. He wanted to understand everything about the world of plants—including how that world was changing as a result of global warming—from every aspect. So, at one and the same time in our lab, there were some grad students working on genetics, some on pollination, some on plant reproduction, some on ecological succession. Looking back on it, I see that our lab was an unusually creative place. Fakhri let us choose what we were interested in. There was no single topic on which Fakhri was a world expert, but he was supreme in his ability to bring them all together.



*Fakhri A. Bazzaz, about 1986.*

How do organisms adjust to their variable environments? Over the very short term, animals have lots of ways of doing so. They can go sit in the shade or perspire if they get too hot. Over a slightly longer term, they can made reversible adjustments to their physiology, for example by producing more red blood cells at higher elevation. (This process is called *acclimation* in response to one environmental factor such as oxygen availability and *acclimatization* in response to their whole environments. I use the term *acclimation* for both.) In addition to acclimation, animals can develop differently over a longer period of time in an irreversible manner. Humans that grow up at high elevations grow bigger lungs. Once you are an adult, you cannot grow bigger lungs or smaller ones. (This process is called *plasticity*.) These processes may involve changes in gene expression, but do not involve genetic change. From one generation to the next, populations of animals can evolve.

Plants can do most of these things as well, although they are rooted to the spot and cannot go hide in the shade if they get too hot. From one generation to another, though, they can run away, so to speak. Their seeds can disperse to new locations, or can remain dormant in the soil until favorable conditions return. Dispersal to new locations is how plants have primarily adjusted to the coming and going of ice ages, and dormancy is the way that weeds can wait until

the sunny, open conditions that they require return after woody plants have grown up in their erstwhile open-field habitat.

Plants have what could be called behavior, as well. They have their own version of perspiration: they can cool their leaves by allowing water to evaporate (a process called *transpiration*). Some plants raise their leaves up during the day and lower them at night. Most plants open their pores *(stomata)* in the day and close them at night. Plants also have acclimation and plasticity. They can, over short periods of time, adjust their gene expression; for example, if another plant grows over them and shades them, they may produce more chlorophyll in their leaves. That is, they can acclimate. They can also adjust how much their leaves, stems, and roots grow relative to one another over their lifetimes. A plant in the shade may produce more leaf area and less root mass than a plant out in the sun. That is, they have plasticity. And, of course, plant populations can evolve.

Plants can also respond to variability in the environment in a way that animals cannot. This is by clonal growth. If you see a field of goldenrods, it may look like hundreds of plants, but it may be only two or three genetically-distinct plants *(genets).* The original two or three plants grew and then sent underground stems throughout the remainder of the field. New plants *(ramets)* then sprouted from these underground runners (known as *rhizomes*). Eventually these ramets are all physiologically separate individuals that acclimate and have plasticity on their own. But at first, a new little ramet remains connected to its "mother" plant through an umbilicus-like rhizome. If a new ramet finds itself in an unfavorable spot (which often happens because plants cannot generally decide where to go), the parent plant can feed it until it is big enough to fend for itself. Evidence that this occurs can be obtained from using radioactive tracers, which demonstrate that sap can flow from one ramet to another. Some of the early work on this concept was done by David Hartnett, another graduate student in Fakhri's lab when I was there, and by Bernhard Schmid, who was a postdoc in the lab at about the same time. The point is that the ramets, instead of being separately blasted by environmental conditions, can share their resources and dampen down the variability of physiological responses, whether these responses are symptoms of stress or are acclimatory or plastic responses.

I wanted a term that encompassed both acclimation and plasticity, but did not include evolution. So I made up the term *phenotypic flexibility.* Phenotypic means the individual and its physiological processes, as opposed to genotypic, which refers to its genes. At the time, no one (except maybe great minds like Carl Woese and Lynn Margulis) even imagined that differences in gene expression could be transmitted from one generation to another, just like the genes themselves. But we now recognize *epigenetic* adjustments, sort of halfway between phenotypic and genetic adjustments. An organism can inherit an inactivated version of a gene from its parents. The gene is there, but might as well not be. In 2014, Svante Paabo's research team indicated that many of the differences between humans and Neandertals were epigenetic, not genetic.

In science, we like to define our terms very specifically. But there was one word that scientists used to mean lots of different things: *adaptation.* This term could mean evolutionary adaptation; it could mean plasticity; it could mean acclimation; it could even mean moment-to-moment physiological adjustments, which is the sense in which the term is often used in medical research (your body adapts to insufficient oxygen by breathing more). Even the evolutionary use of the word could be misleading. Natural selection may favor a certain set of genes (true adaptation), or genetic changes could occur because they were structurally unavoidable or because they got swept along with the genes that were being selected (a process scientists such as Stephen Jay Gould and Niles Eldredge called *exaptation*). The use of a term with so many meanings bothered me, and I wanted to write about it. I finally did, many years later. In the entry on "adaptation" in my *Encyclopedia of Evolution*, I explain the *sixteen* possible meanings of the word. Any word with sixteen meanings is problematic. At least *flexibility* was *a little* less vague.

Plants, in contrast to most animals, are modular. The three organs of plants are leaves, stems, and roots. (Flowers and cones are modified stems.) Plants grow by adding new organs: new stems, new leaves on each stem, new roots). Animals do not do this. An animal grows because all of its organs increase in size. And when an animal adjusts to its environment, its existing organs adjust. In plants, however, the distinction between plasticity and acclimation is less clear. If the environment changes, a plant may alter the structure of its new leaves but cannot alter the structure of its old leaves. The plant as a whole acclimates, but it does so by means of the plasticity of its interconnected leaf, stem, or root modules.

The fact that plants have plasticity and acclimation is now old knowledge. But back in 1987, it had only been a couple of decades since scientists such as Olle Björkman demonstrated that plants could, in fact, adjust their photosynthetic characteristics to the environments (e.g. sun and shade) in which they lived. A modern thesis advisor would respond to my idea by saying, "Sure, plants adjust; what else is new?"

A major idea behind everyone's research into adaptation, in whatever form it takes, is that organisms have limits. A plant can only photosynthesize so much, and an animal can only eat so much. To do more of one thing means to do less of another. This is especially true in the wild world of nature, where neither plants nor animals ever take vacations, unless those vacations are (like hibernation) themselves ways of adjusting to their environments. It seemed to me that phenotypic flexibility must come at a cost, and that plants that live in less variable environments should invest fewer of their resources into flexibility than do plants that live in highly variable environments, in which such investment is necessary. (*Investment* is, in fact, an economic term that botanists use regarding what a plant does with its resources; *allocation* is another such term. Allocation is what a plant does with its resources to survive right now; investment is what a plant does with its resources in preparation for the future.) And that is why, when I used the term *phenotypic flexibility*, I meant not only the plant's ability to alter its phenotype, but also the *growth advantage* that results from this ability.

So there it is: plants that live in highly variable environments have more flexibility than plants that live in less variable environments. That is the (just barely) testable hypothesis of my thesis.

From there it starts to get complicated. The first problem I had to address was, how do you measure phenotypic flexibility? The second was, how do you quantify the growth advantage that may result from it? Those were chapters 1 and 2 of the thesis.

## Vintage References for Further Reading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# CHAPTER 1
# HOW DO YOU MEASURE PHENOTYPIC FLEXIBILITY?

This chapter is about how to quantify plasticity and acclimation in plants. You can see them happen, but how do you assign a number to them?

Phenotypic plasticity is usually quantified by raising a set of plants in a range of environmental conditions. Using a statistical method known as the *analysis of variance* or ANOVA, the total variation in plant traits can be divided up into genetic and environmental components, and the interaction between them. This is a two-way ANOVA, which is extremely difficult to do by hand and calculator. It involves complex matrix algebra. Computers can, however, do it easily. The SAS Institute had developed "PROC GLM" (for general linear models) and it was available to me at a computer terminal in our lab. Had I done my work ten years earlier, it might have been me tearing my hair out over a calculator; thirty years earlier, a slide rule. Or an abacus; they still used the abacus (called *soroban*) in Japan for commercial transactions in the 1970s. Today, the entire analysis can be done on a personal or laptop computer. The environmental component of plant growth responses across a range of conditions is the *norm of reaction* and can be considered a measurement of plasticity.

As an example of the analysis of a norm of reaction, consider plants grown in a range of light conditions. Plants that grow in bright light might have smaller leaves than plants grown in the shade. The amount of variation in leaf size that is directly caused by differences in light (the environmental or E component) can be considered phenotypic plasticity. But if (as is usually the case) the plants are genetically different from one another, there is also a genetic component: some genotypes may produce smaller leaves than others. This is the genetic or G component. Finally, some of the genotypes may respond to bright light vs. shade in different ways: one genotype might greatly adjust its leaf size, another not so much. This is the interaction or G × E component. Whether you use just the E component, and/or the G × E component, or even (as in at least one paper) the leftover unexplained variation as well, as a measure of plasticity, depends on what you want to know.

For ease of calculation, I used just two light levels. I was therefore able to express phenotypic plasticity and acclimation as a simple, unitless ratio of the trait in bright light divided by the trait in low light, or vise-versa. My range of conditions was thereby reduced to just two conditions. I did not use identified genotypes in this work, therefore my G and G × E components were mixed in with the unexplained variation.

To study plasticity, I raised plants in bright light vs. shade. To study acclimation, I raised plants in one light condition and transferred them to another. The resulting four treatments were:

- H: Plants that spent their whole lives in high light;
- HL: Plants transferred from high light to low light;
- LH: Plants transferred from low light to high light;
- L: Plants that spent their whole lives in low light.

You cannot quantify phenotypic flexibility by just measuring something that happens as organisms develop in or respond to different conditions. Think about it. Just because the organism changes does not mean it is adjusting. It might be suffering. One example is the use of weight as a measure of an animal's adjustment to its conditions. The animal has an optimal range of weight, above or below which it suffers. Neither wasting away nor obesity can be considered adjustments to conditions. This is a problem of *construct validity:* weight is not a valid way of measuring health. (Or is it? Being temporarily overweight might be an animal's way of storing up food for the future by eating as much as possible during the brief period when the food is available. This is the standard explanation for why modern humans become obese. Cavemen put on fat during times of feasting, then lived off of the fat during times of famine. But today, with food so readily available to most people, our bodies continue to crave food, thus preparing for a famine that never comes. And when an animal begins to starve, it uses up its bodily resources in the reverse order of their importance: first the fat reserves; next the muscles; last of all the brain. This means that the sequence of events in starvation is adaptive.)

The same is true for plants. Plant responses may be plasticity and acclimation, or they may be stress. This is especially important for LH plants: a sudden transfer from shade into full sunlight can make the leaves overheat and get thirsty, since the stem has developed so as to supply them with just the amount of water they needed in the shade; and the bright light can "solarize" the leaves, actually damaging the photosynthetic machinery, as opposed to indirectly causing the leaves to overheat. All I could do to compensate for this was to use conditions that were not extreme enough to cause any of the easily-recognized symptoms of stress. (As a matter of fact, the LH plants were phenomenally healthy, as you will see.) HL plants might be unhappy as well if they had a lot of tissue that was useful for transporting a lot of water that they no longer needed or absorbing a lot of light that is no longer available. Some of this tissue, now superfluous, can be expensive to maintain.

And sometimes stress and plasticity or acclimation may result in the same phenotypic differences or changes. If a plant experiences drought, it has less water pressure to expand its new leaves. This is stress. But the smaller leaves lose less water. This is acclimation. Sometimes the problem is the solution.

Generally speaking, some traits of a plant or animal need to remain the same—that is, they have to be kept in *homeostasis.* Water content of leaves is a good example. It is stressful for a plant to lose too much water through its leaves. Plasticity and acclimation of some traits can allow homeostasis of others. If, in response to water loss, a plant grows more roots, these extra roots can supply more water to the leaves, helping to maintain leaf homeostasis of water content. Root plasticity contributes to leaf water homeostasis. Although I did not recognize it at the time, I intuitively chose to measure plastic traits rather than those traits that needed to be kept in homeostasis.

This also means that you cannot measure an animal's phenotypic response simply by measuring something about it. Sure, people who grow up at high elevations have bigger lungs, but this is relative to their body size. Big people have bigger lungs just because they are big. The same thing applies to plants. Sure, plants that live in dry soil have a bigger root system (which increases their chances of finding water), but this is relative to their body size. Big plants have bigger root systems just because they are big. So you can't just weigh the roots of a plant as a measure of its phenotypic flexibility in response to soil moisture. And thus is born the science of *allometry.* You study an organism's adjustment to its conditions by measuring its phenotypic responses relative to its body weight. Allometry comes from the Greek for "to measure the same," meaning to use the same basis for comparison—in this case, weight.

As hard as it may be to believe, I wrote a whole chapter about allometry without once using the word (a problem I here rectify). That's because I didn't know what it was. I cannot use the excuse that botanist Karl Niklas had not yet written a book about it; the concept was already well established. Apparently, neither my advisor nor my committee seemed to know about it either. They never once said, as I recall, "Stan, your first chapter is just about allometry." For aught I knew to the contrary, I'd invented the concept. This is always a dangerous thing to think. It would be like saying that the United Nations invented the concept of peace.

Specifically, what this means for plants is the following incomplete list:

- Instead of root weight, you quantify the root weight relative to the plant weight [RWR];
- Instead of stem weight, you quantify the stem weight relative to the plant weight [RSR];
- Instead of leaf weight, you quantify the leaf weight relative to the plant weight [LWR];
- Instead of leaf area, you quantify the leaf area relative to the plant weight [LAR];
- Instead of measuring plant growth in, say, grams per day, you quantify the increase in plant weight relative to its weight on that day [RGR].

These are root weight ratio; stem weight ratio; leaf weight ratio; leaf area ratio; and relative growth rate. The first three ratios are unitless percentages or proportions. The fourth is area per weight, e.g. mg per $cm^2$. The fifth is the unitless proportion of increase per day (e.g. g per g per day, which comes out $day^{-1}$).

It is particularly important to express plant adjustments relative to their weight, more so than for, say, mammals and birds. Mammals have a constant body temperature and expend a lot of calories to keep it so. But plants grow faster when conditions are good (warm; moist; sunny; high soil nutrient levels; all in moderation) than when they are bad. A poor kid who eats half as much food as a rich kid does not develop twice as slowly. A little bit more slowly, e.g. delayed onset of puberty, but not twice as slowly. Animals also have relatively well-defined life spans. Some plants do too, such as annual plants that live only for a year from seed to seed. But our ability to produce bonsai animals is very limited, in contrast to bonsai plants. Bristlecone pines grow extremely slowly in the Inyo Mountains of California, where they experience cold, dry conditions in poor soil. Some of them are over four thousand years old. No animal could do this.

Under good conditions, therefore, a plant grows as if the movie of its life were speeded up like a Keystone Cops flicker, relative to bad conditions. In many cases, a plant that lives only one year may finish up its life more quickly under high resource conditions, or a plant that usually lives for two years may finish up its life in just one, as if it were saying, *Live fast, die young.*

And this was an important insight from my first chapter. If you want to compare plants in different conditions, you cannot just compare their characteristics—*even if* adjusted for plant weight as described above—at the same time. Here's why. As a plant grows, it accumulates roots and stems, but it drops its old leaves. It is therefore inevitable that, during most of its life, a plant's LWR and LAR will decrease. And in a fast-growing plant, this happens sooner than in a slowly-growing plant. If you grow two plants, one in bright light and one in shade, for a month, then compare their leaf weight or leaf area ratios at the end of that month, you will find that the one in bright light has a lower LWR and LAR. You could jump for joy and say that you have demonstrated an adaptation, showing that plants in bright light do not "need" as much leaf material as do plants in low light, when really all you have demonstrated is that the plant in bright light has rushed more quickly through its inevitable, life-cycle-related decline in leaf weight and area. Therefore when you compare different phenotypes of the same kind of plant, you must not only *adjust for* plant weight, but you must *compare them at* the same weight.

This, too, is what allometry does. A typical allometric graph of data shows some plant feature—such as leaf weight—on the vertical axis, and plant weight on the horizontal axis. (Since plant growth is exponential, the graphs are easier to read if the axes are presented in logarithmic terms rather than straight linear terms.) Thus, if you are comparing plants raised in bright light with plants raised in the shade, the bright-light plants form a line of points representing their growth in bright light, and the shade plants form a different line of points representing their growth in the shade. (Each point represents one plant, since you must typically kill a plant in order to measure its total weight or the weights of its component modules.) You can directly compare the two groups of plants at any point along the horizontal axis. Notice that time does not appear on this graph. The fact that plants may grow faster in bright light makes no difference in an allometric comparison.

There is a little trick to doing this, however. If you grow the plants for the same length of time, and then measure them, the weights of the bright-light plants will be greater than those of the shade plants; the line of points of the bright-light plants is shifted to the right, and may not overlap the line of points for the shade plants. You have to think ahead, and plan to measure the bright-light plants earlier than the shade plants. Alas, only after the plants are dead can you know if you have succeeded in getting the ranges of weights of the two groups of plants to overlap.

And this is what I did for the work that appeared in Chapter 1 of the thesis and was published in 1989 in the journal *Oecologia.*

Methods I Used in Chapter 1

The plant species that I used was *Abutilon theophrasti*, a type of mallow (plant family Malvaceae, which includes cotton, okra, and hibiscus). I did so for several reasons, among them:

- The other graduate students, over the years, in Fakhri's lab had accumulated an immense amount of knowledge about how to grow this plant;
- It is an abundant weed in the agricultural fields of central Illinois, therefore I could get lots of seeds;
- It is a weed, so it grows very fast;
- It has few, large leaves, the area of which is easy to measure.

The ease of measuring leaf area is important, since photosynthetic measurements are expressed in terms of how much carbon dioxide a leaf absorbs per unit time relative to its leaf area: a big leaf carries out more photosynthesis than a small leaf.

Also, the fact that the leaves of *Abutilon theophrasti* are heart-shaped made them a standard item in Valentine's Day and anniversary cards between me and my wife. The plant is beautiful, but literally irritating. The stems and leaves are covered with sticky hairs with an irritant in them to which I, fortunately, was not allergic. Hence the common name *velvetleaf.* The fruits look like giant asterisks, or coat buttons, hence the name *buttonweed.* This weed is native to India (hence *Indian mallow*) but was brought over to America to use (I am not making this up) to stamp little asterisk-shaped decorations on slabs of butter, hence the names *stampweed* and *butterprint*. Pigs hate it, so it can grow in pigpens undisturbed, hence the name *pigpenweed*.

*This is an image of* Abutilon theophrasti *taken in Oklahoma in the summer of 2013. Abutilon does not grow very much in Oklahoma, but this plant arrived in a hunk of sod that was imported from the northern Midwest for roadside erosion control.*

I obtained lots of seeds from local field and vacant lot populations of velvetleaf, paying no attention to possible genetic variation. Velvetleaf seeds have physical dormancy. That is, they have hard seed coats, and if you plant them in wet soil they just sit there and do nothing for a few years. But I learned a trick from the Weed Science department over in the agriculture school. Just dip the velvetleaf seeds in boiling water, briefly, then rinse them. This softens the seed coat. The brown seeds turn reddish and sprout—nearly all of them—almost right away.

Then I had to grow the plants under high and low light conditions. Sounds easy? Not really. Environmental conditions interact with one another. In bright light, the air is often warmer and drier. That's three factors, not one. Fortunately our lab had some big, expensive growth chambers that could produce bright light while keeping the temperature and humidity of the air constant. You can't do this very easily in a greenhouse. The solution is not perfect: bright light will cause a leaf to be warmer than it would be in the shade, *even if* the air temperature is kept constant, and may cause the leaf to lose water more rapidly, *even if* the relative humidity is kept constant. But we did the best we could.

The use of growth chambers imposed some limits on the experiment. Space was limited in the chambers. I had to crowd the plants in. One way I did this was by growing them in little plastic Solo cups. Now this presents a problem. Roots need water, but flooding damages them. Roots also need lots of oxygen. A plastic cup allows neither drainage from the bottom nor oxygen penetration from the sides. I partially solved this problem with an advanced piece of technical equipment: a nail on a stick. I heated the nail in a Bunsen burner and used it to melt holes in the sides and bottoms of the cups. The stick, with its low thermal conductivity, kept me from burning my fingers.

The use of little plastic cups presents another problem. Once the roots begin to grow, they quickly run out of space. The plants become "root-bound," the roots forming ropy masses along the bottoms and sides of the soil volume. Eventually, the roots may stop growing and the plant may experience stress. In such a case, root growth of a bright-light plant might slow down while the roots keep growing in the shade. This will tend to dampen the difference between the root allocation in bright light vs. shade. I suspect that this happened a little bit during my experiment. But I measured the plants when they were still small enough that they had not become very root-bound. Undoubtedly, had I used bigger pots, I would have measured a *greater* difference in root allocation between the bright-light and the shade plants. But I still found an easily measurable difference between them. The difference I measured is not a realistic reflection of what happens out in the wild, but the whole system is artificial anyway. And if I used bigger pots I would have needed to use fewer plants, thus reducing my sample size. Every statistical analysis, especially ANOVA, is better if you have a bigger sample size.

I raised all the plants at 27º C in the daytime and 22º C at night. High light (H) conditions were 900, and low light (L) conditions 200. These numbers are measures of photosynthetically active radiation (PAR), which means light that plants can use for photosynthesis. It roughly corresponds to visible light. An incandescent light bulb produces more infra-red radiation (heat) than visible light. What the plant "cares about" is the PAR, not the total photon output. The units are in $\mu E\ m^{-2}\ s^{-1}$, or micro-Einsteins per square meter per second. An Einstein is a mole of photons. To measure light intensity in moles has an advantage over measuring it in lumens or candelas or something else like that. Since photosynthesis uses sunlight energy to fix carbon dioxide molecules into sugar, you can directly calculate the *quantum efficiency* of photosynthesis by determining how many molecules of carbon dioxide the leaf uses relative to the number of photons that it absorbs. The theoretical maximum is about one molecule of carbon dioxide for each twelve photons, or a quantum efficiency of about 8 percent. Whole plants, of course, come nowhere close to this.

How do you measure a plant's weight? Sounds easy, but there are some things you have to remember to do. First, it is more useful to know the plant's dry weight—that is, the organic and mineral components—rather than the amount of water in the tissues. Otherwise, tissue hydration is an uncontrolled variable. Second, to measure root weight, you have to wash the soil away very thoroughly, since just one little rock can weigh as much as a whole bunch of roots, introducing a large error. We used commercial potting mix, which has few rocks in it, but we still had to be careful. You have to be delicate as well, since you don't want to dislodge any roots and watch them wash down the drain. I spent many hours washing roots. These were not all unpleasant times, since I often did it at the greenhouse sink right in front of the murmuring and soothing circulation fan.

How do you measure leaf area? There are lots of ways, some better than others. The cheapest way is to trace the outline on graph paper and count the squares. But fortunately Fakhri's lab had a leaf area meter. You put the leaf on a conveyer belt of clear plastic. The meter reads the number of little beams of light interrupted by the leaf. Nowadays computer images are used. Automated methods are better for leaves that have complex shapes. Velvetleaf, however, is not one of them.

In order to get the weights of the plants in all four treatments to overlap as much as possible, I began chopping the plants up on day 15 for high light and on day 28 for low light. This was a guess but it worked. The grand mean mass of all the plants in the experiment was 679 mg, and the means of each of the four treatments were pretty close to this. I continued chopping up a few plants each day in each treatment until they stopped producing full sized leaves.

I had not before, nor have I since, conducted such an elegant experiment. All the plants were at almost exactly the same height and developmental stage when I started each phase of the experiment. Never before or since has the natural world been so obedient to my expectations or so clear in its patterns.


RICE

*My 1985 Abutilon experiment. In the second photograph, green stakes indicate L plants grown in shade and red stakes indicate HL plants transferred from sun to shade. The unlabeled plant is an H plant of the same age for comparison. In the third photograph, the middle plant is an LH plant, transferred from shade to sun, which shows obvious symptoms of stress but which, nevertheless, experienced a prodigious growth spurt upon transfer.*

## Results of Chapter 1

Differences or changes in light intensity produced markedly different plants. I will summarize these differences below. Remember that I compared the plants at the same mass (679 mg) not at the same time, except for SLW (specific leaf weight), which was leaf weight divided by leaf area. I used this as an indirect measure of leaf thickness, which can only be accurately measured after careful preparation and with a microscope, or density.

Differences due to plasticity (H vs. L):

| | |
|---|---|
| Height | Shade plants were 3.73 times taller |
| Leaf area | Shade plants had 2.36 times as much leaf area |
| SLW | Sun plant leaves were 2.36 times as "dense" |
| Roots | Sun plants had 4.57 times as much fine (absorptive) root weight and 56 percent more taproot (storage) weight |
| Stems | Shade plants had 51 percent more stem weight |
| Leaves | Shade plants had only 7 percent more leaf weight |

Therefore, in the shade, plants had more leaf area and grew bigger and taller stems. This allows plants to reach for the sun in competition with other plants. To afford this, however, the shade plants had thinner leaves and less root weight. The thinner leaves also helped in low light, because what is the point in having a thick leaf if there isn't enough light to penetrate it? Also, in the shade, plants are transpiring less water and do not require as much root.

Differences due to acclimation from sun to shade (H vs. HL):

| | |
|---|---|
| Height | Shaded plants were 2.71 times taller |
| Leaf area | Shaded plants had 2.41 times as much leaf area |
| SLW | Leaves of plants that remained in the sun were 2.34 times as "dense" |
| Roots | Plants that remained in the sun plants had 2.29 times as much fine (absorptive) root weight and 35 percent more taproot (storage) weight |
| Stems | Shaded plants had 30 percent more stem weight |
| Leaves | Shaded plants and plants that remained in the sun had exactly the same leaf weight |

Therefore, in all these cases, the plants acclimated the same way and almost as much when transferred to shade as their plasticity to shade. They produced more leaf area and grew taller. They stopped allocating as much mass to their roots as they had been before. The leaves, I assume, did not actually become thinner, but they didn't have as much organic material stored in them.

Differences due to acclimation from shade to sun (L vs. LH):

| | |
|---|---|
| Height | Shade plants were 95 percent taller than plants transferred to sun |
| Leaf area | Shade plants had 65 percent more leaf area than plants transferred to sun |
| SLW | Leaves of plants transferred to sun were 2.04 times as "dense" as shade leaves |
| Roots | Plants transferred to sun had 69 percent more fine (absorptive) root weight and 25 percent less taproot (storage) weight than shade plants |
| Stems | Shade plants had 12 percent more stem weight than plants transferred to sun |
| Leaves | Plants transferred to sun had only 9 percent more leaf weight than shade plants, but this was not statistically significant |

Therefore, in all these cases, the plants acclimated the same way when transferred to high light as their plasticity to high light. They produced less leaf area and stopped growing as tall and started allocating more mass to their roots. The leaves did not actually become thicker but they had more organic material stored in them. However, in all these cases, acclimation from shade to sun was more modest than plasticity to sun. This probably occurred because, when first thrust into high light, the shade plants experienced some stress, which limited their ability to respond. But they did respond. The reduction, rather than increase, in taproot weight was the only way in which plasticity and acclimation differed in this set of measurements. Did this occur because the shade plants, finding themselves suddenly in bright light, needed to use some of their stored energy to produce more absorptive roots? I can only speculate.

Much to my astonishment, the paper that was based on this chapter has been cited often, even decades after its publication. When I checked up on some of these citations, I found that there were two major reasons for this.

- First, it is apparently one of the best examples of how light intensity, quite apart from soil moisture, can induce the production of more roots. Even if they should not have been, many scientists were surprised that plants produce relatively more roots in high light even under conditions of adequate moisture.
- Second, there were some good examples here of how comparing phenotypes at the same age gives very different results from comparing them at the same weight.

One of the latter examples is plant height. For any given plant *weight*, shade plants were taller than sun plants (Figure 1); plants transferred from sun to shade were taller than plants that remained in the sun (Figure 2); and plants transferred from shade to sun were shorter than plants that remained in the shade (Figure 3). But at any given *time*, throughout the over forty days on which the plants grew, all the plants were about the same height, regardless of the light intensity at which they grew or were transferred to (Figure 4). Is height therefore a flexible trait? If you simply compared the plants on any given day, you would conclude that it is a stable trait, not a flexible one. But on an allometric basis it is clearly a flexible trait.

Figure 1

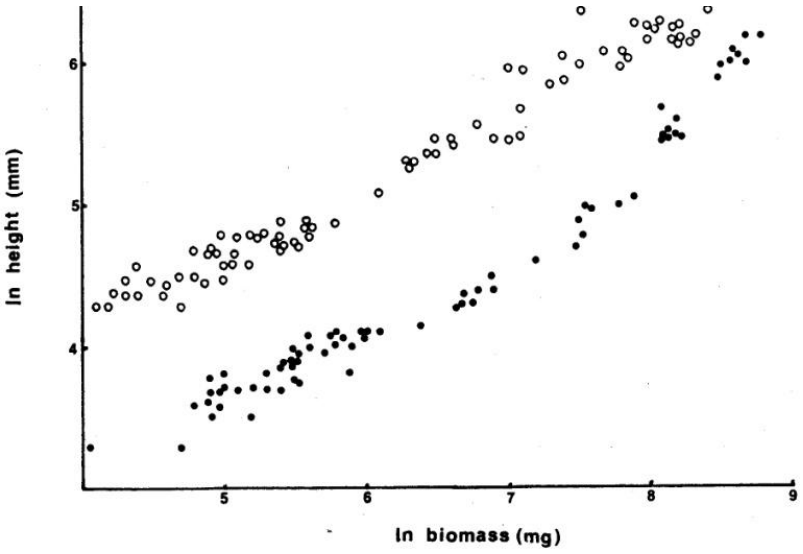



Figure 2

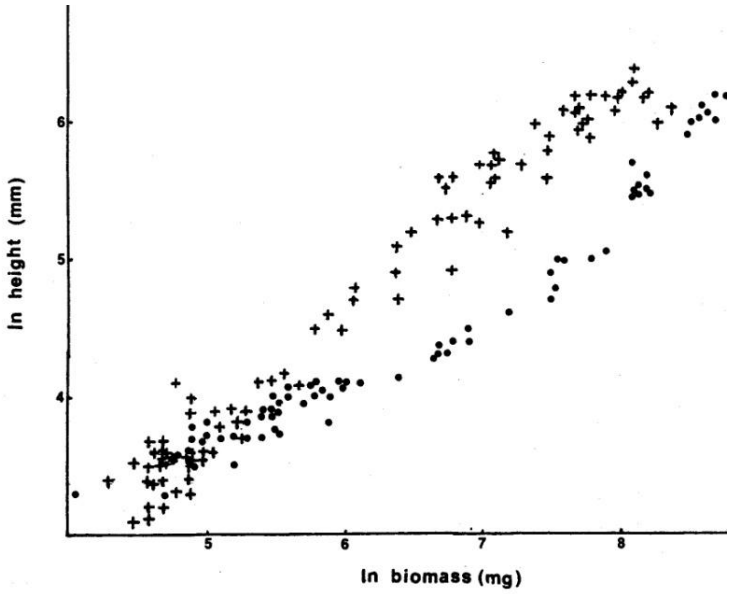


Figure 3

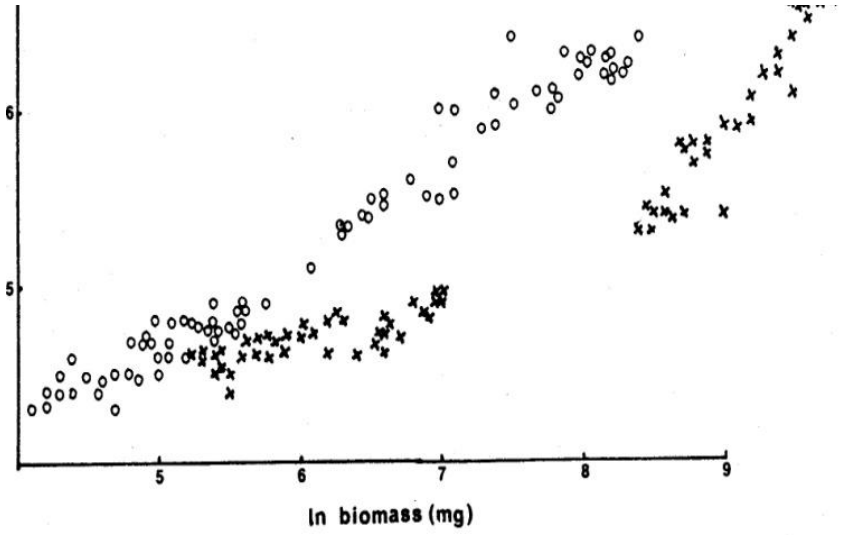
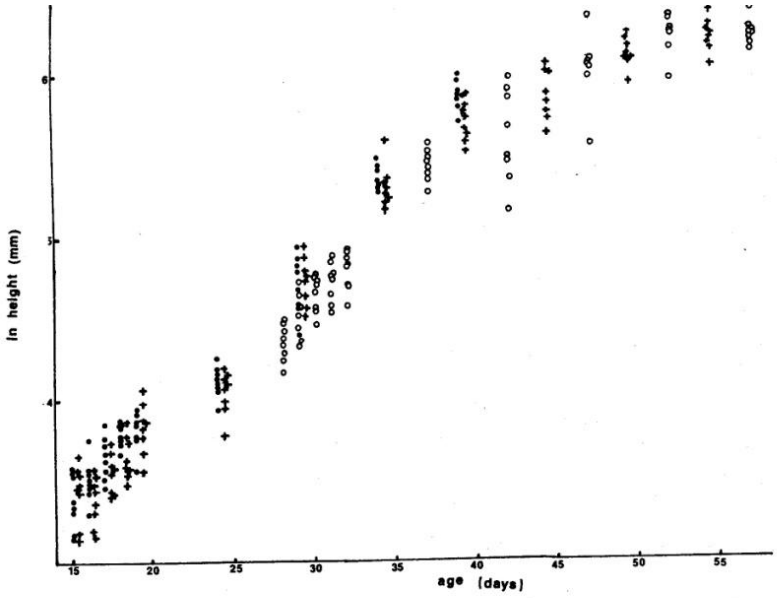



Figure 4




*Figures 1-4. When compared on any given date, all the plants were about the same height (Figure 1). But in an allometric comparison, shade plants (L; open circles) are taller than sun plants (H; closed circles) (Figure 2); plants transferred from sun to shade (HL; +) are taller than plants that remained in sun (H; closed circles) (Figure 3); and plants transferred from shade to sun (LH; x) are shorter than those that remained in the shade (L; open circles) (Figure 4). Incomplete scans are the result of a malfunctioning scanner.*

Another example is relative leaf weight. At any given *time,* sun plants had a lower leaf weight ratio than the plants that grew in or were transferred to the shade (Figure 5). Whoopee, you might say, plants in shade need more leaf weight. But not so fast. For any given *weight,* sun and shade plants had virtually indistinguishable leaf weights (Figure 6), as did plants transferred from sun to shade (Figure 7) or from shade to sun (Figure 8). Leaf weight is, therefore, a stable trait, not a flexible one.

Figure 5

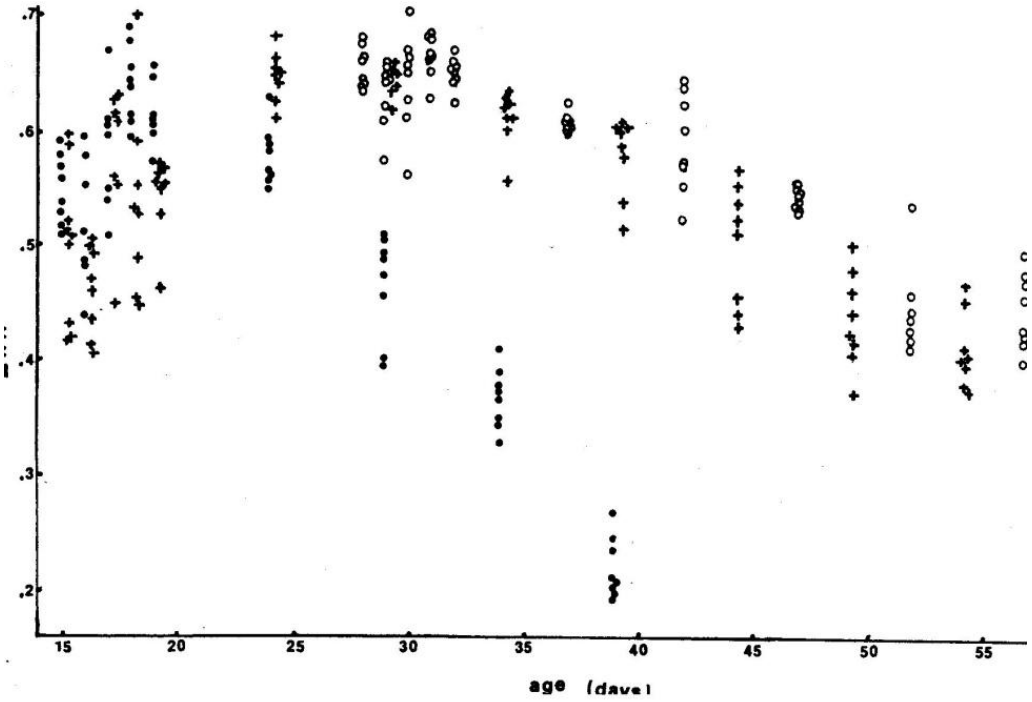


Figure 6

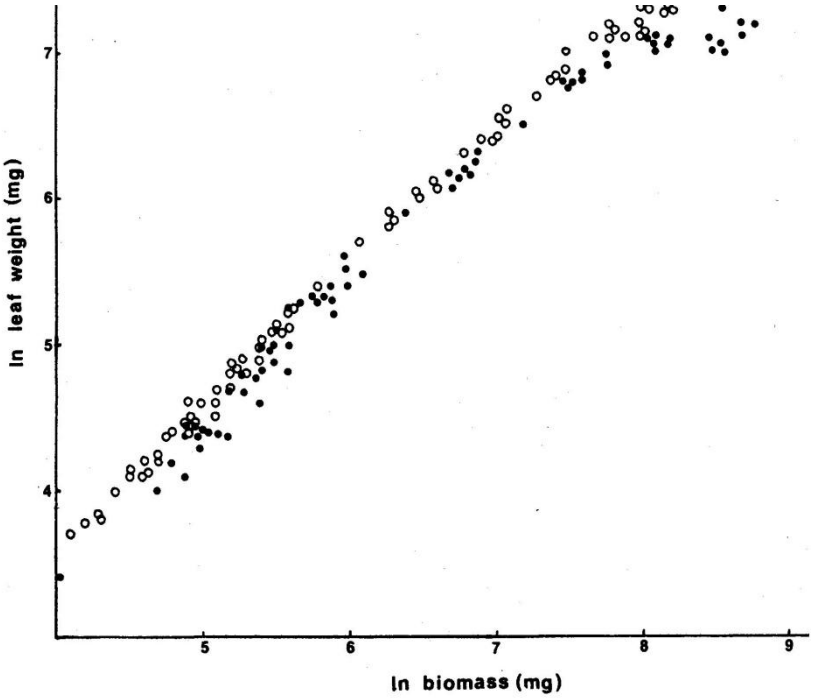



Figure 7

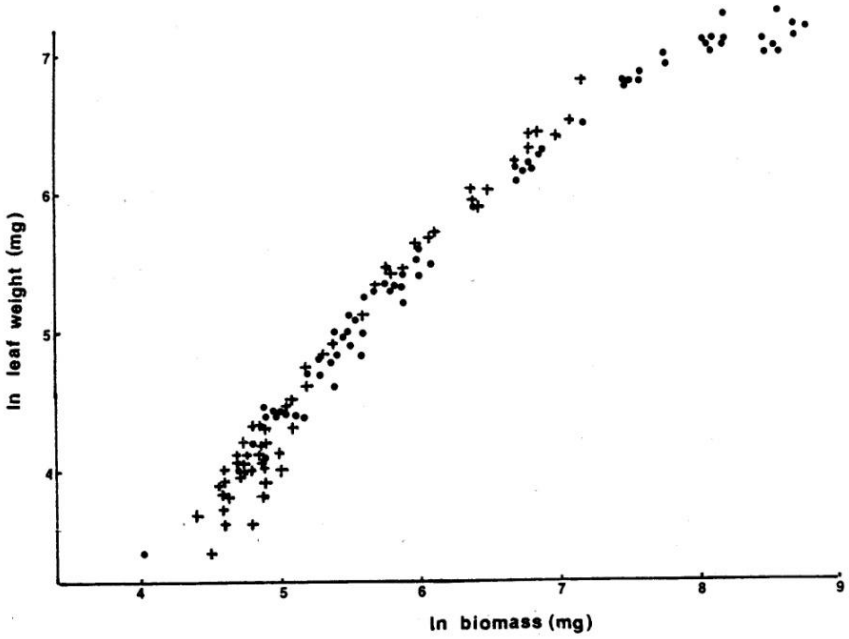


Figure 8

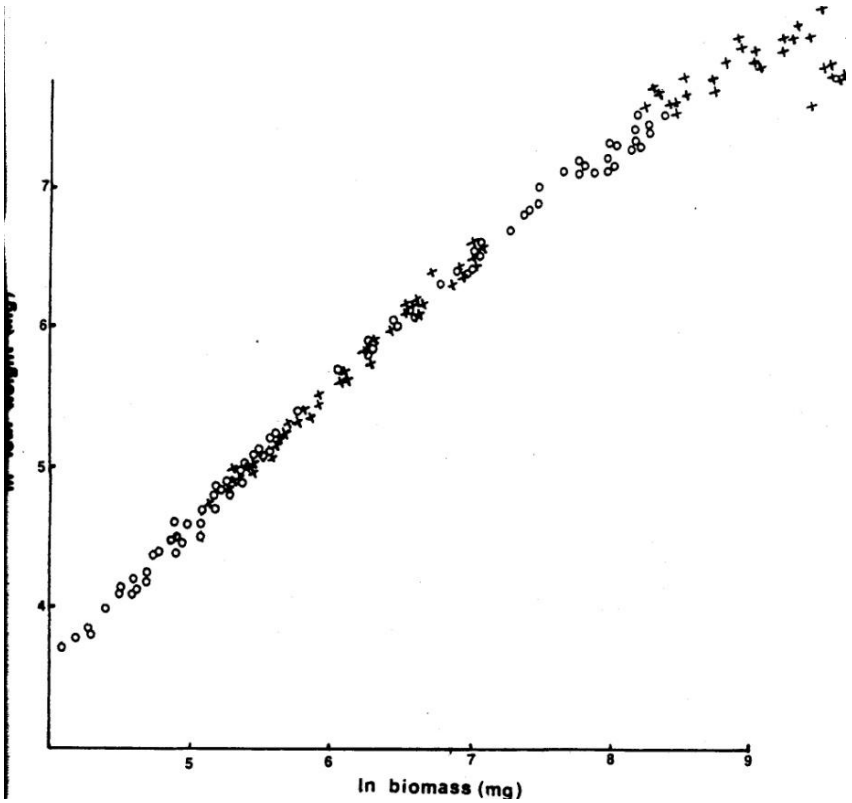



*Figure 5-8. At any given time, the plants differed dramatically in leaf weight ratio (LWR). In particular, the plants that remained in sun had lost many leaves by day 40 (Figure 5). But in an allometric comparison, all the plants had a constant allocation to leaf weight, whether you compare plants in sun (H; closed circles) and shade (L; open circles) (Figure 6), plants that remained in sun (H; closed circles) and those transferred to shade (HL; +) (Figure 7), or plants that remained in shade (L; open circles) and those transferred to sun (LH; x) (Figure 8). Incomplete scans are the result of a malfunctioning scanner.*

Therefore the basis on which you make comparisons between treatments can make all the difference in the conclusion you draw from them. Therefore, in my thesis, I made all comparisons between treatments at a common weight, not at the same time.

Vintage References for Further Reading

Bradshaw, Anthony D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13: 115-155.

Chain, Richard K., and Daniel I. Arnon 1977. Quantum efficiency of photosynthetic energy conversion. *Proceedings of the National Academy of Sciences USA* 74: 3,377-3,381.

Niklas, Karl J. 1994. *Plant Allometry: The Scaling of Form and Process.* University of Chicago Press.

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# CHAPTER 2
# HOW DO YOU KNOW WHETHER FLEXIBILITY MATTERS?

It is one thing to assign a number to how flexible a trait is. It is quite another to know whether the flexibility of the whole set of traits in a plant confers a growth benefit upon it. In this chapter, I developed a way of calculating the growth benefit of plasticity and acclimation. I cannot do these calculations for any individual trait, but only for the entire set of them.

The idea is quite simple. If bright light doubles the immediate metabolism of a plant, and then the plant grows twice as much, this means that the plastic or acclimatory features do not confer any growth benefit. The traits, just as they are, allow the plant to grow twice as much. If the changes (growing more roots or more leaf area) confer a benefit, then they should cause the plant to grow *more than* twice as much. I used, as my measure of metabolic stimulation, the light-induced stimulation of photosynthesis. The benefit conferred by phenotypic flexibility in response to light intensity is the amount of growth that occurs *in excess of* the amount that the light stimulates its photosynthesis.

## Methods I used in Chapter 2

I have already explained how I raised the velvetleaf plants at 200 and at 900 micro-Einsteins per square meter per second and transferred some of the plants between the two light levels. Growth is straightforward, if tedious, to measure. Big plants grow faster than small plants, but not necessarily relative to their weight. A small plant can, relative to its size, grow a lot faster than a large plant. What we need to know is therefore the relative growth rate (RGR), or the growth rate relative to the weight of the plant. RGR (for example, in grams per gram per day, or just $day^{-1}$) can be calculated as the slope of the increase in the natural logarithm of plant weight per day.

You can measure RGR without complex equipment, but you cannot measure photosynthesis without some advanced equipment. The most common way that scientists quantify photosynthesis is by measuring how much carbon dioxide a leaf absorbs. Therefore if you know how big a closed chamber with a leaf in it is, and how much the concentration of carbon dioxide decreases over time, then you can readily calculate the amount of carbon dioxide the leaf has absorbed. You can measure carbon dioxide concentration with infra-red light. Carbon dioxide absorbs infra-red light; the more carbon dioxide is in the air of the closed chamber, the more it depletes the infra-red light. This is the whole basis of the greenhouse effect: carbon dioxide absorbs infra-red light and warms up as a result.

Infra-red gas analyzers (IRGA) are fairly expensive. You can now buy equipment with everything built into it. But back in my day we had to piece together our own systems. I say "we" even though I am not very mechanically-minded and I used the system that others had designed. The IRGA gives a voltage readout, which could be traced onto a moving roll of paper (a servo mechanism). You first have to calibrate the IRGA by flushing gas through it with a

specified concentration of carbon dioxide. That way you know which number, on the paper, corresponds to which concentration of carbon dioxide. You have to calibrate it each day, because even little things can change the IRGA output.

*This photograph shows the Bazzaz lab IRGA in use. The late Art Zangerl is in the background, assisted by Mark Boudreau.*

But that is just the beginning of what you have to consider. The most important factor that determines how much carbon dioxide a leaf absorbs is how open its pores (stomata) are. The stomata are pretty skittish; they will close up if the temperature or the relative humidity are unfavorable. Only if the stomata are all the way open can you know that the leaf is absorbing as much carbon dioxide as its internal cells are capable of using. Therefore, in the little chamber with the leaf in it, you have to control the temperature and the humidity. Furthermore, you have to keep the air in the chamber stirred up; otherwise there might be plenty of carbon dioxide in the chamber but the leaf depletes the carbon dioxide from the air in a thin layer (known as a boundary layer) right next to it and its photosynthesis might slow down as a result. The leaf has to be small enough that the fan inside the chamber can stir up the air underneath it but also on top of it. If the leaf is too big for the chamber, and the air at the top of the chamber is not stirred up properly, that air at the top overheats and can scorch the leaf. Go ahead, ask me how I know. I know this the same way that scientists usually know things: by doing it wrong the first few times.

One final factor that I will mention is that leaves go to sleep every night. Their photosynthesis shuts down. Well, of course, I hear you saying. If there's no light, of course there will be no photosynthesis. But this is not what I mean. I mean that leaves shut down their photosynthesis—primarily by closing their stomata—every night even artificial light is still shining at night. And not just at night, but in the early evening even when (in the high summer,

when velvetleaf likes to grow) the sun is still shining. In our lab we referred to this as five-o'clock shutdown. Plants are union plants; at 5:00 they go home. You can't get them to keep photosynthesizing even if you offer them overtime pay. So if a graduate student measuring photosynthesis gets behind in his or her work, he or she cannot just stay up late to finish it. In this sense, the leaves are sleeping at night. Sleep is not the same as inactivity. When you are asleep you are largely unresponsive to your surroundings. Sleeping leaves are unresponsive to light. Some plants even put their leaves down, from a horizontal to a vertical position, or even close up their leaves, at night. Velvetleaf plants hold their leaves straight out in the day, but at night, the leaves literally clasp up next to the stem.

This hardly needs to be mentioned but the leaf has to be attached to the stem. A detached leaf sitting in a chamber will just die, and pretty quickly. The way to seal up the chamber is by having the top and bottom of the chamber meet in a layer of foam rubber that can be clamped shut without constricting the leaf stalk.

So each morning I would start up the equipment and calibrate it. I would then position the plant so that I could adjust the leaf at a right angle to the light source, which was a big, very bright halogen bulb. I could adjust the light intensity by raising or lowering the light source with a rope on a pulley.

## Results of Chapter 2

Now, with all of that introduction, prepare yourself for a surprise and an anticlimax.

First, the surprise (though in retrospect it should not be surprising). Plants that have developed in the shade grow faster upon transfer to the sun than do plants that have developed in the sun! This sounds like reverse plasticity! If the shade phenotype grows faster in both sun and shade than the sun phenotype, why don't the plants just produce the shade phenotype? Why bother with plasticity?

The reason that shade phenotypes grow faster than sun phenotypes in the sun is almost certainly due to its higher LAR: that is, it has a lot more photosynthetic (leaf) surface area. Even if, per unit area, a shade leaf does not have as high of a photosynthetic rate as a sun leaf, the shade plant has a lot more leaf area. That is what happened in this experiment. In sunny conditions, the sun phenotype had a photosynthetic rate of 23.6 (units given above) while the shade phenotype managed only 12.4. But the shade phenotype more than made up for this with its greater relative leaf area. In shady conditions, the sun phenotype (at 6.7) and the shade phenotype (at 6.9) had virtually indistinguishable photosynthetic rates. This is a well-known phenomenon; shade plants do not necessarily photosynthesize faster in the shade than sun plants. What is going on here?

The answer probably lies with factors other than light. A high LAR allows one heck of a big whole-plant growth rate, but it is risky. Out in the sun, temperatures can get hot and soil can dry out, with the result that a plant with a big LAR can suffer out in the sun as a result mainly of insufficient water supply. Remember plants use transpiration to cool their leaves.

So the apparent reverse plasticity of the shade phenotype in the sun is not surprising after all. And we should just take it into account in our calculations. And this leads us to the anticlimax.

In the following table, I present my calculations for the advantage of plasticity in velvetleaf. I do so in two ways for each of the phenotypes. First, I calculate how much faster or slower the actual plants grew than they would have grown based on the *predicted* RGRs (based on photosynthetic response). Second, I calculate how much faster or slower the actual plants grew than they would have grown based on the *measured* RGRs. Shade plants grew *faster* in the shade than the sun plants would have or did in the shade. Sun plants grew *more slowly* in the sun than the shade plants would have or did in the sun.

| **Sun plants** | |
|---|---|
| Sun plant measured RGR | 25.7% (0.257) per day |
| Sun plant photosynthesis in shade compared to photosynthesis in sun | 28% (0.28) as much |
| *Predicted* RGR of sun plants transferred to shade (product of previous two numbers) | 7.2% (0.072) per day |
| *Measured* RGR of sun plants transferred to shade | 7.4% (0.072) per day |
| Shade plant measured RGR | 27.0% (0.270) per day |
| Factor by which shade plants actually grew compared to the *predicted* RGR of sun plants in the shade | (0.257 ÷ 0.072) = 3.57 times as fast |
| Factor by which shade plants actually grew compared to the *measured* RGR of sun plants in the shade | (0.257 ÷ 0.074) = 3.47 times as fast |
| **Shade plants** | |
| Shade plant measured RGR | 27.0% (0.270) per day |
| Shade plant photosynthesis in sun compared to photosynthesis in shade | 1.81 (81% more) |
| *Predicted* RGR of shade plants transferred to sun (product of previous two numbers) | 48.9 % (0.489) per day |
| *Measured* RGR of shade plants transferred to sun | 37.3% (0.373) per day |
| Factor by which sun plants actually grew compared to the *predicted* RGR of shade plants in the sun | (0.257 ÷ 0.489) = 53% as fast (0.53) |
| Factor by which sun plants actually grew compared to the *measured* RGR of shade plants in the sun | (0.257 ÷ 0.373) = 69% as fast (0.69) |
| Plasticity advantage based on predicted RGRs | 2.05 (average of 3.57 and 0.53) |
| Plasticity advantage based on measured RGRs | 2.08 (average of 3.47 and 0.69) |

In the shade, the shade phenotype actually grew more rapidly than the sun phenotype (3.47 times as fast). My prediction was that they would grow 3.57 times as fast. Those two numbers are pretty close. In the sun, the sun phenotype grew more slowly than the shade phenotype (only 69 percent as fast). My prediction was that they would grow only 53 percent as fast. These two numbers are not too different either. The plasticity advantage based on *predicted* RGRs (2.05) is very similar to the plasticity advantage based on *measured* RGRs (2.08). Notice that from both predictions and measurements, the shade phenotype appeared to be superior in the sun. But all I am trying to do here is to demonstrate that calculating a plasticity advantage based on predicted values is not too different from using measured values.

Why is this important? Because in chapter 4 I will be measuring twelve species of plants. To get a sample size adequate to actually measure RGR responses of all twelve species would require a sample size large enough that I could not have fit them in the growth chambers. I was using velvetleaf as a proof-of-concept plant upon which I could base my work with the twelve species.

From this, you can see why this confusing chapter, though published back-to-back with the first chapter, has not been cited. I even got myself confused as I re-read it. Of course, it has been almost thirty years since I wrote it.

## Vintage Reference for Further Reading

Rice, Stanley A. and F.A. Bazzaz 1989. Growth consequences of plasticity to light conditions in *Abutilon theophrasti*. *Oecologia* 78: 508-512.

# CHAPTER 3.
# THREE HABITATS

All habitats are variable in space and time. They all have spatial heterogeneity on different scales, from large disturbances (fires, etc.) to small molehills. And they all have temporal variability, changing from year to year, month to month, moment to moment. How do you quantify environmental variability? Variability of conditions can be even more important than the average conditions. As the old farmer said, in fifty years of farming, he'd seen only two average years.

Well, you can't measure everything all the time, and if you did, what would you do with all of the data? But you *can* measure *some* things *some* of the time and, if you make your measurements *in the same way* in all of the habitats, you can compare the variability in each habitat with the others. I limited my measures to light and moisture, ignoring temperature and nutrients. I did this because light and moisture affect one another very closely, and light is the variable I manipulated in my experiments for chapters 1, 2, and 4.

All habitats are variable, but not all equally variable. I decided to compare three habitats common (or formerly common) in east central Illinois: the agricultural old field, the tallgrass prairie, and the forest floor.

*Agricultural old fields.* It seemed to me that the agricultural old field would have the most variable light and moisture conditions. An agricultural old field is an abandoned farm that the weeds, then the shrubs, then the trees take over by a process of *ecological succession*. As the shrubs move in, they create shaded microhabitats among the weeds; as trees move in, they create more shaded microhabitats among the shrubs. Even the weeds are all different. Some of them, like *Setaria faberii*, a foxtail grass, are short (except when they aren't), while others, like *Ambrosia trifida*, the giant ragweed, are, well, giant.

*On the left, I am standing beside some giant ragweeds* (Ambrosia trifida) *in a field in 1983. On the right, I am standing amidst some tall goosefoot* (Chenopodium album) *and foxtail* (Setaria faberii)*, which are plants that do not normally grow tall, in 1985, but 1985 was wetter than 1983.*

The earliest weeds to dominate during ecological succession are annuals, which live only one year, and they are generally smaller than the later, perennial weeds. During the first year of succession, the annual weeds may be in full sun, but in the second year, when the annual weeds sprout from seeds, they may find that the perennial weeds (which had started growing, slowly, the first year) have already outgrown them and are casting shade on them.

*In this photo, tiny goosefoot* (Chenopodium album) *seedlings are beginning to grow in spring 1984, amidst corn cobs from when the field was cultivated, and foxtail seed heads from 1983. The goosefoot seedlings are crowded and are already being shaded by perennial weeds that are beginning to grow around them.*

Therefore the annual weeds may experience sunny conditions the first year and shady conditions the second. Meanwhile, the perennial weeds may experience shady conditions the first year and sunny conditions the second.

Furthermore, succession does not occur everywhere in an old field at the same rate. Plant invasion of the abandoned, empty field occurs faster in some places than others. Back when the old field was a farm, it had uniformity imposed on it by the farmer; but let the farm be abandoned and all variability breaks loose.

And as if these were not enough reasons, there are other processes at work. Within an old field, the weed species differ in rooting depths, creating a vertical patchiness of soil moisture, as described by Fakhri and his former grad student Nancy Wieland. Moreover, old fields in which overwintering annuals have established can be quite different from those in which only summer annuals are growing, as described by Fakhri and his former grad student Dudley Raynal.

This is why I expected that an agricultural old field would be very patchy in terms of sun vs. shade and all other environmental variables associated with them.

The patchy plant cover creates patchiness (variability) of light but also of soil moisture, because big weeds withdraw more water from the soil than do small plants. The soil moisture in a patchy old field can be even patchier in dry years than in wet years. In contrast, in a forest, even dry years are fairly uniformly shaded and not much more likely to be patchy than in wet years.

*Tallgrass prairie.* In contrast, the tallgrass prairie seemed like it would be less variable than an old field. The prairie soil is filled with dense roots of grasses and forbs, creating a more or less uniform fabric. Yes, there are prairie fires, but usually when a prairie fire burns the whole prairie, leaving a uniform layer of ashes in the wake of the fire. Most of the grasses and forbs are about the same height—not as different from one another as giant ragweed and foxtail. Sure, a prairie can be disturbed by prairie dogs and bison (which have to wallow in the soil in order to scratch their itches; they don't have hands) but the variability in an old field is created by the diverse assortment of plants themselves, in addition to what animals may do to them. The prairies we used in this study were artificial, or restored, prairies, because there are hardly any natural ones left. Restored prairies might be less variable than natural ones, but they were all we had. But here is a photo of a native prairie in Oklahoma, and it looks just about as uniform as the restored prairies that we used in this study.

*A native Oklahoma prairie, never plowed or grazed.*

*Forest floor.* Then there is the forest floor. Forests have lots of variability, all right, but much of it is up in the canopy. When you are away from the forest edge and down on the forest floor, you are in a hushed atmosphere of relative uniformity. While it is true that stray sunflecks find their way through the canopy, they travel across the forest floor without staying in one location long enough to induce phenotypic changes, although they may induce transient opening or closing of stomata. Besides, all habitats have sunflecks. You just don't see them unless you get underneath the canopy—easy enough to do by walking through a forest, but in a weedy field, you have to crawl through the weeds. Having done this on several miserable days, I can assure you there are sunflecks in a weedy field too.

*Sunflecks down underneath giant ragweeds (Ambrosia trifida) in an old field.*

I therefore expected to find that of these three habitat types, the old field was the most variable, the prairie the second most variable, and the forest floor the least variable. And I set out to discover whether this was so.

Right away you may notice a problem. It is confirmation bias. A scientist who sets out to prove what he already believes runs the risk of seeing only those things that confirm his idea. Politicians, lawyers, and preachers do it all the time and don't have a problem with it. But scientists try to avoid confirmation bias as much as possible. How could I avoid this bias? By using the same sampling technique in all three habitats, that's how.

## Methods I used in Chapter 3

*Choosing the habitat locations.* Variation in soil moisture between two sites can result if one of the sites gets rained on and the other does not. Therefore I chose sites that were all geographically close together, to minimize the risk that rain would fall on one and not another.

*Defining habitat boundaries.* Then there is the problem of defining what you mean by field, prairie, and forest. In nature, no habitat has clear boundaries. I defined habitat boundaries by assuming that a certain set of species could be found anywhere within that habitat. Therefore, in the forests, I avoided large gaps caused by disturbance. These large gaps contained a recognizably different set of species from the undisturbed forest floor. In fact, a large enough gap is actually more or less a field.

*Choosing when to make measurements.* I made all measurements in summer, after the forest canopy had closed and the summer herbaceous plants had grown. I did this because the twelve species I used in Chapter 4 are all summer-active species.

*Choosing the habitat sites.* I made measurements in five old field sites, three restored prairie sites, and three forest floor sites. I made these measurements in the summers of 1984 and 1985. Here are the sites, with the dominant herbaceous species listed for each. The ecological importance values are based on the leaf areas (LAI) I measured as described below.

*Old field #1* was in the Philips Tract Ecological Research Area of the University of Illinois, about seven kilometers northeast of Urbana. This field was used for maize cultivation, then was abandoned and left unplowed in Fall 1982. By 1984 and 1985, here were the dominant herbaceous species:

| Old field #1 | Ecological importance values | |
|---|---|---|
| Species | 1984 | 1985 |
| Goldenrod *(Solidago canadensis)* | 19.3% | 39.4% |
| Aster *(Aster pilosus)* | 17.4% | 27.8% |
| Fescue *(Festuca eliator)* | 15.0% | 12.0% |
| Canada fleabane *(Conyza canadensis)* | 13.6% | |
| Daisy fleabane *(Erigeron annuus)* | 12.3% | 1.2% |
| Goosefoot *(Chenopodium album)* | 9.0% | |
| Curly dock *(Rumex crispus)* | 1.5% | |
| Bluegrass *(Poa pratensis)* | 1.2% | |

*Old field #2* was in the same location. It was used for maize cultivation until Fall 1983. It was plowed in May 1984 then abandoned. By 1984 and 1985, here were the dominant herbaceous species:

| Old field #2 | Ecological importance values | |
|---|---|---|
| Species | 1984 | 1985 |
| Knotweed *(Polygonum pensylvanicum)* | 33.9% | |
| Foxtail *(Setaria faberii)* | 24.3% | |
| Giant ragweed *(Ambrosia trifida)* | 19.9% | |
| Aster *(Aster pilosus)* | 4.8% | 23.4% |
| Curly dock *(Rumex crispus)* | 2.9% | 3.7% |
| Daisy fleabane *(Erigeron annuus)* | | 25.2% |
| Thistle *(Cirsium arvense)* | | 13.4% |
| Evening primrose *(Oenothera biennis)* | | 7.3% |
| Goldenrod *(Solidago canadensis)* | | 5.9% |
| *Potentilla* sp. | | 3.0% |

*Old field #3* was in the same location. It was abandoned from cultivation after a fall plowing in 1968. By 1984 the following herbaceous species and tree seedlings dominated it:

| Species | Ecological importance value |
|---|---|
| Bluegrass *(Poa pratensis)* | 37.2% |
| Goldenrod *(Solidago canadensis)* | 16.4% |
| Snakeroot *(Sanicula gregaria)* | 13.1% |
| Fescue *(Festuca eliator)* | 6.4% |
| Parsnip *(Pastinaca sativa)* | 6.4% |
| Violets (*Viola* sp.) | 2.6% |
| Blackberry *(Rubus occidentalis)* | 2.2% |
| Trumpet creeper *(Campsis radicans)* | 1.7% |
| Black cherry tree *(Prunus serotina)* | 1.1% |

In addition, field #3 had some trees (black cherry, *Prunus serotina;* honey locust, *Gleditsia triacanthos;* and white ash, *Fraxinus americana*).

*Old field #4* was in the same location. It was abandoned from cultivation after being plowed in May 1985. I measured the vegetation only in 1985.

| Species | Ecological importance value |
|---|---|
| Foxtail *(Setaria faberii)* | 82.7% |
| Velvetleaf *(Abutilon theophrasti)* | 19.0% |
| Goosefoot *(Chenopodium album)* | 4.9% |
| Resprouting corn *(Zea mays)* | 1.5% |
| Bindweed *(Ipomaea hederacea)* | 1.1% |

*Old field #5* was adjacent to field #4. It was plowed in fall 1984 then abandoned from cultivation.

| Species | Ecological importance value |
|---|---|
| Daisy fleabane *(Erigeron annuus)* | 30.6% |
| Foxtail *(Setaria faberii)* | 25.3% |
| Canada fleabane *(Conyza canadensis)* | 21.7% |
| Wild lettuce *(Lactuca serriola)* | 12.9% |
| Goosefoot *(Chenopodium album)* | 2.5% |
| Dandelion *(Taraxacum oficinale)* | 1.5% |
| Aster *(Aster pilosus)* | 1.1% |

*Field #5, with lots of daisy fleabane (Erigeron annuus).*

In addition, fields 4 and 5 had quite a few sugar maple seedlings *(Acer saccharum).*

These old fields differed considerably from one another in age and in the species that chanced to dominate them.

*Prairies #1, #2, and #3* were in the Trelease Woods Ecological Research Area, right across the road from Philips Tract. It had been cultivated until 1938 when it was restored into a prairie using native grass seeds. It had been burned repeatedly, most recently in May 1983, prior to my measurements. I avoided areas with weed invasion, although sericea lespedeza had invaded everywhere. I made these measurements in 1984.

| | | Ecological importance values | |
|---|---|---|---|
| Species | Prairie #1 | Prairie #2 | Prairie #3 |
| Indian grass *(Sorghastrum nutans)* | 48.7% | 57.0% | 62.5% |
| Big bluestem *(Andropogon gerardi)* | 20.9% | 32.2% | 11.9% |
| Bluegrass *(Poa pratensis)* | 17.8% | 2.5% | 9.7% |
| Sericea *(Lespedeza cuneata)* | 8.3% | | |
| Goldenrod *(Solidago canadensis)* | 2.7% | | 1.3% |
| Panic grass *(Panicum virgatum)* | 0.9% | | 1.7% |
| Foxglove *(Penstemon digitalis)* | | 2.9% | 1.7% |
| Milkweed *(Asclepias syriaca)* | | 1.8% | |
| Unidentified composite | | | 5.5% |

*Forest floor #1* was at the Brownfield Woods Ecological Research area, about 6 km north-northeast of Urbana and about 1.5 km from the other locations. The dominant trees in this forest were:

| | |
|---|---|
| *Acer saccharum* | sugar maple |
| *Carya ovata* | shagbark hickory |
| *Celtis occidentalis* | hackberry |
| *Juglans nigra* | black walnut |
| *Quercus muhlenbergii* | chinkapin oak |
| *Quercus rubra* | red oak |
| *Tilia americana* | linden |

The understory contained small buckeye (Aesculus glabra) and pawpaw (Asimina triloba) trees.

*Forest floors #2 and #3* were in Trelease Woods (see above) and had a canopy and understory composition similar to forest floor #1. Here are the importance values of the forest floor herbs:

| Species | Forest floor 1 | Forest floor 2 | Forest floor 3 |
|---|---|---|---|
| Nettle *(Laportea canadensis)* | 21.9% | 52.8% | 46.2% |
| Waterleaf *(Hydrophyllum virginianum)* | 31.2% | 3.4% | |
| Wild ginger *(Asarum canadense)* | 19.9% | 12.2% | 3.7% |
| False solomon's-seal (*Smilacina* sp.) | 8.0% | 2.2% | 3.1% |
| Snakeroot *(Sanicula gregaria)* | 4.7% | 8.4% | 21.0% |
| Sweet cicely *(Osmorhiza longistylis)* | 5.3% | | |
| *Begonia* sp. | | 6.9% | |
| Grass *(Hystrix hirsuta)* | | 3.1% | |
| Poison ivy *(Toxicodendron radicans)* | | | 6.5% |

I just thought I might mention that the forest floor was not necessarily a pleasant place. It was hot and humid and buggy, and was dominated by nettles. In only one site, however, was there a lot of poison ivy.

*This photo was taken in Trelease Woods about 1983. Fakhri tells an undergraduate plant ecology student to measure wind velocity in the woods as former graduate student Ed Reekie looks on. In fact, there was almost no wind, in contrast to the fields outside of the forest.*

How did I make my light and moisture measurements? Well, with a little help from undergraduate assistants and later from my wife. Within each of the habitats described above, I used random numbers to choose eight sampling sites.

*Lisette Clarkston, now Lisette Rice, helped me in most of my field measurements of light and moisture.*

*Light measurements.* I measured spatial heterogeneity and temporal variability (variation over space and time) in light conditions. But what techniques did I use? At first, I tried to measure light directly with what is known as a *quantum sensor*. This small sensor measures photosynthetically useful light (PAR). I used a quantum sensor to measure light intensity at many points within several quadrats within each of the habitats. But these measurements were not too useful. For one thing, they were influenced by clouds passing overhead, as they almost always did. I remember one day, predicted to be clear, in which a swath of clouds covered just a tiny bit of sky—the bit between my study site and the sun. And if the wind is blowing, sunflecks—in all three habitat types—flitter around causing the quantum sensor readings to jump all over the place. Clearly, measuring light directly was an exercise in frustration.

*The quantum sensor measures PAR (photosynthetically active radiation) or visible light. The sensor is clamped to a pole at mid-canopy height in a prairie.*

What I ended up doing is a testament to the primacy of clever ideas over expensive technology. I made my measurements with nine sticks, a string, and a nail.

The most important determinant of variability in light intensity in a community of plants is the presence of the leaves of other plants. So why not measure these leaf areas directly? So I put together (with help from duct tape) a meter-square quadrat (four sticks) mounted on four legs (four more sticks); the legs held the quadrat above the herbaceous canopy. I then had a meter stick (a metric version of a yardstick) with notches filed every 10 centimeters. I then tied a nail to a string that had indelible ink marks on it every 15 centimeters. I would move the meter stick along the quadrat 10 centimeters at a time to sample 25 points (a five-by-five grid) covering 0.25 square meters. At each point of a grid, I would lower the string 15 centimeters at a time. If the nail touched a leaf, I would call out which species it was, and my wife would write it down on a piece of paper.

*I made my best "light" measurements with nine sticks, a string, and a nail.*

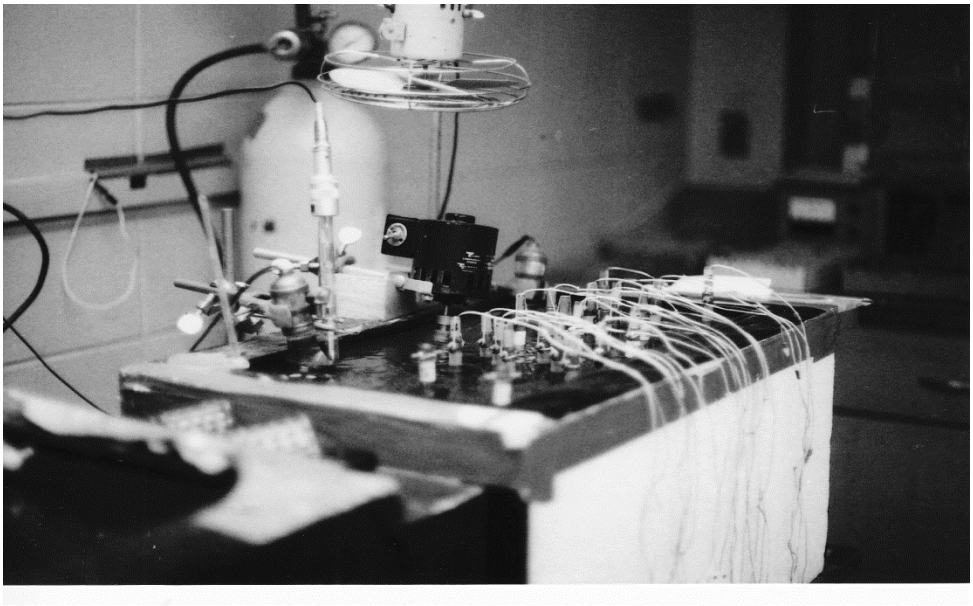
Back in the lab, I could reconstruct a three-dimensional model of the leafy herbaceous canopy. Small-scale variability was how different each point on the grid within a square meter was in terms of total number of leaf layers below it; large-scale variability was the differences among the grids spaced out in the habitat. What we were measuring was large- and small-scale variability in *leaf area index* (LAI). If a square meter of ground area has an average of two square meters of leaf area above it, LAI = 2. This was tedious work but wonderful in a way; it made me look at a field, prairie, and forest floor more closely than I had ever done before. It's amazing what you can see when you look closely.

There are all kinds of other possible ways of measuring light intensity integrated over time. I even briefly considered a technique in which I would place some single-celled algae in a little bottle of nutrient solution, and leave these little bottles scattered all over the habitats. Greater sunlight intensity, integrated over time, would cause more algae to grow. I cannot remember why I decided against using this, but it might have been because I would have to leave them there for several days, and nocturnal animals may have disturbed them or even carried them off in their curiosity to know what that interesting human scent was.

*Soil moisture measurements.* I also measured spatial heterogeneity and temporal variation in soil moisture. What you really want to know, to understand what the plants are experiencing, is how tightly held the water is to the soil particles rather than simply how much water was present in the soil. That is, you want to measure the potential energy of the water *(water potential).* But especially back then this would have been an expensive proposition. It also takes a long time for each sample. If you wanted to make lots of measurements, you would have to wait hours to measure at most a half dozen samples at a time. Do the math and you will see that this approach was not practical. What you gain in quality of the data you lose by having a smaller sample size.

So I used another cheap trick. My wife and I took soil samples from lots of places in each habitat, weighed them, dried them, and weighed them again. This was a simple measure of soil water content. And within certain limits, water content and the potential energy of the water are closely related. It would not be true in very dry desert soils, where a very slight difference in water content could cause a major difference in water potential (the tightness with which the soil holds the water), nor would it be true in soggy soil where too much water is actually bad for the roots. The difference between soggy soil and soil that is twice as soggy is, in water content terms, a factor of two, but to the plant it might make no difference at all. But in the moderate soil moisture conditions of the habitats I was studying, this difference was not crucial. We could get many dozens of soil samples each evening (when the soil moisture was not changing as rapidly), and then take our time (within reason) weighing, drying, and re-weighing them, so long as they were stored in airtight metal soil cans.

As a matter of fact, I did check to make sure that, in a subset of my samples, the soil moisture content and the potential energy were similar. From samples of known water content, I removed small cores of soil and measured the potential energy with which the water was held using a home-made device rigged up by plant physiologist John Boyer.



*John Boyer's isopiestic technique for measuring water potential. Samples were inside of metal chambers kept in a water bath the temperature of which could be controlled to within a tiny fraction of a degree. Diffusion of water vapor to or from a drop of solution on a thermocouple indicated whether the sample had a higher or lower water potential than the solution; diffusion of water out of the drop caused a measurable cooling of the drop.*

It was wonderful to see how elegantly Boyer's *isopiestic technique* worked. Using an equation I calculated from these measures, I could transform all of my soil moisture content data into terms of potential energy of water.

I took five soil samples from each of the eight sample areas of each habitat (the same ones used for LAI) on each of 12 days in 1983 and each of seven days in 1984. As described above, not all measurements were made in both years.

*Effects of vegetation on soil moisture.* At each of eight randomly selected sites within fields #4 and #5, I removed the vegetation from one square meter and left the vegetation intact in an adjacent square meter. Once in June and once in July 1985, I took five soil samples from each of the pairs of plots.

*Biological significance of variation in light.* Does the measured spatial heterogeneity in light and shade have any biological significance? I decided to investigate this by the use of phytometers.

*Phytometers* are where you use plants to measure environmental conditions rather than by measuring them directly. They will give you, so to speak, a plant's-eye view of what is going on in the environment. You grow some plants, place them out in the habitat you are studying, and measure their response. In this study, I measured the transpiration response of velvetleaf plants:

- I grew the velvetleaf plants in plastic pots in the greenhouse.
- I took the plants out into the eight locations in Field #4, eight locations in Field #5, and eight locations in Prairie #1. Within each location, I used eight potted plants, for a total of 192 plants.
- I wrapped aluminum foil around the pots and soil surface (to make sure that all the water lost by the plant and pot was from the leaves).
- I weighed the pots.
- I left the pots in various locations in the fields and prairie at mid-canopy level for a few hours.
- I retrieved the pots and weighed them again. The weight difference represented how much water each plant had transpired.
- I determined the leaf area of each plant, so that I could calculate transpiration rate per unit leaf area.

My notes are unclear about the details of what I did. For example, my thesis contains no sample sizes for these measures. However, I did run across a photograph that showed eight phytometers suspended with clamps, four each on two metal poles, at one of the locations. The only problem is that this was in a forest, from which I presented no data in my thesis.

*Eight phytometers (potted velvetleaf plants) are suspended above the forest floor in 1985.*

While the use of phytometers sounds great, it has some disadvantages:

- It is a lot of work. You have to grow and measure a lot of plants.
- Transpiration can be affected by lots of factors other than the one you think you are studying (in my case, shading).
- The plants themselves begin to acclimate. They probably would not do so during the course of such measurements, however.

## Results of Chapter 3

*Overall weather conditions.* The three summers on which I made measurements were very different from one another.

| Month | Temperature departure | Rainfall departure |
|---|---|---|
| June 1983 | +1.4 C | +13.1 cm |
| July 1983 | +3.2 C | -5.5 cm |
| June 1984 | +3.3 C | -7.0 cm |
| July 1984 | -1.9 C | +2.8 cm |
| June 1985 | -1.6 C | +3.9 cm |
| July 1985 | -1.2 C | +1.5 cm |

In 1983, a very wet and moderately warm June was followed by a very dry and hot July. July 1983 included a 19-day drought that caused considerable agricultural loss. In 1984, a warm dry June was followed by a cooler, wet July. In 1985, both June and July were cool and wet. All departures are from the 1890-1980 average. All data came from the Illinois State Water Survey.

*Light measurements.* I present here only the variances of the LAI measures as described above. I used ANOVA to partition variation into between-habitat, between-sample-site, and within-sample site-components. Rather than to present all the data, I will present only the variance in shading *that occurred among the eight sample sites within each of the habitats* and the variance in shading *that occurred within each of the eight sample sites*. The habitats (e.g. the four old fields, the three prairies, the three forest floors) did not differ from one another in variability. I also present the average (mean) LAI for each of the groups of habitats.

| | Three fields 1984 | Four fields 1985 | Three prairies 1984 | Three forests 1984 |
|---|---|---|---|---|
| Mean LAI | 3.32 | 2.77 | 3.23 | 1.62 |
| Variance between sample sites | 1.70 | 1.16 | 0.76 | 0.20 |
| Variance within sample sites | 3.86 | 3.57 | 2.37 | 1.08 |
| Total variance | 5.62 | 4.64 | 3.21 | 1.26 |

Even though the light intensity was only about two percent as great above the forest floor herbaceous layer as above the prairie and field herbaceous layers, the mean LAI was about half as great. Those forest floor herbs were using the light very efficiently. On the average, both field and prairie herbaceous layers had about three layers of leaf above each point on the ground.

The results had to be analyzed numerically. But the following graphs provide a dramatic visual representation of just how much more variable the fields are than the prairies and the prairies than the forest floor in terms of vegetation cover. Each graph represents one of the habitats in one or both years 1984 and 1985. Each line represents the cumulative total LAI as you go from the top to the bottom of the vegetation layer within one of the eight sample plots in each habitat. It is obvious that the lines differ greatly from one another in the fields, less so in the prairies, and very little on the forest floors. Fakhri used these figures as an example of how old field plant species experience a great deal of variability in light conditions.

Figure 9

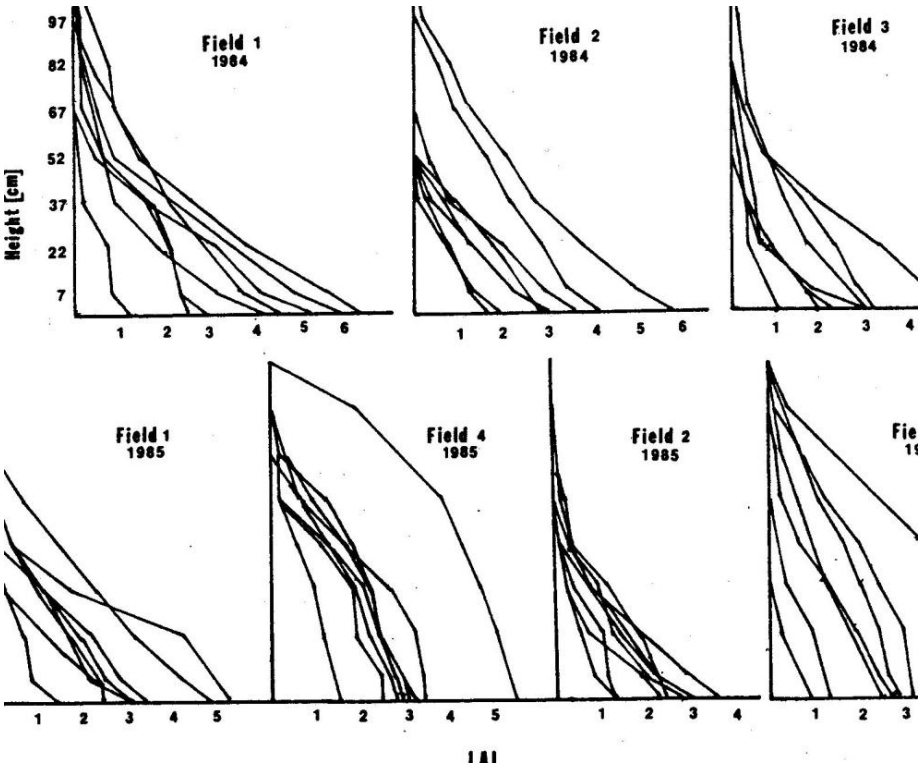


Figure 10

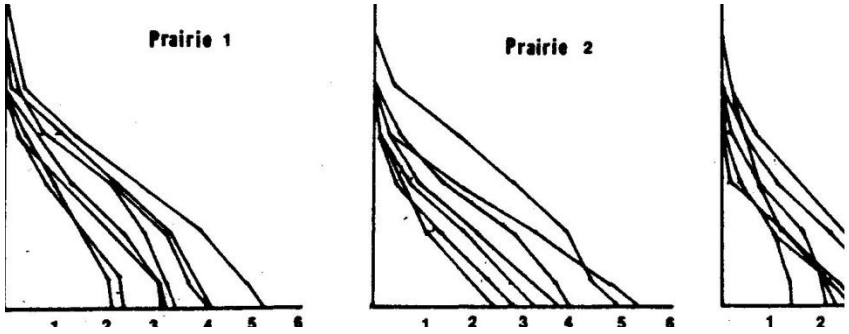
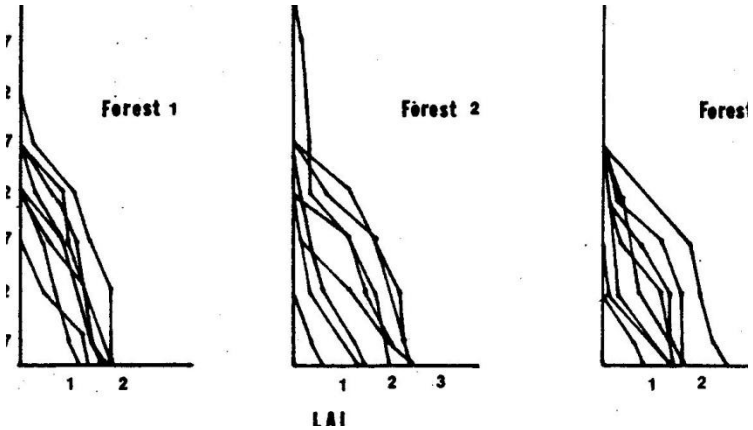



Figure 11




*Cumulative leaf area index (LAI) in fields, prairies, and forests in 1984 and 1985. The dependent axis is height in cm, therefore the lines represent shading vs. height for 8 locations in each habitat. Incomplete scans are the result of a malfunctioning scanner.*

The results here are very clear: the fields (in both years) had greater variation in shading caused by leaves, both between sample sites and within them, than the prairies, and the prairies had more variation than the forest floors. The four numbers for the between-sample variance all differed significantly from one another, as did the four numbers for total variance.

*Soil moisture measurements.* I made measurements in 1983 and 1984. I present here variances in soil moisture variation between different sample locations; within sample locations in each of the habitat types; and total variance. I also calculated other variance components, not here presented.

| Variance component | Fields 1983 | Fields 1984 | Prairies 1983 | Prairies 1984 | Forests 1983 | Forests 1984 |
|---|---|---|---|---|---|---|
| Between sample sites | 0.62 | 1.39 | 0.70 | 1.51 | 0.21 | 0.38 |
| Within sample sites | 7.61 | 5.81 | 3.64 | 2.19 | 0.34 | 0.55 |
| Total | 18.2 | 23.7 | 11.5 | 14.4 | 0.74 | 1.90 |

These results are also very clear. In both years, almost all variance components are greater in the fields than in the prairies, and in the prairies than in the forests. For both years, total variation in soil moisture differed significantly among the three habitat types.;

*Effects of vegetation on soil moisture.* Within fields #4 and #5 in June and July of 1985, plots from which vegetation had been removed had more water (average water potential -0.247 MPa) than plots in which vegetation was left intact (average water potential -0.287 MPa). Negative numbers mean dry soil. These differences are statistically significant.

*Phytometers.* The phytometers showed significant place-to-place variation in transpiration rate in all three habitats:

| Variance component | Field #4 | Field #5 | Prairie #1 |
|---|---|---|---|
| Large-scale | 25.41 | 18.57 | 16.82 |
| Small-scale | 13.24 | 14.08 | 6.89 |
| Total | 36.44 | 31.03 | 20.06 |

The variances were calculated from transpiration rates in mg per $cm^2$ of leaf area per hour. Each of the large-scale variances is significantly different from the other. Overall, this also shows that conditions in old fields are more variable than in prairies. But what can you say from just two fields and one prairie? The two fields differ from one another as much as either does from the prairie. The only point I need to make, and the only point I can make, is that the variation in shading within these habitats is big enough to affect transpiration.

All of these measurements confirm a general trend: environmental conditions, measured directly or as experienced by plants, is greatest in old fields, intermediate in prairies, and smallest on the forest floor.

## Vintage References for Further Reading


Bazzaz, Fakhri A. 1996. *Plants in Changing Environments: Linking Physiological, Population, and Community Ecology*. Cambridge University Press.

Raynal, Dudley J., and Fakhri A. Bazzaz 1975. Interference of winter annuals with *Ambrosia artemisiifolia* in early successional fields. *Ecology* 56: 35-49.

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# CHAPTER 4
# DO PLANTS IN MORE VARIABLE ENVIRONMENTS HAVE GREATER PHENOTYPIC FLEXIBILITY?

In chapter 2, I explained that you can calculate a plasticity advantage that reflects the growth advantage that results from the plant's ability to develop different phenotypes in sun and shade. In chapter 3, I presented evidence that old fields are more variable in light conditions than prairies, which are more variable than forest floors. It follows directly, then, that plant species that live in old fields should have a greater plasticity advantage than plant species that live in prairies, and prairie plant species should have a greater plasticity advantage than plant species that live on the forest floor.

I chose four plant species from each habitat type. They were all herbaceous perennials. I considered these species to represent all the herbaceous perennial species in their habitats. Of course this limits the external validity of the study, but it was a pretty good number anyway. I wanted to avoid the extraneous effects of life cycle (annual vs. perennial) and the production of woody tissue. I did the following:

- I grew these plants in bright and in shady conditions inside of growth chambers.
- I measured their relative growth rates (RGR) in those two conditions.
- I measured their photosynthetic responses to light.
- I used the photosynthetic responses to estimate what the RGR in bright and in shady conditions would be in the absence of phenotypic flexibility.
- I compared these estimated growth rates with the actual ones to calculate a plasticity advantage for each of the phenotypes of each plant species.
- I averaged these two plasticity advantages together to get a plasticity index for the species.

I had no idea how lucky I was to be able to do this experiment. It is just about the closest thing I have ever experienced to a miracle. I gathered seeds and grew seedlings for each species. In order to do this, I had to break the dormancy of the seedlings. I assumed I knew how to do this: gather the seeds in the fall, keep them in a refrigerator under cool conditions over the winter, and then raise them in plastic pots in the growth chamber in the spring. Easy, right? Well, it turned out that one of the species (sweet cicely, *Osmorhiza longistylis*) doesn't work that way. When you gather the seeds, you first have to store them in warm moist conditions, to get the embryo to develop, and then store them in cool moist conditions, to break dormancy. I would not have known this had it not been for a chance encounter with the gurus of seed germination, Carol and Jerry Baskin, at an Ecological Society of America meeting in 1984. Had it not been for this encounter, I would have failed to grow *Osmorhiza* seedlings for this last experiment. I suppose the experiment would have continued, but the elegance of the experiment—four species from each of three habitats—would have been eleven-ized.

The twelve species were:

Old field herbaceous perennials:

- *Aster pilosus,* the old field aster
- *Solidago canadensis,* the goldenrod
- *Cichorium intybus,* the chicory
- *Taraxacum oficinale,* the dandelion

Each of these species forms a dense rosette of leaves next to the ground in its first year, then they produce upright leafy stems beginning their second year—except for the dandelion, which produces only an aerial stem for dispersing its seeds. Chicories and dandelions are introduced Eurasian weeds. All four species are in the plant family Asteraceae.

Prairie herbaceous perennials:

- *Aster laevis,* the prairie aster.
- *Ratibida pinnata,* one of the prairie coneflowers.
- *Silphium terebinthinaceum,* the prairie dock, which produces (when it is older) huge and extremely sandpapery leaves without lobes.
- *Silphium laciniatum,* the compass plant, which produces leaves similar to prairie dock but it has deep lobes. It is called compass plant because its leaves tend to face east and west.

Each of these species grows as a dense rosette of leaves its first year, and all four are in the plant family Asteraceae.

Forest floor herbaceous perennials:

- *Aster shortii* (Asteraceae), a forest aster.
- *Laportea canadensis* (Urticaceae), the abundant nettle species.
- *Sanicula gregaria,* the snakeroot (Apiaceae).
- *Osmorhiza longistylis,* the sweet cicely (Apiaceae).

Each of these species, except the nettle, produce a dense rosette of leaves during their first year of life. The nettle produces an upright leafy stem its first year. The American species of the genus *Aster* are now classified in a separate genus, but were not at the time of this thesis. I do not have good photographs of these plants growing in their native habitats, but here are some photos of some of them in the growth chamber:

*These photographs show some of the herbaceous perennial plant species I used in the experiment for this chapter, including Osmorhiza longistylis and Silphium laciniatum (top) and Sanicula gregaria, Laportea canadensis, and Silphium terebinthinaceum (bottom).*

I gathered these seeds from either the same ecological study areas used in Chapter 3 or, for prairie species, a remnant, high-diversity railroad prairie, just before it was bulldozed by a railroad company. (Much good this did them. The bulldozed area, once held in place by a beautiful tapestry of prairie forbs, was promptly filled in by ragweeds and poison ivy.)

Results of Chapter 4

And now, the moment you've all been waiting for, the climax that I haven't thought about in 27 years. We are now going to calculate predicted RGRs (assuming that the RGR of the "wrong" phenotype changes to the same extent that the photosynthesis changes), and then compare this predicted RGR to the actual RGR. Let's start with the H phenotype.

| | RGR of L phenotype | Photosynthetic light response of L phenotype in high light | Predicted RGR of H phenotype (from L phenotype in high light) | Actual RGR of H phenotype | Advantage or disadvantage of actual relative to predicted H phenotype |
|---|---|---|---|---|---|
| **Old field** | | | | | |
| *Aster pilosus* | 0.026 | 1.913 | 0.050 | 0.134 | 2.680 |
| *Solidago* | 0.178 | 1.218 | 0.217 | 0.215 | 0.991 |
| *Cichorium* | 0.142 | 1.849 | 0.263 | 0.211 | 0.802 |
| *Taraxacum* | 0.083 | 1.706 | 0.142 | 0.246 | 1.732 |
| **Prairie** | | | | | |
| *Aster laevis* | 0.143 | 1.715 | 0.245 | 0.202 | 0.824 |
| *Ratibida* | 0.082 | 1.433 | 0.117 | 0.199 | 1.700 |
| *Silphium terebinthinaceum* | 0.044 | 1.525 | 0.067 | 0.070 | 1.045 |
| *Silphium laciniatum* | 0.008 | 2.004 | 0.015 | 0.021 | 1.400 |
| **Forest floor** | | | | | |
| *Aster shortii* | 0.109 | 1.590 | 0.173 | 0.261 | 1.509 |
| *Laportea* | 0.145 | 1.735 | 0.251 | 0.153 | 0.609 |
| *Sanicula* | 0.115 | 1.510 | 0.174 | 0.139 | 0.800 |
| *Osmorhiza* | 0.065 | 1.500 | 0.098 | 0.094 | 0.960 |

Let's use *Aster pilosus* for an example. The L phenotype grows in low light at a rate of 0.026 per day. But its photosynthesis can respond to high light by a factor of 1.913. We could therefore predict that the H phenotype could grow in high light at a rate of 0.026 x 1.913 = 0.050 per day. The H phenotype actually grows, however, at 0.134 per day, so the H phenotype grows in high light 2.680 times as fast as we would predict.

In six of these species, the H phenotype has at least a slight advantage over the L phenotype in high light. In six of them, however, the H actual phenotype actually grows worse than the predicted H phenotype in high light. But those are the results that I got.

Now let's do the same thing with the L phenotype.

| | RGR of H phenotype | Photosynthetic light response of H phenotype in low light | Predicted RGR of L phenotype (from H phenotype in low light) | Actual RGR of L phenotype | Advantage or disadvantage of actual relative to predicted L phenotype |
|---|---|---|---|---|---|
| **Old field** | | | | | |
| *Aster pilosus* | 0.134 | 0.266 | 0.036 | 0.026 | 0.722 |
| *Solidago* | 0.215 | 0.380 | 0.082 | 0.178 | 2.171 |
| *Cichorium* | 0.211 | 0.295 | 0.062 | 0.142 | 2.290 |
| *Taraxacum* | 0.246 | 0.237 | 0.058 | 0.083 | 1.431 |
| **Prairie** | | | | | |
| *Aster laevis* | 0.202 | 0.410 | 0.083 | 0.143 | 1.723 |
| *Ratibida* | 0.199 | 0.419 | 0.083 | 0.082 | 0.989 |
| *Silphium terebinthinaceum* | 0.700 | 0.357 | 0.025 | 0.044 | 1.760 |
| *Silphium laciniatum* | 0.021 | 0.294 | 0.006 | 0.008 | 1.258 |
| **Forest floor** | | | | | |
| *Aster shortii* | 0.261 | 0.413 | 0.108 | 0.109 | 1.009 |
| *Laportea* | 0.153 | 0.524 | 0.080 | 0.145 | 1.806 |
| *Sanicula* | 0.139 | 0.563 | 0.078 | 0.115 | 1.469 |
| *Osmorhiza* | 0.094 | 0.440 | 0.041 | 0.065 | 1.571 |

In most of these species, the L phenotype grows better in low light than the H would grow, based upon its photosynthetic response.

Now let's bring these numbers together to calculate an average plasticity for each species. The first two numerical columns come from the final columns of the previous two tables:

| | Plasticity advantage of the H phenotype | Plasticity advantage of the L phenotype | Average | Rank |
|---|---|---|---|---|
| **Old field** | | | | |
| *Aster pilosus* | 2.680 | 0.722 | 1.701 | 1 |
| *Solidago* | 0.991 | 2.171 | 1.581 | 3 |
| *Cichorium* | 0.802 | 2.290 | 1.546 | 4 |
| *Taraxacum* | 1.732 | 1.431 | 1.582 | 2 |
| **Prairie** | | | | |
| *Aster laevis* | 0.824 | 1.723 | 1.274 | 8 |
| *Ratibida* | 1.700 | 0.989 | 1.345 | 6 |
| *Silphium terebinthinaceum* | 1.045 | 1.760 | 1.403 | 5 |
| *Silphium laciniatum* | 1.400 | 1.258 | 1.329 | 7 |
| **Forest floor** | | | | |
| *Aster shortii* | 1.509 | 1.009 | 1.257 | 10 |
| *Laportea* | 0.609 | 1.806 | 1.208 | 11 |
| *Sanicula* | 0.800 | 1.469 | 1.135 | 12 |
| *Osmorhiza* | 0.960 | 1.571 | 1.266 | 9 |

Therefore, the ranks of the indices of phenotypic flexibility of the species line up perfectly with the ranks of the variability of their habitats:

| | Rank of flexibility | Rank of habitat variability |
|---|---|---|
| **Old field** | | |
| *Aster pilosus* | 1 | 1 |
| *Solidago* | 3 | 1 |
| *Cichorium* | 4 | 1 |
| *Taraxacum* | 2 | 1 |
| **Prairie** | | |
| *Aster laevis* | 8 | 2 |
| *Ratibida* | 6 | 2 |
| *Silphium terebinthinaceum* | 5 | 2 |
| *Silphium laciniatum* | 7 | 2 |
| **Forest floor** | | |
| *Aster shortii* | 10 | 3 |
| *Laportea* | 11 | 3 |
| *Sanicula* | 12 | 3 |
| *Osmorhiza* | 9 | 3 |

I still marvel that these results came out so perfect. I checked and rechecked my calculations to make sure they were correct. At first I thought I had found an error, then I discovered that my recalculations were in error. These results show that the average plasticity advantage of the two phenotypes is greatest for the old field species, intermediate for the prairie species, and smallest for the forest floor species. There actually is a way to analyze whether this is a significant correlation. Spearman's rho calculates a correlation coefficient for ranks. These data were correlated at rho = 0.946, and were significant at the $p < 0.01$ level.

What do these flexibility indices actually mean?

- A plasticity advantage of the H phenotype means the H phenotype actually grew faster than you would expect an L phenotype to grow in high light.
- A plasticity advantage of the L phenotype means the L phenotype actually grew faster than you would expect an H phenotype to grow in high light.

But I cannot see an immediate pattern in these results:

| | H phenotype | L phenotype |
|---|---|---|
| **Old field** | | |
| *Aster pilosus* | Grew more than predicted | Grew less than predicted |
| *Solidago* | Grew about the same | Grew more than predicted |
| *Cichorium* | Grew less than predicted | Grew more than predicted |
| *Taraxacum* | Grew more than predicted | Grew more than predicted |
| **Prairie** | | |
| *Aster laevis* | Grew less than predicted | Grew more than predicted |
| *Ratibida* | Grew more than predicted | Grew about the same |
| *Silphium terebinthinaceum* | Grew about the same | Grew more than predicted |
| *Silphium laciniatum* | Grew more than predicted | Grew more than predicted |
| **Forest floor** | | |
| *Aster shortii* | Grew more than predicted | Grew about the same |
| *Laportea* | Grew less than predicted | Grew more than predicted |
| *Sanicula* | Grew less than predicted | Grew more than predicted |
| *Osmorhiza* | Grew about the same | Grew more than predicted |

These patterns were not evident from any individual traits, such as the plasticity of LAR or of root weight.

| | LAR (L relative to H) and rank | Root weight (H relative to L) and rank |
|---|---|---|
| **Old field** | | |
| *Aster pilosus* | 1.72 (10) | 0.77 (12) |
| *Solidago* | 2.03 (6) | 1.32 (6) |
| *Cichorium* | 1.55 (11) | 1.09 (9) |
| *Taraxacum* | 3.13 (2) | 6.51 (1) |
| **Prairie** | | |
| *Aster laevis* | 2.97 (3) | 1.09 (9) |
| *Ratibida* | 2.60 (4) | 2.62 (2.5) |
| *Silphium terebinthinaceum* | 2.02 (7) | 1.24 (7) |
| *Silphium laciniatum* | 7.64 (1) | 1.44 (5) |
| **Forest floor** | | |
| *Aster shortii* | 2.35 (5) | 2.62 (2.5) |
| *Laportea* | 1.96 (9) | 1.48 (4) |
| *Sanicula* | 1.97(8) | 0.83 (11) |
| *Osmorhiza* | 1.20(12) | 1.09 (9) |

However, there was a slight tendency for plasticity of photosynthetic light response to follow the same order (old field more than prairie more than forest floor). I calculated plasticity of photosynthesis in this way:

- Plasticity to high light: How much higher photosynthesis the H phenotype had than the L phenotype in high light. These numbers were usually greater than one.
- Plasticity to low light: How much higher photosynthesis the L phenotype had than the H phenotype in low light. These numbers were usually greater than one.
- I then averaged these two plasticities.

| | Plasticity to high light | Plasticity to low light | Average (rank) |
|---|---|---|---|
| **Old field** | | | |
| *Aster pilosus* | 1.268 | 1.547 | 1.408 (4) |
| *Solidago* | 1.327 | 1.629 | 1.478 (2) |
| *Cichorium* | 1.191 | 1.536 | 1.364 (5) |
| *Taraxacum* | 4.217 | 0.588 | 2.403 (1) |
| **Prairie** | | | |
| *Aster laevis* | 1.045 | 1.361 | 1.203 (10) |
| *Ratibida* | 1.591 | 1.046 | 1.319 (6) |
| *Silphium terebinthinaceum* | 1.919 | 0.957 | 1.438 (3) |
| *Silphium laciniatum* | 1.288 | 1.316 | 1.302 (7) |
| **Forest floor** | | | |
| *Aster shortii* | 0.975 | 1.564 | 1.270 (8) |
| *Laportea* | 0.795 | 1.380 | 1.088 (12) |
| *Sanicula* | 1.352 | 0.870 | 1.111 (11) |
| *Osmorhiza* | 1.445 | 1.040 | 1.248 (9) |

Photosynthetic flexibility did not correlate as well with habitat variability as did total phenotypic flexibility (rho = 0.828, significant at $p < 0.01$) but it was still significant. Photosynthetic flexibility and phenotypic flexibility, of course, correlated pretty closely (rho = 0.909, $p < 0.01$). I say "of course," but when I started this work there was no way to know that I could have just measured photosynthetic light response and ignored all the tedious work with relative growth rates.

The overall message, then, is as follows:

- Recently disturbed old fields are the most variable habitats, and their plant species have the greatest growth advantage due to plasticity.
- The forest floor is the least variable habitat, and its plant species have the least growth advantage due to plasticity.
- The prairies are intermediate in variability and in the growth advantage that plasticity confers on their plant species.

Each species of plant seems to have arrived at its own way of achieving a growth advantage due to plasticity. Frequently one of the phenotypes (but never both) had a negative growth advantage. This would imply that it would be better if the plant expressed just one phenotype instead of two. But the average of the growth responses was always greater than one.

This fits in perfectly with an evolutionary expectation: plasticity of each trait is an evolved characteristic because it confers a growth advantage. Since these plants were all long-lived perennials, it is not possible to calculate their fitness, but undoubtedly greater growth resulting from plasticity can allow greater fitness. Plasticity isn't just something that happens; it evolved. Of course, plasticity and stress both occur at the same time and may be inseparable. But plasticity is part of the irrepressibility of life.

This rewritten thesis is almost as long as the original, but it is in plain English and is intended to be accessible and educational. I hope that I have rescued it from oblivion and that it might prove to be of some benefit to people who want to understand the natural world a little better.