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SPOTLIGHT

Wolpert's French Flag: what's the problem?

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ABSTRACT

Two phrases attributed to Lewis Wolpert – 'positional information' and 'The French Flag Model' – have become so intertwined that they are now used almost interchangeably. Here, I argue that this represents an unfortunate oversimplification of Wolpert's ideas that arose gradually in the developmental biology community, some significant time after his key papers were published. In contrast to common belief, Wolpert did not use the phrase French Flag 'Model' but instead introduced the French Flag 'Problem'. This famous metaphor was not a proposal of how patterning works, but rather an abstraction of the question to be addressed. More specifically, the French flag metaphor was an attempt to de-couple the problem from the multiple possible models that could solve it. In this spirit, Wolpert's first article on this topic also proposed (in addition to the well-known gradient model) an alternative solution to the French Flag Problem that was self-organising and had no gradients, and in which each cell 'cannot compute where it is in the system', i.e. there is no positional information. I discuss the history and evolution of these terms, and how they influence the way we study patterning.

KEY WORDS: French flag model, Positional information, Wolpert

A much celebrated anniversary this year is the 50th birthday of 'positional information' – an influential concept that was introduced by Lewis Wolpert in his famous paper of 1969 (Wolpert, 1969). This was his first comprehensive discussion about the ways that spatial gradients might specify patterns of cell fates in a tissue. There are a number of interesting points sometimes overlooked about this seminal paper. For example, it is often suggested that Wolpert only discussed molecular concentration gradients to encode positional information, while in fact he clearly stated that other kinds of spatial variables could also be used, such as gradients of timing or even oscillator phase differences – an idea proposed to him in the same year by Goodwin and Cohen (Goodwin and Cohen, 1969; Wolpert, 1989). Indeed, many of Wolpert's early ideas deserve more attention. However, in this Spotlight I wish to discuss a different but related phrase that is commonly, but mistakenly, attributed to him: the 'French Flag Model'.

This phrase has become so closely associated with the concept of positional information that the two phrases are sometimes used interchangeably. The most common statement of this idea, repeated over many decades now, can be paraphrased as follows: to achieve a rigorous definition of a developmental field, Lewis Wolpert introduced the concept of positional information illustrated by his French Flag Model, in which a signalling gradient across the field is converted into a pattern of gene expression domains by the concentration-specific response of target genes. (Kraut and Levine, 1991; Briscoe and Ericson, 1999; Panman and Zeller, 2003; Ephrussi and St Johnston, 2004; Jaeger and Reinitz, 2006; Umulis et al., 2008; Dahmann et al., 2011; Restrepo et al., 2014). Within the developmental biology community, we have replicated this idea so often that the 'French Flag' (Fig. 1A) can be considered an icon of the field, and the gradient-threshold mechanism itself as a 'dogma', with both the positive and negative connotations that this implies. On the one hand, Wolpert is often praised for introducing a powerful paradigm, while on the other hand his ideas are sometimes considered too naïve, simplistic and static. Anniversaries are good moments to reflect on history. So here I highlight how re-reading some of Wolpert's earlier theoretical papers reveals that our simplified recollections of his early ideas have lost some of the important insights, which were broader and more subtle than he is credited for. I will start by working through some of the discrepancies to see where this takes us – from apparently pedestrian observations, to progressively more interesting ones.

First, I should point out that we have missed the 50th anniversary of the French Flag! Although Wolpert's famous introduction of the term 'positional information' was indeed published 50 years ago, in 1969, the French Flag was first introduced to us the year before, in a book about theoretical biology edited by Waddington. The article was entitled: 'The French Flag Problem: A Contribution to the Discussion on Pattern Development and Regeneration'. Second, the phrase French Flag 'Model' never appeared in the article – only the French Flag 'Problem' – an observation that is equally true for his subsequent more famous paper of 1969 (Wolpert, 1969). In fact, Wolpert did not use the phrase French Flag Model in any of his subsequent research articles (over almost three decades). Is this important, or just the usual evolution of language over time? Is there any importance to the distinction between 'French Flag Model' and 'French Flag Problem'? Let's consider his 1968 French Flag article in more detail to re-examine what he was trying to say.

By the late 1960s, discussions about spatial patterning, and how gradients might be invoked to explain them, had been going on for a while (Rose, 1952; Webster, 1966), but were rather vague. The focus was more on individual fate regions, and how they influenced each other, rather than global mechanisms to span and coordinate a whole field. A rare example of a concrete hypothesis came from Sylvan Meryl Rose, who was trying to understand how a series of structures (the hypostome, distal tentacles, gonophores, proximal tentacles and then stolon) could be formed in the correct sequence during regeneration of *Tubularia* (Rose, 1957). His patterning model suggested that while the first region (the hypostome progenitor region) is forming at the distal end it inhibits the formation of the next region (the distal tentacles) until its formation is finished. Once the second region is specified, it then allows the third to form, and so on until the whole sequence is created. This resulted in an apparent gradient in the rate of differentiation, but there was no global coordination – there was a sequential domino-like effect propagating through the tissue.

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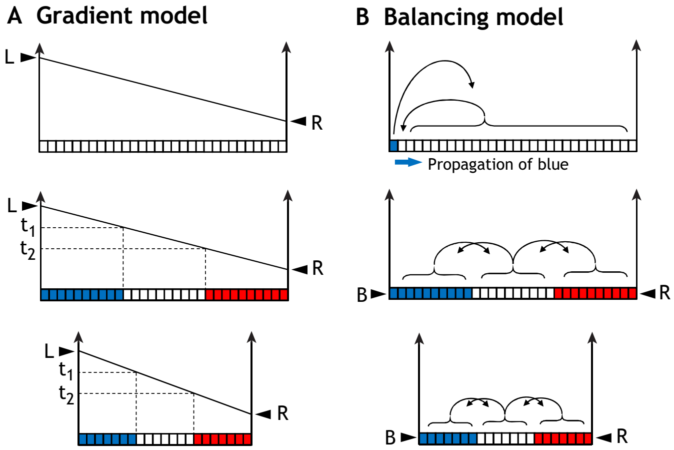

Fig. 1. Two models to solve the French Flag Problem. (A) The first model that Wolpert proposed (the 'gradient model') has become an icon for the developmental biology community. In this model, a variable (*y*-axis) displays a spatial gradient across the field (top panel), and different cellular fates (colours) are specified at different values of this gradient (middle panel). The positions where fates switch from one colour to the next are specified by threshold values (t_1, t_2). A boundary condition must be imposed to ensure scaling of the pattern: the value of at the left (L) and right (R) boundaries must be kept fixed. If the tissue size changes (by cutting or growing, bottom panel), provided the left and right boundary values are maintained, then the full range of positional values will also be maintained across the altered tissue. (B) The second model that Wolpert described (the 'balancing model') has no gradients and no positional information. It functions by the following simple rule: if a cell has a neighbour with a different colour, it will adopt that colour if the substance produced by that other fate has a lower concentration than its own. In the scenario shown (top panel), the greater number of white cells means that the white diffusible substance is at a far higher concentration; this stimulates the white boundary cell to switch to the blue fate. Repeating this process multiple times will result in the blue/white boundary shifting to the right, until equal amounts of the blue and white substance are produced. A different set of rules can produce the French Flag (middle panel), provided the boundary conditions are maintained, i.e. with a blue cell (B) on the left and a red cell (R) on the right, the pattern can exhibit perfect scaling on different sized domains (bottom panel).

Wolpert stated that his goal was to construct some concrete models that exhibit the key behaviours seen in real experiments. But before imagining models, his first step was to create a definition of the problem to be solved. The key features he considered were: (1) that the pattern should be defined by the proportions of its different regions, rather than their absolute sizes (scale invariance within a certain range); and (2) that sub-regions of the pattern were competent to re-form the whole pattern, to reflect the process of tissue regeneration. The popular models of domino-like specification were unsatisfactory because a temporal sequence of events, each step dependant on the one before, cannot be fully regulative, i.e. earlier steps cannot retrospectively be altered by changes to later ones. Wolpert thus recognised that a fully regulative pattern would require some form of 'dynamic equilibrium' (Wolpert, 1969) across the whole pattern. Only in this way could changes at one point in the tissue (e.g. extra growth at one end or cutting out a sub-region) feed back to adjust the whole pattern. This naturally incorporated the idea that cells anywhere in the field were not irreversibly committed to a given fate, but remained in an equilibrium state that could be changed at any moment.

Wolpert's second step was to capture some of this behaviour in a memorable metaphor. Describing patterned tissues as flags was ingenious and quite specific because a flag has no intrinsic size. Projecting the French tricolore onto a huge palace wall, or printing a small version on a business card does not alter what it is. Large or small, the definition of a French flag lies purely in maintaining the correct proportions of blue, white and red. As such, it describes the outcome of a process – the final state – without specifying the 'mechanism'. Although the Union Jack was mentioned in this first article as an example of a very complex 2D pattern (and the Stars and Stripes in the subsequent paper), the French Flag (which is essentially a 1D pattern) remained the key metaphor for fairly intuitive reasons. The process of induction (the earlier focus of embryologists) could be captured by a two-coloured flag, which would represent the induced fate and the default uninduced fate (which is out of reach of the inducer molecule). However, to go beyond this – to capture the concept of a single integrated field divided into multiple coordinated regions – requires at least three colours. Going further to include a fourth colour does not alter the concept, and so is unnecessary.

Now that the problem had been clearly defined, Wolpert's third step was to find some solutions, i.e. to devise some 'concrete models' that would satisfy the required patterning behaviour. He noted very clearly that 'From our attempts at the French Flag Problem, there now seems to be two main ways for solving it' (Wolpert, 1968). He called the first solution a 'fixed gradient model', and this appears to be his first published description of the famous idea alluded to as a dogma above (Fig. 1A). This popular idea is so embedded in the minds of developmental biologists that it needs little explanation here. I will only emphasize that the phrase 'positional information' typically implies that every cell has access to information about its position in the field, and therefore this concept is necessarily linked to a spatial gradient of some type. Positional information must be a variable of something that is detectable by each cell and that indicates position (accurately or roughly). If one considers a line of cells at sequential positions through a tissue, their distances from the edge vary smoothly and monotonically along the line. The positional variable sensed by the cells (if it exists) should also vary monotonically, and therefore must be, by definition, a spatial gradient. Two years later, Francis Crick used the term 'morphogen' to describe such a molecule (Crick, 1970), although the word had actually been invented by Alan Turing 18 years earlier: 'the word being intended to convey the idea of a form producer' (Turing, 1952).

A key assumption of Wolperts's fixed gradient model concerned the boundary conditions. A primary goal was to explain size invariance, or scaling, e.g. regeneration of the normal pattern if smaller pieces were cut out from the original tissue. This was achieved by postulating that specific boundary conditions are imposed onto the tissue, i.e. that the variable has two distinct fixed values at the two ends of the field. In truth, this aspect of scaling was never explained within the gradient model itself – it was simply acknowledged as a necessary requirement for scaling. However, recent proposals for self-scaling gradients, e.g. the 'expansion-repression feedback control model' of Ben-Zvi and Barkai (2010), do effectively satisfy this condition. This model reliably ensures that the level of morphogen at each end of the gradient maintains fixed values independently of the size of the field (although it functions by a more plausible and explicit feedback reaction throughout the whole tissue, rather than the simplistic idea of externally fixing boundary conditions, which would be hard to explain). Irrespective of the specific model considered, the consequence is the same – the full range of positional values will be evenly distributed across the tissue, no matter how long or short the field is (Fig. 1A), and thus the different coloured/fate regions of the pattern will always maintain their correct proportions. So far, no surprises.

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However, Wolpert also proposed a second solution, called the 'balancing model', which could not have been more different (Fig. 1B). Rather than using a gradient, it postulated two basic rules. First, that cells of different states (blue, white and red in the case of the French Flag Problem) would each produce a different substance that was highly diffusible and rapidly degraded. The fast diffusion of the substances ensures that they do not form spatial gradients, but instead form uniform overall concentrations that act as a measure of the relative size of that region. Second, cells could only change fate if they were at the boundary between two regions, and would adopt the fate that produced the substance at lower concentrations. It is easiest to illustrate this model by imagining a flag with just two regions, e.g. blue and white. The initial condition may be that all cells are white, except for one blue cell specified on the left edge of the tissue (Fig. 2B). Both the blue and the white diffusible substances are produced, but a far higher concentration is reached for the white substance. The leftmost white cell, which is touching the single blue cell, follows the rule that if it detects a higher concentration of the white substance than the blue, then it switches to a blue fate. Thus, the blue region expands cell-by-cell at the expense of the white region until the two regions are the same size (when the concentrations of blue and white substances are equal). Wolpert pointed out that the coloured regions of the pattern can easily be specified to have different sizes (e.g. with the white region being twice the size of the blue region) simply by adjusting the production rates of the diffusible substances (e.g. by producing the blue substance twice as fast as the white).

This is the most forgotten, and perhaps the most surprising, aspect of Wolpert's landmark paper. Contrary to popular belief, in this original article which introduced the French Flag to the developmental biology community for the first time, Wolpert created and demonstrated a model in which there is no gradient and no positional information, and which relies on a form of local self-organisation. As with the fixed gradient model, this model was capable of size regulation: if the tissue was halved in size, the pattern would reorganise to create the correct 1:1:1 ratios of blue, white and red (Fig. 2B). Also like the fixed gradient model, tissue polarity in the balancing model needs to be imposed at the boundaries, e.g. by specifying blue on the left boundary and red on the right boundary. Although this does constitute some external positional input to the system, the majority of cells have no clear information about their position. In Wolpert's own words, such a model can 'form the French Flag, which will regulate perfectly. This type of model may be contrasted with the previous one. Not only can a unit never compute where it is in the system, but the system has no gradient. This latter point is of great interest as it shows that gradients are not required for pattern regulation.'

Effectively, this was a gradient-free model to solve the French Flag Problem. So where does this leave the phrase French Flag 'Model'? Wolpert's motivation for introducing the French Flag was clear. It was not to describe a model or mechanism or solution – that came later – but rather to define the problem. In fact, the real value of the flag metaphor was specifically that: to emphasize the distinction between problems and models. The French Flag captures perfectly what has to be achieved by the end of the process (e.g. equal proportions of blue, white and red), without saying anything about how the coloured proportions could be achieved (e.g. by painting, printing, or projecting the flag), or indeed anything about the size of the flag. This represents a crucial concept in science – that we should strive to clearly define what we are trying to understand, independently of the mechanism that may explain it. It is this de-coupling that frees us to search for multiple alternative hypotheses, which can then each be contrasted and tested experimentally.

With the distinction between problems and solutions now clearly in mind, let's reconsider a couple of well-known examples of tissue patterning. As we've seen, the French Flag Problem may be solved using a model that employs positional information (specifically a morphogen gradient) or, alternatively, a model that uses neither a gradient nor positional information (Fig. 2). What about other patterns? While the French Flag may be a good metaphor for regionalisation (with the three vertical bands of different colour representing, for example, the head, trunk and tail of an embryo), a historical flag known as the The Rebelious Stripes (a forerunner of the US Stars and Stripes) is a good representation of periodic patterning (where the repeated vertical red stripes may symbolise, for example, axial segmentation of early embryos). If we consider solutions to this Striped Flag Problem, again we can find at least two types of model. In similar fashion to the French Flag Problem, we can imagine a first model based on positional information (Fig. 2). This could be driven by the gradient of a molecular morphogen, which is interpreted by multiple threshold responses, each positioning the left or right boundary of a stripe. An alternative model to solve the Striped Flag Problem involves the already very famous self-organised diffusion-driven dynamics of a Turing system (Turing, 1952) (Fig. 2). Like the balancing model for the French Flag Problem, this model is self-organised with no global gradients or positional information. It should be noted that although a simple Turing system does not satisfy one key criterion of a flag problem – scale invariance – modified Turing systems have been discovered in which the wavelength of the pattern does scale with domain size (Ishihara and Kaneko, 2006).

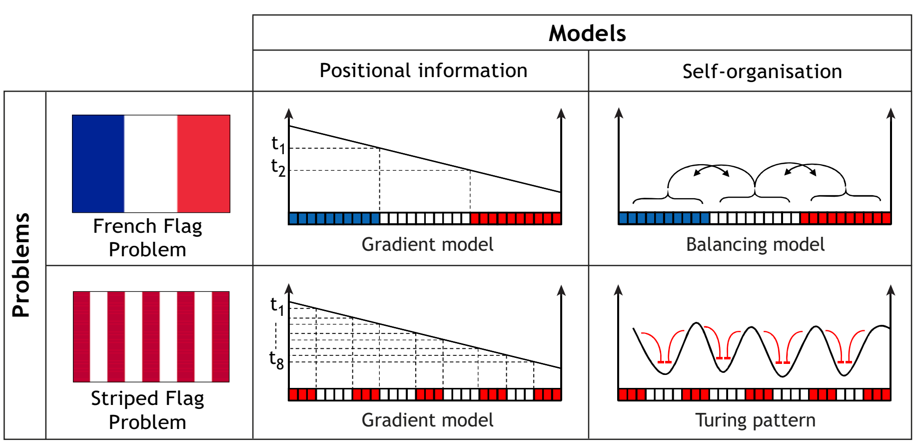

Fig. 2. Flags as metaphors for patterning outcomes. An ingenious idea of Wolpert was to use flags as metaphors of patterning problems, thereby de-coupling them from their multiple theoretical solutions. Thus, problems/flags can be viewed orthogonally to models (rows versus columns in the table shown). The French Flag Problem represents regionalisation, while the Striped Flag Problem can represent periodic patterning. Both flag problems can theoretically be solved by using either a gradient model or a gradient-free self-organising model.

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Reviewing all four cases (the intersection of two types of problem, and two types of model; Fig. 2) highlights why we should question the phrase 'French Flag Model'. Flags are patterns, and therefore outcomes. They represent the behaviour that a model should display (both the proportions of the target pattern and the scale invariance), rather than the mechanism itself. Keeping the concepts separate helps to emphasize that for each problem there may be multiple, very different, solutions. In particular, even the famous French Flag Problem has a solution with no gradients or positional information, while conversely Turing systems are not the only way to create a stripy patterns.

If Wolpert's idea was so clear in his original article, how did we end up with the current strong association of the French Flag with a particular gradient-based mechanism? It is hard to answer such a question with certainty, but a number of observations are interesting. Surveying the literature reveals that, for the first 15 years after its publication, there was plenty of discussion about the French Flag Problem, and almost no mention of the phrase French Flag Model – the community was largely adhering to Wolpert's proposal to use flags as a metaphor for the outcome rather than the mechanism (Fig. 3). Interest in both versions of the concept seemed to lie dormant during the 1980s and 1990s (probably owing to the phenomenal success of molecular biology, which emphasized temporal aspects of gene regulation at the expense of thinking about spatial phenomena) and then a dramatic revival of interest in the French Flag boomed at the turn of the century. However, from this point on, references to the French Flag 'Model' significantly overtook discussion of the French Flag 'Problem' (Fig. 3), and they all referred specifically to gradient-based models. Both the resurgence of interest in this metaphor and the focus on gradients was due to an exciting period of experimental successes in finding molecular evidence for this mode of patterning (e.g. Driever and Nüsslein-Volhard, 1988; Struhl et al., 1989).

How did the 'model' phrase first come into existence? Intriguingly, the first recorded mention was just 5 years after Wolpert's article, by Garrod (1973) who wrote 'The idea of positional information and the French Flag Model are really statements of what a pattern-specifying mechanism has to achieve'. So at this early point the original concept was still preserved – the flag was what must be achieved, not how it is achieved – nevertheless through presumably a slip of the typewriter it was accidentally cast for the first time as the French Flag Model. But by the 1990s, the phrase was being used specifically to mean a mechanism using spatial gradients and threshold responses (Kraut and Levine, 1991; Huang et al., 1997; Briscoe and Ericson, 1999). Many papers from then onwards cite Wolpert's 1969 paper rather than the original introduction of the term in 1968, and therein may lie the key to the switch in terminology. In this second more famous paper, Wolpert focused almost exclusively on gradient-based models. He did not use the term 'French Flag Model', and he did briefly mention the gradient-free balancing model from the previous article, but for the rest of his analysis (and in many subsequent articles) he explored only the gradient-based models in detail. Over the years, the experimental community also found increasing evidence of positional gradients performing regionalisation, and, in the *Drosophila* blastoderm, long-range gradients even appear to control a periodic pattern (Ingham, 1988), while evidence for self-organised regionalisation appeared more elusive. So as time went by, focus shifted from a problem to a solution, and the French Flag label, being visually appealing and memorable, shifted with it.

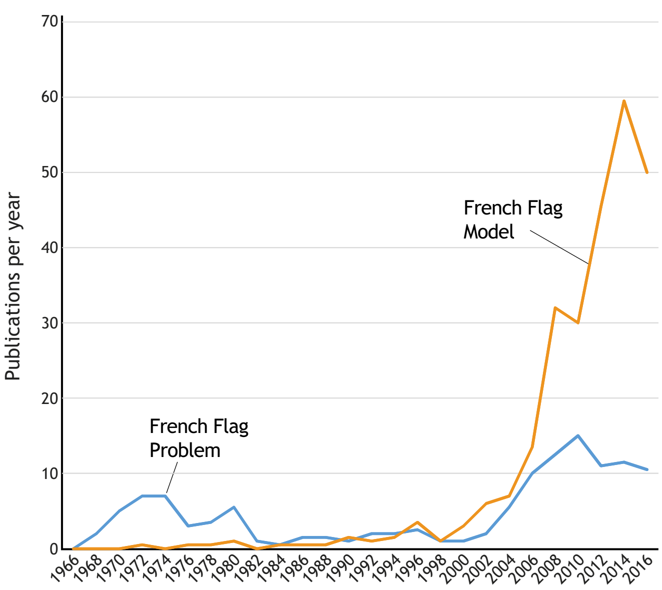

Fig. 3. From problem to model. Literature survey of the use of the terms 'French Flag Problem' versus 'French Flag Model'. For the first 15 years after the publication of Wolpert's article (Wolpert, 1969), discussion focused on the former concept. However, at the turn of the century there was a dramatic increase in attention on the so-called French Flag Model.

Does it matter? It is not technically a contradiction to give the same name to both a problem and a model. Language evolves, and as long as researchers continue to understand each other, no harm is done. It appears that even the language of Wolpert himself eventually slipped, because later in life the phrase 'French Flag Model' crept into a few of his own articles, although they were mostly in interviews and musings about his life (Richardson, 2009; Vicente, 2015; Wolpert, 2018) and a couple of co-authored reviews (e.g. Kerszberg and Wolpert, 2007). It could therefore be argued that, as gradients have indeed been found to underlie well-studied examples of regionalisation, it is reasonable to abandon the French Flag as a problem – a motivation for seeking new types of solution – and focus instead on the French Flag as a gradient-based model. However, I would disagree for multiple reasons. First, simple lack of clarity – giving the same name to two different concepts – could easily lead to confusion. Secondly, this could cause ambiguity: we have seen clearly that gradient models can produce many patterns other than a French Flag and, conversely, that non-gradient models can produce the French Flag. But perhaps most importantly is the danger of allowing a kind of confirmation bias into our research. If you only search for positional gradients you will only find positional gradients. Abandoning the French Flag as a problem reduces the drive for conceptual exploration. We might narrow our questions only to address molecular implementation (e.g. how does the morphogen diffuse/move through the tissue, how are threshold responses implemented at the molecular level?) and thereby forget to also ask, for example, is this diffusible molecule really acting as a positional gradient or could its behaviour also be compatible with an alternative patterning strategy? This bias may well have hindered the discovery of self-organising systems (such as Turing patterns), which received a surge of interest from the community much later than gradient models (Marcon and Sharpe, 2012).

In summary, I believe these early classic articles by Wolpert gave us at least three important insights, some of which were appreciated more than others. First, a new specific model – the gradient-based model – which was indeed a very influential paradigm shift. Its intention was not to ponder the molecular or dynamic details but rather to broaden the minds of the community to think beyond temporal sequences of local interactions which could not easily self-regulate, and to provide instead a concrete proposal in which the whole pattern found a 'dynamic equilibrium' and thus could re-adjust itself by a simple but global coordinate system. Second, and more importantly, Wolpert strongly emphasized the desirability of searching for fundamental and potentially universal principles, rather than cataloguing details. This view cannot be better stated than in his own words: 'A feature of developmental processes which is not often discussed is the extent to which there are, or will emerge, general or universal principles which are applicable to development… It is too often implicit in embryological thinking that each step in development is a unique or special phenomenon with little general significance…Viewed in this light, the possibility of obtaining a set of general principles enabling one to deal with the translation of genetic information into cellular patterns and forms would seem almost hopeless…I would like to suggest that such a view is quite misleading and that there is good reason for believing that there are a set of general and universal principles involved in the translation of genetic information into pattern and form.' (Wolpert, 1969). Finally, and returning back to the core thesis of this article, he gave us a visual icon whose original intention was precisely to help us focus at this more conceptual level of universal principles. It seems unfortunate that a great metaphor for a developmental problem became attached instead to just one of its multiple theoretical solutions. For, in Wolpert's own words again: 'The key to the problem of pattern formation lies in the correct posing of the problem'. Happy 51st birthday, French Flag Problem.

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