Graft 4 states in reverse positions.
Which creates two unfriendly contacts
Pennsylvania-South Carolina & Maryland-Georgia

		New Jersey
		Pennsylvania
		MARYLAND
New Jersey	New Jersey	VIRGINIA
Pennsylvania	Pennsylvania	NORTH CAROLINA
Maryland	South Carolina	South Carolina
Virginia	North Carolina	North Carolina
North Carolina	Virginia	Virginia
South Carolina	Maryland	Maryland .
Georgia	Georgia	VIRGINIA
Florida	Florida	NORTH CAROLINA
		SOUTH CAROLINA
		Georgia
		Florida

These unfriendly contacts are fixed by creating 2 new Virginias,
2 new North Carolina, 1 new Maryland & 1 new South Carolina

Presumably a new "South of the Border" will open
And 1 or 2 new Districts of Columbia.

But no state will be adjacent to any state it wasn't previously been next to!
(at the cost of creating six new states)

I am not sure if you need to reverse four states in order to get 3 branches.
But if you only reverse 3 states, then you get only 4 new magically-created states.

			New Jersey
			Pennsylvania
New Jersey	New Jersey	New Jersey	Maryland
Pennsylvania	Pennsylvania	Pennsylvania	Virginia
Maryland	North Carolina	North Carolina	North Carolina
Virginia	Virginia	Virginia	Virginia
North Carolina	Maryland	Maryland	Maryland
South Carolina	South Carolina	South Carolina.	Virginia
Georgia	Georgia	Georgia	North Carolina
Florida	Florida	Florida	South Carolina
			Georgia
			Florida

One North Carolina is between two Virginias,
And one Maryland is between two Virginias.

But if you only reverse two states, then you only have to duplicate those 2 states.
(That's not a very good triple branch, however.

New Jersey	New Jersey	New Jersey
Pennsylvania	Pennsylvania	Pennsylvania
Maryland	Maryland	Maryland
Virginia	North Carolina	Virginia
North Carolina	Virginia	North Carolina
South Carolina	South Carolina	Virginia
Georgia	Georgia	North Carolina
Florida	Florida	South Carolina
		Georgia
		Florida

Many cases of "intercalary regeneration" are known, like this

Oklahoma, Arkansas, Tennessee, North Carolina
Oklahoma, Arkansas, Tennessee, North Carolina
Oklahoma, *Arkansas, Tennessee,* North Carolina
Oklahoma, Arkansas, Tennessee, North Carolina

Therefore, perhaps we should think of the triple branching in response to rotated grafts as resulting from two intercalations.
(close-range interactions, instead of long-range radial coordinates)

Oklahoma, Arkansas, Tennessee, North Carolina
Oklahoma, Tennessee, Arkansas, North Carolina
Oklahoma, Arkansas, Tennessee, Arkansas, Tennessee, North Carolina
You would need three Mississippi Rivers, however; one for each Tennessee-Arkansas border

You could think about this as a kink in the intercalation regeneration, maybe?

Most of the scientists were mentally invested in graph paper types of explanation, instead of unfriendly neighbors trying to intercalate familiar intermediates.

How can we visualize John Saunders quadruple wing experiment?
Will we need to think in three dimensions?

He grafted a reversed axis wing tip onto the side of a normal axis wing tip

ABCDEFGHI
IHGFEDCBA

This creates two broad discontinuities, nearer the ends.
A next to I, B next to H, G next to C, F next to D, and then repeats of all these.

Also, it starts with two anteriors and two posteriors.

How do they compare with the situation of a reversed tip being grafted in place of a cut off tip? Are the discontinuities worse? Are there more anteriors and posteriors? There is "one more" wing tip, after all; Is that why 4 wings form instead of 3?

ABCDEFGHI
ABCFEDGHI

ABCdeFEDefGHI is a way to visualize a triple-branched wing bud.

Anterior bump Anterior
Posterior Posterior

Posterior Posterior
Anterior Anterior

connection
to the
body
of the chicken

Anterior *Intercalate?* Posterior

Intercalate? *Intercalate?*

Posterior *Intercalate?* Anterior

But this seems to predict eight wings?
Or does it. Maybe only two of the intercalation occur.
Or maybe you get just one wing per intercalation event?

It would really help to have seen some of the intermediate stages between the limb tip with the reversed limb tip grafted to its side, and the end result with the four wings.

In particular, where do the two planes of reflection symmetry come from

Notice that the four wings have two planes of reflection symmetry,
But the initial condition has no planes of symmetry,
Except maybe a rotational version of glide reflection symmetry
If you reflect one wing tip, but not the other,

then they acquire reflection symmetry relative to each other.

The material below was added on April 22 at 7 pm:

Virginia		Virginia			South Carolina	
North Carolina	is OK!	North Carolina	is also OK!	And so is	North Carolina	perfectly OK!
South Carolina		Virginia			South Carolina	

QUESTION: Do you suspect this has anything to do with why conjoined twins have mirror-image symmetry to each other,
And are joined hip to hip, or head to head, or stomach to stomach. Notice that all the cells and organs along where such twins are conjoined are like the North Carolina that is next to two the two Virginias, and the North Carolina that is next to two South Carolinas. These tissues are not next to any other tissues that it is not normal for them to be next to. In fact, they are next to TWO copies
(one on each side) of some of the neighbors, even though entirely lacking the neighbors they would usually have on the other side!

Please notice that this is not a logical necessity, for tissues to be intolerant of abnormal neighbors, but not to be disturbed by lacking one set or normal neighbors, and also not to be disturbed by being flanked on both sides by duplicate copies of one of their normal neighbors. It is an observable fact about embryos, with many examples, but not logically necessary. It must mean something about how the control mechanisms work.

Virginia South Carolina	cannot be allowed to continue? A new North Carolina would have to be regenerated.

This regeneration could be done by taking some of the counties from southern Virginia, and combining them with some of the counties of northern South Carolina. Using these, a new replacement for the missing North Carolina could be constructed.

Would you think of this replacement process as probably resulting from close range interactions, instead of long range signals?

Next consider the effect of reversing positions of several states, for example…

Maryland South Carolina North Carolina Virginia Georgia	How would this reversal be repaired? As above, you could take a few counties from Virginia and construct a new North Carolina, and also take a few Georgia counties and modify them to make a replacement South Carolina. That would fix the lower discontinuity. To fix the upper discontinuity, where Maryland touches South Carolina, would require constructing duplicates of Virginia and North Carolina

Maryland
VIRGINIA

NORTH CAROLINA
South Carolina
North Carolina
Virginia
NORTH CAROLINA
SOUTH CAROLINA
Georgia

This lower discontinuity, where Virginia has been pushed up against Georgia, can be made more tolerable by converting some of their newly-adjacent counties into duplicates of North and South Carolina (probably with a new "South of the Border" between them)

These imaginary geographical changes are meant as analogies to the Bryants' rotations of regenerating newt limb bud tips.

Would you interpret such experimental results as supporting long-range "positional information" types of signal?

That was how the Bryants interpreted their results, except that they proposed using polar graph paper instead of the x-y kind.

My own intuition is different; these kinds of results seem to fit close-range interactions, recognize when they are adjacent to the wrong neighboring tissue, and respond by changing gene expression so as to fill in the gaps, despite this having the result of duplicating tissues, even to the extent of making 3 branched legs. (Or 4 branches, in one of John Saunders' experiments).

I hope that I am not being unfair to the Bryants if I suggest that their training in departments where "Positional Information" had recently been invented had the effect of causing them to interpret these limb-triplification results in terms of a variation on "Positional Information" (Specifically, polar coordinates). Most other embryologists (and all textbooks) have accepted this interpretation.

More than that, these experimental results have been generally accepted as <u>having been predicted</u> by "Positional Information".

One reason that can't be correct is that triple branching had already been described a few years before "Positional Information was proposed. In particular, John Saunders described triple branching of chicken wing buds in 1968, or before.

Another reason is that it is a separate question whether tissues can detect being next to the wrong neighbors, as opposed to whether the different tissues "learned" their differences by means of a shared set of long-range signals.

Maybe I am wrong, but I think that has been the basic logic, apart from the details of the two clock faces and the idea or radial coordinates. Tissues that can detect that they ought not to be next to each other, must (therefore!?) have learned from the same source what tissues they <u>should</u> be next to. By extension, those shared signals must be long range. Don't get me wrong: I have never heard or read this explicit reasoning. But it seems to be what people were thinking. If any students in this course (or anyone else) can explain to me how I am wrong, I will be very positively impressed.

Much of the reasoning of embryology is intuitive, and not explicit. This is true now as much or more than in the past. People visualize gradients, etc. and imagine what effects they could have. It is difficult to visualize even one set of causes. Seldom does anyone visualize two or more alternative sets of causes. Unless you consider two or more

alternatives, that make different predictions, then you can't do real experiments. For those whose minds are stuck in the groove of one theory, all experimental results seem to confirm that theory, unless these results are absolutely contradictory to the favored theory, and there is absolutely no way to adjust the theory to make it consistent. In the limb tip reversal experiments, the adjustment was substituting radial coordinates in place of x-y coordinates.

Suppose someone had developed a theoretical framework according to which each part of the body decides its geometry based close-range interactions. For example, B develops because that tissue is just beyond A, or because it is between A and C; and A develops where tissues are between B and the end; and D develops from whatever tissue is just beyond C, or between C and E; and E develops from whatever tissue finds itself next to D and F, etc. Lots of close range interactions, which could go around in a loop. Maybe Z develops from any tissue located between Y and A.

Next, imagine these tissues can replace missing tissue and fill in gaps. That's not really an extra assumption: 1) because many tissues really can fill in gaps, and 2) because the advocates of positional information also assume the ability to fill in gaps.

If CDE can be filled in between AB and FG, then what ought to happen if CDE is not just removed, but grafted back into position with its orientation reversed, so as to produce ABEDCFG? One possibility is that E would change its behavior and become like C, and vice versa, with the misplaced C converting itself into E. That would fix the problem.

Alternatively, cells at the newly created BE boundary might duplicate what should be between B and E, by forming a new C and D.
And cells at the imposed, abnormal boundary between C and F might duplicate the D and E that are supposed to be between C and F.

Either one of these responses could have been "predicted" from either the long-range type of theory just as much as from the short- range type of theory. Neither result disproves either category of theory, although they may seem to. For a person who starts out believing in long-range signal types of theory, either experimental result could be fitted into their theory, and would therefore seem to confirm what was already believed. The same is true for persons who started out visualizing development in terms of combinations of many close-range interactions.

The most difficult stage of experimentation is to be able to visualize more than one alternative theory, at the same time. Or you could decide to believe one theory on Mondays, Wednesdays and Fridays, but believe the alternative theory on Tuesday, Thursday and Saturdays, taking Sundays off to doubt both of them, and try to think of a third. You need to sympathize with both theories enough to figure out what one predicts should or can happen, that the other predicts should not happen, or be impossible.

Saunders' discovery of the 4-branching chicken wings is difficult to predict either based on long range, positional information theories, or on the basis of close range interactions. Incidentally, the long-range quality of Positional Information is less central to that way of thinking than the idea that coordinated development of different tissues is because all these tissues are getting quantitative variations of one set of signals. That is as opposed to neighboring tissues signaling each other how to behave.

Perhaps Saunders' 4-branching chicken wings somehow disprove either the long-range, shared signal type of control mechanism,
or alternatively disprove explanations in terms of many close range interactions.
Honestly, I can't visualize how to interpret (or to have predicted) the 4 branches based on either type of theory. It would probably help to know what the intermediate stages were like.
Is one of the four a continuation of the original tip of the limb bud, while one of the others is a continuation of the grafted tip, and the remaining two are intercalations between the original two. Alternatively, maybe the graft and the original tip both branched into two. Are either of these sequences predicted by either of the kinds of theoretical assumptions (long-range common signal, etc.)?

I should find Saunders' papers and re-read his interpretations. My impression was that he was not theoretically inclined.

Sometimes unjustified theories become widely accepted (e.g. Positional Information) because there no alternative theories are proposed and or not advocated strongly enough.

Many good scientists regard theoretical papers as idle guessing, intellectually or morally equivalent to betting on a lottery, instead of as a necessary stage in working out logical alternatives, and figuring out experimentally testable alternatives.

Ask yourself whether physics could have succeeded, except by respecting the process of developing theories.
In my opinion, the greater complexity of biological phenomena means that we need good, careful theories even more than physics did in the years since Newton. This is a minority opinion, which you should not feel pressured to agree with. I respect good counter-arguments.