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AN EXTREMAL CRITERION FOR EPIMORPHIC REGENERATION

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Many developing systems obey the principle of continuity: a morphogenetic field, when perturbed, tends to restore the normal local pattern of structures in its organ district. We have investigated physical field theories for a morphogenetic field, seeking constraints which would make a field theory produce the principle of continuity. We assume that during embryonic (ontogenetic) development a leg develops a pattern of positional values and a length which extremize a time-independent functional—the integral, over the length of the leg, of a function of positional values and position. For a single state variable which represents positional value, if a unique extremizing solution for the ontogenetically generated pattern and the length exists, and if no position-dependent functions other than the state variable appear in the integrand, then the principle of continuity is valid: in any regenerated leg the state variable is continuous and each region is locally identical to a region of the ontogenetically generated leg. This proposition is applied to three simple examples. For an exponential gradient and a Jacobi elliptic function there is a set of parameter values and boundary values for which a functional is minimized and the ontogenetically generated leg has an optimal length. Thus a leg which meets these constraints will obey the principle of continuity. However, a functional which when extremized gives a sinusoidal pattern does not in general provide a unique extremal length. Mathematical conditions are discussed under which an ontogenetically generated limb or a regenerated limb represents an asymptotically stable steady state. For a specific model of the transient dynamics in the exponential gradient case, the steady state gradient is asymptotically stable.

1. *Introduction.*

"The problem is whether mathematical tools, based on principles of optimality, may or may not be applied to problems of ontogenetic development."

R. Rosen (1967).

A strategy for analyzing development is to characterize rules for starting, performing, and stopping its component processes. Such rules can be interpreted in terms of processes at the cellular and molecular levels. Classes of models compatible with the rules can be defined mathematically, to interpret the rules in terms of the dynamics of the system. The present work contributes to this enterprise. We discuss a class of models which can produce a known stopping rule for the regeneration of limbs.

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Rules for development characterize the mode of operation of a morphogenetic field. A morphogenetic field regulates the course of development in a region of an animal. A limb field governs the development of a limb, not only during embryonic development (ontogeny) but also when the limb regenerates after it is perturbed by addition, deletion, or rearrangement of its parts (Hinchliffe and Johnson, 1980; Muneoka and Bryant, 1982; Stocum, 1984). During regeneration, as in ontogeny, mitosis adds cells to construct a limb of approximately normal size; that is, regeneration of limbs is epimorphic. Some other systems can regenerate by reforming the system from the available cells without mitosis; such regeneration is morphallactic (Morgan, 1901).

It is desirable to derive rules for development from a physical field theory. A field theory allows one to predict the temporal evolution of a spatial pattern of state variables in a region, given (1) the dynamic of the field—an algorithm which specifies allowed transitions of state; (2) boundary conditions—sufficient information about the values of state variables at the boundary of the region; (3) initial conditions which provide sufficient information about the history of the state variables. It seems likely that a field theory describes the operation of a morphogenetic field.

Many models have been devised to describe the development and regeneration of limbs. Some models have used a reaction–diffusion or mechanochemical dynamic, operating in a region of specified size and shape, to predict the pattern of state variables which develops in the region. Among these are models for siting limbs during ontogeny, and models for siting intercalated structures, including supernumerary limbs, after a grafting operation (the polar co-ordinate model of French *et al.*, 1976 and subsequent models—e.g. Meinhardt, 1983; Papageorgiou, 1984; Tevlin and Trainor, 1985; Totafurno and Trainor, 1987). Models for patterning a specified region include models for siting cartilage condensations in developing vertebrate limbs (Wilby and Ede, 1975; Newman and Frisch, 1979; Goodwin and Trainor, 1983; Oster *et al.*, 1983; 1985). Other models have generated the developing shape of a vertebrate limb bud by using local growth rules which give an appropriate sequence of shapes. Growth rules have been posited for the mesenchyme which forms the core of the bud (Ede and Law, 1969; Mitolo, 1971) or for the epithelium which covers its surface (Barrett and Summerbell, 1984).

One would like a field theory which models a growing limb, using a mechanochemical mechanism to generate the sequence of external forms and internal structures which appear. Such a model is likely to require a complex interplay among many state variables, including levels of gene activity, concentrations of gene products, and movement and affinity of cells. However, it may be possible to predict the morphology of a fully developed limb without knowing the field dynamic which governs the course of its development. Limbs can regenerate aspects of normal size, shape and pattern after wounding or surgery disrupts the normal arrangement of cells. Thus a normal limb, or local features of its organization, may represent a stable steady state which is an attractor set for the field dynamic.

In treating a limb as attaining a steady state in which it is "fully developed" we will be neglecting its slow growth during growth of the animal. A regenerating limb undergoes morphogenesis, forming the structures of the fully developed limb. During and after morphogenesis there is a period of rapid "catch-up growth", at a rate which decreases gradually to the growth rate normal for the corresponding ontogenetically generated limb. We shall regard morphogenesis and catch-up growth as aspects of the transient approach to a fully-developed limb, which represents a quasi-steady state that changes relatively slowly during normal growth.

We have considered the possibility that the field dynamic can be derived from a functional of the state variables. The dynamic is stated as Euler–Lagrange equations which are obtained from the functional by variational methods. We assume that in the limit as time goes to infinity, the functional asymptotically becomes time-independent. Variation of the asymptotic functional will then give a set of time-independent, space-dependent differential equations with boundary conditions. These equations govern the steady state shape of the limb and the pattern of state variables in it. Thus a variational principle could specify the location of boundaries, as well as the values of state variables on and within the boundaries, in a fully developed leg.

Minimization of a time-independent functional has been used to determine the shapes of arthropod limbs in a fluid elastic shell model for the shaping of epithelia (Mittenthal and Mazo, 1983). In this model the limb field establishes a spatial pattern of intercellular affinities in the epithelium covering the leg. Adhesive energy associated with cell–cell interactions, and energy of mechanical strain associated with the curvature of the epithelium, contribute to an energy functional. The stable shape of the leg corresponds to a minimum of the energy functional. This model can predict the proportions of segments in arthropod legs.

One may object that the derivation of a field dynamic from a variational principle is appropriate for conservative systems in physics, but not for dissipative systems such as a developing organism. Indeed, the variation which gives the field dynamic minimizes a functional for conservative systems, but not in general for dissipative systems. However, extremization of a functional can also provide dynamical equations for a dissipative system (cf. the principle of virtual work in mechanics: Malvern, 1969). Thus a variational formulation of problems in development may be valid. Moreover, models which treat biological systems as conservative, from the Lotka–Volterra predator–prey equations of population biology (Abraham and Shaw, 1982) to the cellular dynamics of Goodwin (1963), have stimulated further mathematical inquiries, even though the systems are not conservative. We hope that our analysis will serve such heuristic and motivational functions.

We have used a variational formulation to distinguish field dynamics which are compatible with an empirical rule for stopping regeneration. The rule assumes that as development proceeds, morphogenetic fields provide each cell with information about what types of cells should be its neighbors. After a perturbation alters the connectivity among cells, the field tries to restore the normal local pattern of structures—to restore normal neighbors to every cell. This hypothesis has been called the rule of normal neighbors, or the principle of continuity (review: Winfree, 1984).

Figure 1 illustrates the principle of continuity in a simple context. Consider a cylindrical arthropod leg which is axially symmetric. The epithelium underlying the cuticle bears positional information (Wolpert, 1971) in an axially symmetric pattern of state variables. Suppose that an arbitrary axially symmetric region of this pattern is deleted, and the distal and proximal remnants are grafted together at their wound margins. The positional values at the junction provide boundary conditions for regeneration. During regeneration cell division at the junction intercalates additional cells which assume new positional values. According to the principle of continuity, regeneration is complete when the intercalated region is as long as the deleted region was, and when it bears the same pattern of state variables as the deleted region did. In arthropod legs the lengths of segments containing the intercalated region, and the pattern of cuticular structures in them, are nearly normal after such operations, so far as these variables have been assayed (cockroaches: Bulliere and Bulliere, 1985; crayfish: Mittenthal, 1980, 1985).

Under some conditions the normal local pattern can only be restored by producing a regenerated structure with large-scale abnormalities, including multiple copies of structures normally present in a single copy. To see this, consider a grafting experiment in which a region of one leg is grafted with proximodistally reversed orientation to the stump of a host leg. As before, the legs and the levels of cutting are axially symmetric (Fig. 2). Normal neighbors are restored to nearly all cells if two copies of the grafted region with normal length and orientation regenerate, one on either side of the graft, and if the leg is distally completed. (It is appropriate to say "nearly all cells" because the regenerated leg has two planes of local mirror symmetry perpendicular to the proximo-distal axis, one plane at each end of the intercalated region. Cells on these planes have mirror-symmetric neighbors rather than normal neighbors.)

We assume that during ontogeny a leg develops a pattern of state variables and a length which approach steady state values that are extremals (Elsgolts, 1970) of a time-independent functional. After the grafting operations just discussed, the asymptotic result of regeneration is an intercalated region for which the length and the pattern of state variables are also extremals of the

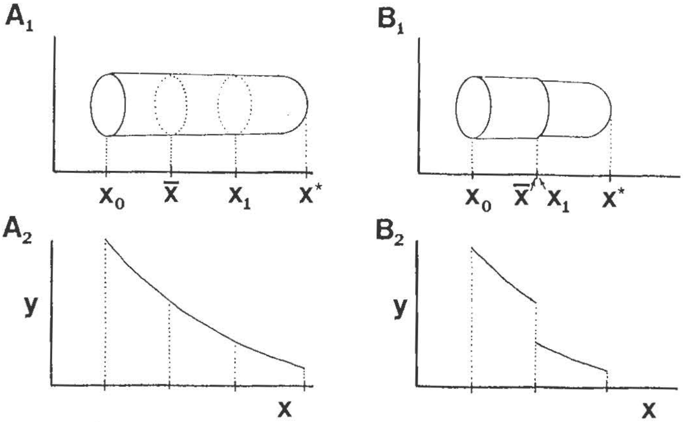

Figure 1. Regeneration of a leg with normal morphology and pattern after a central region of the leg is deleted. The upper diagrams show the leg; the lower diagrams show the pattern function. The proximo-distal axis of the leg corresponds to the horizontal axis. The leg extends from x_0 to the extremal value x^*. The values for the pattern function are displayed on the vertical axis. The value $y_0 = y(x_0)$ is given; $y^* = y(x^*)$ is an extremal value. (A) The ontogenetically generated limb. The region from $\bar{x}$ to x_1 will be removed to provoke intercalary regeneration. (B) The limb immediately after the grafting operation. The discontinuity of the pattern function represents the discontinuity of positional information which intercalation eliminates.

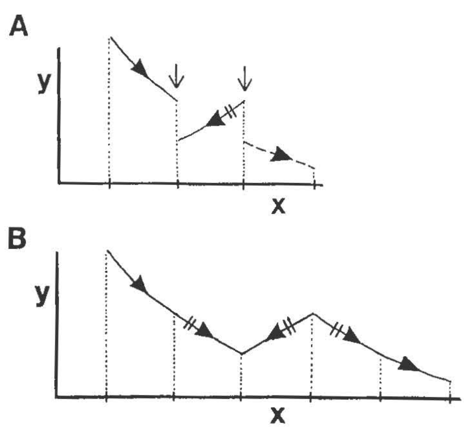

Figure 2. The pattern function after a grafting operation in which a region of the leg is grafted with proximo-distally reversed orientation (arrowheads) onto a stump. (A) Just after the operation, discontinuities of pattern exist between the graft and the host, and at the distal end of the graft (arrows). (B) After regeneration is complete the discontinuites have been eliminated. The graft is flanked by two regenerated regions which are mirror-symmetric to it.

functional. We have asked, what constraints on the functional guarantee that the principle of continuity is obeyed, in that size and pattern are locally normal in intercalated regions? We have found that in one spatial dimension, for a functional which is an integral over a function of position and of a single state variable, the principle is obeyed if variation of the functional yields a unique ontogenetic pattern of the state variable, and if the position variable does not appear explicitly in the integrand. The latter condition means that the state variables are not affected by functions of position which might, for example, represent hidden state variables.

We have applied this proposition to three simple examples. If an exponential gradient is an extremal of the functional, and if the distal boundary value is specified, then (with a minor exception) the extremalization uniquely defines the location of the distal tip and the length of the fully developed limb. If any portion of this limb is deleted, then restoration of the deleted region and its pattern re-extremizes the functional. By contrast, a functional which when extremized gives a sinusoidal pattern does not provide a unique extremal length, except in one very restricted case. A functional which gives Jacobi elliptic functions when extremized provides an ontogenetic pattern of defined extremal length, but only for a restricted range of distal boundary values which depends on parameters appearing in the differential equation.

The proposition which guarantees the principle of continuity does not guarantee that an ontogenetic or regenerated limb represents an asymptotically stable steady state (in mathematical terms, an equilibrium state). The theory of stability of solutions for partial differential equations provides a sufficient condition for uniform and asymptotic stability: any solution sufficiently near the equilibrium state must tend toward it uniformly—the maximum distance between the current state and the equilibrium state must decrease monotonically with time. The crucial theorem which guarantees this form of stability is the generalized Liapunov stability theorem (Henry, 1981). For ordinary differential equations the Liapunov stability theorem requires the existence of a Liapunov function; the generalization to partial differential equations requires the existence of a Liapunov functional.

A Liapunov function or functional, when evaluated on any time-dependent solution, decreases monotonically as time increases. It is possible that a time-dependent Liapunov functional asymptotically approaches a time-independent functional which has a minimum value at the equilibrium point. In view of this possibility, we have investigated whether the extremum is a minimum in the exponential and Jacobi cases. (There is no extremal solution for the sinusoidal case.) For the Jacobi case there is a set of parameter values and boundary values for which the functional is minimized. For a specifiable range of distal boundary values, the negative exponential minimizes its functional. We have used this case to illustrate the application of the generalized Liapunov stability theorem, following work in fluid dynamics (Pritchard, 1968). We chose a time-dependent Liapunov functional in such a way that it asymptotically approaches the time-independent functional which was minimized to obtain the exponential gradient.

2. *General Procedure. Analysis of the Problem.*

2.1. Existence of the specified type of solution. Assume that a functional is obtained by integrating a density over the surface. Further, assume the integrand depends only on the position x on the cylinder axis, on the pattern function $y(x)$ and on its derivative. Thus one may write:

$$I=\int_{x_0}^{x_1} F(x, y, y')\,dx,$$

where x_0 is the value of x at one end of the cylinder axis, which we may choose as we like because the results must be independent of the coordinate system. x_1 is the value of x at the other end of the cylinder. $y(x_0)=y_0$, $y(x_1)=y_1$ are the values of the pattern function at the ends. Our procedure will produce $y(x)$, the extremal solution itself, and extremal values for any two of the four variables x_0, y_0, x_1 and y_1. We assume that x_0 and y_0 are specified in advance. Of course, either x_1 or y_1 may also be specified in advance, thus simplifying the extremization problem.

To determine x_1 and y_1 we shall use a pair of equations called the transversality conditions. They provide necessary conditions which an extremally chosen boundary point and an extremally chosen boundary value must satisfy. To determine the unknown pattern function $y(x)$ we shall impose the solution to the transversality conditions, $y_1=y(x_1)$ and solve the Euler–Lagrange equation generated by the functional. Taken together, the transversality conditions and the Euler–Lagrange equation are necessary conditions for an extremum (Elsgolts, 1970).

For functionals of the form I given above the Euler–Lagrange equation is $F_y-(d/dx)F_{y'}=0$ and the transversality equations are $F_{y'}|_{x_1}=0$ and $(F-y'F_{y'})|_{x_1}=0$, where $|_{x_1}$ indicates evaluation at x_1. The transversality equations allow extremalization over endpoints of the domain of the solution curves, and over values of the solution curves at the endpoints of the domain, respectively. As will be seen in the first two examples, one can also find the extremal domain for the solution curve and the extremal values at the endpoints of that domain by substituting the explicit form for a general solution to the Euler–Lagrange equation into the functional and then differentiating with respect to the limit of integration (the endpoint of the domain) and the boundary value appearing in the general solution. This will give the same result as applying the transversality conditions. In fact, the derivation of the transversality equations is the same limit calculation as is used in differentiation. Thus these conditions offer an easy and quick way to extremize and are a more powerful technique because they do not require the general solution. Only the roots of the transversality equations need to be approximated.

Unfortunately, it is more difficult to show that real roots exist for pairs of differential relations such as transversality equations than it is to show they exist for analytic functions. This means it is very difficult to guarantee simultaneous existence of extremal lengths and boundary values. Of course, with one of x_1 or y_1 given, for some choices of the integrand F one may invoke existence and uniqueness of solutions for the differential equations $F_{y'}=0$, or $F-y'F_{y'}=0$ to show that a single transversality equation can be solved to give a unique solution.

Many generalizations of the Euler–Lagrange equations to larger classes of functionals have been derived; these often result in one equation for each unknown function. Generalizations of the transversality equations exist also (Courant and Hilbert, 1965).

As the following proposition shows, it is the second transversality equation which allows one to prove that if a certain pattern is optimal over a domain then deleted portions are regenerated exactly.

PROPOSITION. 1. *Suppose the value y_0 of the pattern function $y(x)$ at x_0 is given. If the position variable x does not appear explicitly in F and a unique extremizing solution for the ontogenetic pattern exists, then the same solution curve extremizes the functional over any regeneration domain within the ontogenetic domain.*

Proof. Suppose that by exteremization of:

$$I(\hat{x}, \hat{y}, y(x)) = \int_{x_0}^{x} F(y, y')\,\mathrm{d}x,$$

an extremal pattern function $y(x)$ is found and that extremal values for the right hand endpoint of the ontogenetic region and the value of the pattern function there, here denoted by $\hat{x}$ and $\hat{y}$, are found to be x^* and $y^*=y(x^*)$, respectively. That is, the ontogenetic pattern develops as the limb tip grows out to x^*. Fix $\bar{x}\in(x_0, x^*)$ and select $x_1\in(\bar{x}, x^*)$. (See Fig. 1). Now, suppose the region between $\bar{x}$ and x_1 has been removed. It must be shown that a region of this size regenerates and that the pattern function on the regenerated region is the same as on the normal region. The left hand endpoint of the removed region is $\bar{x}$; we assume that the value of the pattern function there is its normal value $y(\bar{x})$. Also, the value of the pattern function at the right endpoint of the regenerated region is taken as $y(x_1)$, as in the ontogenetic pattern. Now, we must examine a slightly different functional

$$\bar{I}(\hat{x}, y(x)) = \int_{\bar{x}}^{\hat{x}} F(\bar{y}, \bar{y}')\,\mathrm{d}x$$

to find the extremal value x_1^* of $\hat{x}$ and the extremizing pattern function $\bar{y}(x)$ defined on the interval $(\bar{x}, x_1^*)$ such that $\bar{y}(\bar{x}) = y(\bar{x})$ and $\bar{y}(x_1^*) = y(x_1)$. If it can be shown that $x_1^* = x_1$ and $\bar{y} = y$ on the common domain of definition then the result will be proved because the extremalization procedure will have reproduced the deleted region. Note that the Euler–Lagrange equation in $\bar{y}$ is the same as the Euler–Lagrange equation for y. Therefore to complete the proof it is sufficient to show that the transversality condition is satisfied by $\bar{y}$. This is so because it was assumed that a unique extremizing solution exists and the only variable which remains to be specified to determine the solution is x_1^*. x_1^* will be determined by the second transversality equation. Note:

$$\frac{\mathrm{d}}{\mathrm{d}x}(F - y'F_{y'}) = F_y y' + F_{y'} y'' - F_{y'} y'' - y' \frac{\mathrm{d}}{\mathrm{d}x} F_{y'}$$

$$= y'\left(F_y - \frac{\mathrm{d}}{\mathrm{d}x} F_{y'}\right) = 0.$$

So, there is a real constant C such that $F - y'F_{y'} = C$. For both y and y the transversality condition is satisfied, i.e., $C = 0$. The solution is unique, so $x_1^* = x_1$. ■

The proof of this proposition shows that the transversality quantity $F - y'F_{y'}$ is constant on solution curves. In fact, the appearance of the constant C in the above argument means that the system is conservative. This will be used further in the Jacobi case. The mathematical assumptions used in proving this proposition are fairly strong: there is only one unknown function $y(x)$, and the integrand explicitly depends only on it and on its derivative. The physical content of this hypothesis is that the positional values, specified by $y(x)$, are not affected by any other functions of x which might, for example, represent hidden state variables. Also, the formulation of the problem neglects changes of positional value at the cut edges which may accompany dedifferentiation and subsequent redifferentiation after surgery.

2.2. Uniqueness of solutions to the transversality equations. There are many integrands which lead to the same Euler–Lagrange equation (Rosen, 1967). These integrands may give different transversality equations which in turn can give different solutions for the extremal values x^* and y^*.

To specify a class of Euler–Lagrange equations which uniquely determine a functional we restrict attention to the class of integrands of the form:

$$F=y_2'^2+f(y), \tag{1}$$

where we assume f is analytic and has zero constant term in its Taylor expansion. Each integrand in the form of equation (1) generates an Euler–Lagrange equation of the form:

$$y''=f'(y). \tag{2}$$

This class of differential equations includes all reaction diffusion equations.

Since each F uniquely determines its transversality equation it is enough to prove that each element in the class of Euler–Lagrange equations of form (2) uniquely determines an F of form (1).

Suppose we have two integrands:

$$F_1=y'^2+f_1(y)$$

$$F_2=y'^2+f_2(y)$$

and they have the same Euler–Lagrange equation. Then:

$$f_1(y)=f_2'(y).$$

By integrating there is a $c\in R$ so that:

$$f_1(y)=f_2(y)+c.$$

Since both f_1 and f_2 have zero constant term, $c=0$. This means that F_1 and F_2 are the same so an Euler–Lagrange equation of form (2) uniquely determines an integrand of form (1).

This result is quite weak, but the three examples in the next section have integrands of the form (1).

2.3. Testing the character of the extremum. If a minimum exists it will be a solution to the Euler–Lagrange equations and transversality conditions. To ensure that a solution minimizes the functional some form of the second variation must be examined. The extremization is conducted over endpoints, values at endpoints, and functions over the domain. So, for a sensible solution, the functional should assume a minimal value with respect to variations in all of those arguments. Thus the second variation must be calculated with respect to some class of functions on the domain with variable endpoints and variable boundary values.

There are several techniques to test if a solution which satisfies the necessary conditions for extremization minimizes the functional (Bolza, 1904). Two techniques will be used here. One is the method of vector calculus; in the other a formula for the second variation is derived.

The method of vector calculus requires use of an explicit form of the general solution of the differential equation. This form and its derivative are used in the integrand of the functional I to give a function dependent only on the parameters of the solution function. Thus the extremization is conducted over the space of solutions to the differential equation, of which the dimension equals the order of the Euler–Lagrange equation. The functional is differentiated twice with respect to the parameters and the Jacobian criterion for extrema is used. The argument of the second derivative is a point (x_1, y_1), and the method tests whether that point gives a minimum. This method is sensitive to the orientation of the leg: if the proximal end of the leg is at $x=0$ and the function is minimized with the distal tip on the right, it will be maximized with the distal tip on the left, unless the limits of integration are reversed along with the position of the distal tip.

The other criterion for a minimum evaluates the second variation of the functional. It does not require an explicit form of the general solution. It shows an optimum over a much larger set of functions than the method of vector calculus—all twice differentiable functions defined on the domain, allowing variation of the endpoint of the domain and of the boundary value there. That is, the argument of the second variation formula is a function, and the method tests whether that extremizing function minimizes the functional. Because the two methods optimize over different sets of functions they may give different results. In all but the simplest cases the second variation is easier to apply. However it tests for a stronger minimum and so is a harder criterion to satisfy. It will not detect whether a solution is a minimum over a smaller class of functions than the set for which it is defined.

There are several versions of the second variation. The one presented here is particularly suited to the problem because it only requires the solutions to the transversality equations and the values of the solution and its derivative at certain points. The equation for the second variation includes the effects of variation in x_1 and y_1 as well as that of $y(x)$. The transversality conditions emerge as the first order terms in the variation. To begin the derivation some notation must be introduced. Let $\delta x_1, \delta y_1$ be small real increments in x_1 and y_1. Assume δy is a variation of y with domain $[x_0, x_1+\delta x_1]$, such that $(\delta y)(x_0)=0$ and $(\delta y)'=\delta(y')$.

PROPOSITION 2. *Consider a functional of form:*

$$I(y(x), x_1, y_1) = \int_{x_0}^{x_1} F(x, y, y')\,\mathrm{d}x,$$

where $x_0, y_0 = y(x_0)$ are given but $x_1, y_1 = y(x_1)$ are allowed to vary. The second variation of I is:

$$(\Delta I) = F|_{x_1}\,\delta y_1 + (F - y'F_{y'}|_{x_1}\,\delta x_1 + (\tfrac{1}{2})\int_{x_0}^{x_1}\left[\left(F_{yy} - \frac{d}{dx}F_{yy'}\right)\delta y^2 + F_{y'y'}\,\delta y'^2\right]dx + (\tfrac{1}{2})F_{yy'}|_{x1}\,\delta y_1^2 + (F_y - y'F_{yy'}|_{x_1}\,\delta x_1\,\delta y_1 + \tfrac{1}{2}(F_x - y'F_y + y'^2F_{yy'}|_{x_1}\,\delta x_1^2$$

Proof. The derivation is as follows. Start by considering the contribution to the value of the functional by variation from the solution curve y by an increment δx_1 and δy. This gives:

$$\Delta I = \int_{x_0}^{x_1+\delta x_1} F(x, y+\delta y, y'+\delta y')\,\mathrm{d}x - \int_{x_0}^{x_1} F(x, y, y')\,\mathrm{d}x$$
$$= \int_{1}^{x_1+\delta x}{}_{x_1} F(x, y+\delta y, y'+\delta y')\,\mathrm{d}x$$
$$+ \int_{x_0}^{x_1} F(x, y+\delta y, y+\delta y') - F(x, y, y')\,\mathrm{d}x.$$

Expanding the first of these two integrals to second order gives:

$$\int_{x_1}^{x_1+\delta x_1} F + F_y\,\delta y + F_{y'}\,\delta y'\,\mathrm{d}x + R = F\Big|_{x_1}\,\delta_{x_1} + (\tfrac{1}{2})\frac{\mathrm{d}}{\mathrm{d}x}F\Big|_{x_1}\,\delta x_1^2 + F_{y'}\,\delta y'|_{x_1}\,\delta_{x_1} + F_y\,\delta y|_{x_1}\,\delta x_1 + R_1.$$

Dropping the remainder terms R and R_1 gives a second order approximation. Expanding the second integral to second order gives:

$$\int_{x_0}^{x_1} [F_y\,\delta y + F_{y'}\,\delta y' + (\tfrac{1}{2})F_{yy}\,\delta y^2 + F_{yy'}\,\delta y\,\delta y' + (\tfrac{1}{2})F_{y'y'}\,\delta y'^2]\,\mathrm{d}x + E$$
$$= F_{y'}\,\delta y|_{x_1} + \int_{x_0}^{x_1}\left(F_y - \frac{\mathrm{d}}{\mathrm{d}x}F_{y'}\right)\delta y\,\mathrm{d}x + (\tfrac{1}{2})\,(F_{yy'}\,\delta y^2|_{x_1}$$
$$+ (\tfrac{1}{2})\int_{x_0}^{x_1}\left(F_{yy} - \frac{\mathrm{d}}{\mathrm{d}x}F_{yy'}\right)\delta y^2 + F_{y'y'}\,\delta y'^2\,\mathrm{d}x + E_1.$$

Dropping the remainder terms E and E_1 gives a second order approximation. Adding the two approximations gives an approximation $(\Delta I)_2$. To complete

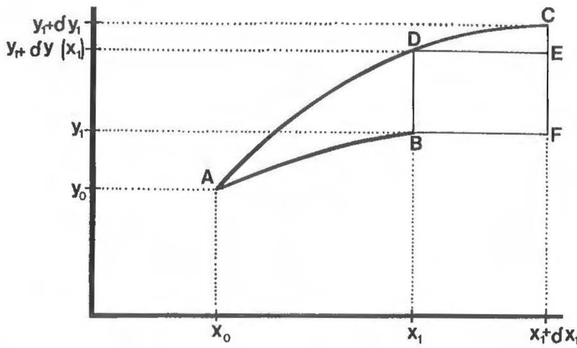

Figure 3. A variation in the endpoint (x_1, y_1) of the argument of the functional (from Elsgolts, 1970), shown in order to motivate a second order expansion for the length CE. Note that $FC = \delta y_1$ and that $CE = [y(x_1+\delta x_1)+\delta y(x_1+\delta x_1)] - [y(x_1)+\delta y(x_1)]$. We assume that $\delta y''$ is negligible.

the derivation the evaluation terms must be approximated. From Fig. 3 one sees $\delta y|_{x_1} = BD$. So,

$$\begin{aligned} CE &= (y+\delta y)(x_1+\delta x_1) - (y+\delta y)(x_1) \\ &= (y+\delta y')|_{x_1}\, \delta x_1 + (\tfrac{1}{2})(y+\delta y)''|_{x_1}\, \delta x_1^2 \\ &= y'(x_1)\, \delta x_1 + \delta y'(x_1)\, \delta x_1 + (\tfrac{1}{2}) y''(x_1)\, \delta x_1^2 . \end{aligned}$$

Now, $\delta y|_{x_1} = BD = FC - EC$

$$= \delta y_1 - y'(x_1)\, \delta x_1 - \delta y'(x_1)\, \delta x_1 - \tfrac{1}{2} y''(x_1)\, \delta x_1^2 .$$

Differentiating both sides the last approximation gives, to first order,

$$\delta y'|_{x_1} = -y''(x_1)\, \delta x_1 .$$

A first order approximation is sufficient because $\delta y'|_{x_1}$ only appears to first order in a second order term. Finally one obtains:

$$\begin{aligned} (\Delta I)_2 = {} & F\Big|_{x_1} \delta x_1 + (\tfrac{1}{2}) \frac{\mathrm{d}}{\mathrm{d}x} F\Big|_{x_1} \delta x_1^2 + F_y|_{x_1}\, \delta x_1 (\delta y_1 - y'\, \delta x_1) - F_{y'} y''|_{x_1}\, \delta x_1^2 \\ & + F_{y'}|_{x_1} (\delta y_1 - y'\, \delta x_1 + y''\, \delta x_1^2 - (\tfrac{1}{2}) y''\, \delta x_1^2|_{x_1} \\ & + (\tfrac{1}{2}) F_{yy'} (\delta y_1^2 + y'^2\, \delta x_1^2 - 2y'\, \delta y_1\, \delta x_1)| \\ & + (\tfrac{1}{2}) \int_{x_0}^{x} \left[\left(F_{yy} \frac{\mathrm{d}}{\mathrm{d}x} F_{yy'} \right) \delta y^2 + F_{y'y'}\, \delta y'^2 \right] \mathrm{d}x \\ = {} & F_{y'}|_{x_1}\, \delta y_1 + (F - y' F_{y'}|_{x_1}\, \delta x_1 + (\tfrac{1}{2}) F_{yy'}|_{x_1}\, \delta y_1^2 \\ & + (F_y - y' F_{yy'}|_{x_1}\, \delta x_1\, \delta y_1 + \tfrac{1}{2}(y'^2 F_{yy'} - y' F_y + F_x|_{x_1}\, \delta x_1^2 \end{aligned}$$

$$+\left(\tfrac{1}{2}\right)\int_{x_0}^{x_1}\left[\left(F_{yy}-\frac{\mathrm{d}}{\mathrm{d}x}F_{yy'}\right)\delta y^2+F_{y'y'}\,\delta y'^2\right]\mathrm{d}x. \qquad \blacksquare$$

Note that in the last formula in the proof the first two terms are the transversality equations and the last term is the second variation of the function on the domain. In the derivation the Euler–Lagrange equations were used; they emerge from the first variation on the domain. The extra terms are the contribution to the total second variation of the functional from the second variations of x_1 and y_1. When the second variation at an extremally chosen value is positive a minimum is indicated; when it is negative a maximum is indicated. If the second variation is zero it gives no information: the point may be a minimum, a maximum, or neither. If it may change sign depending on the size of δx_1 and δy_1 then a saddle point is indicated. The formula in the proposition will be applied in the next section to the exponential and Jacobi examples. In the exponential example we note the difference between the conclusions of this formula and those from the methods of vector calculus. In the Jacobi example, although explicit forms of the general solution to the Euler–Lagrange equations can be identified, they are too complicated to allow one to directly ascertain whether or not the functional has been minimized. So the second variation method is used.

3. Specific Examples. The procedure outlined above will be applied to three functionals. Their extremizing curves are exponential, trigonometric, and Jacobi elliptic functions. These are the simplest cases we found to study. In each case the system represented by the functional is conservative. Furthermore, it is convenient to choose $x_0=0$.

In each of the following examples, we examine three cases to see if a unique ontogenetic curve exists and minimizes the functional. Note that if this is so, then because the system is conservative it will regenerate any deleted portion, by the first proposition. In ontogeny the positional value at the proximal boundary, $y(x_0)$, is fixed. The limb develops to length x_1, which is determined by the dynamic of the morphogenetic field. During development the positional value at the distal boundary, y_1, may be fixed as a boundary condition or may be generated by the dynamic. The three cases we examine are: (1) the least constrained case, that is, finding both x_1 and y_1 by extremalization; (2) the epimorphic case, that is finding x_1 given y_1; (3) the morphallactic case, that is finding y_1 given x_1. The morphallactic case is of least interest here; it is given primarily for completeness. The reader may skip to the end of each example for a qualitative summary of the conclusions from the formal analysis.

Example 1. *Exponential functions.* We present this example for two reasons. One is that case two will exemplify the key features of the modelling strategy we are advocating. The other is that exponential gradients of morphogen concentrations have been used in models of morphogenesis. In relation to the first of these reasons, the stability of the solution to the extremization problem, under a conveniently chosen field dynamic, will be examined in a separate section.

Let $k \in R$, and consider the functional:

$$I[x_1, y_1, y(x)] = \int_{x_0}^{x_1} [(y')^2 + k^2 y^2] \, dx.$$

(i) Exponential example, least constrained case: find x_1 and y_1. After rescaling by replacing x with kx the Euler–Lagrange equation becomes

$$y'' - y = 0.$$

The solutions to this are of the form:

$$y(x) = C_1 \exp x + c_2 \exp - x,$$

where $c_1, c_2 \in R$. The transversality conditions give $y'(x_1) = y(x_1) = 0$ and the boundary condition gives $y_0 = c_1 + c_2$. If $y_0 = 0$ it is easy to show there is no non-trivial extremal solution. If y_0 is assumed to be non-zero then the solution y may be replaced by y/y_0. Now the solution is of the form:

$$y(x) = \alpha \exp x + (1 - \alpha) \exp - x, \tag{1}$$

where $\alpha \in [0, 1]$. The Euler–Lagrange equation is unaffected by replacing x with $-x$ so without loss of generality one may restrict attention to the positive side of $x_0 = 0$. Now the transversality equations imply $\alpha = 0$ and $x_1 = \infty$. So, the solution is $y(x) = \exp - x$.

Now that a solution has been found one can check whether or not it minimizes the functional. If the interval size and boundary values are fixed the solution is a strong minimum (Elsgolts, 1970). This says nothing about minimality with respect to varying the boundary and boundary value. In this case the second variation formula is:

$$(\Delta I)_2 = \tfrac{1}{2} \int_{x_0}^{x} [\delta y^2 + dy'^2] \, \delta x + 2y(x_1) \, \delta x_1 \, \delta y_1 - y(x_1) y'(x_1) \, \delta x_2^2,$$

which gives no further information because the last two terms are zero.

To employ the method of vector calculus an explicit form of the general solution is required. Making use of the earlier rescaling it is convenient to write the solution in the form:

$$y(x, x_1, y_1) = C \sinh(x) + \cosh(x),$$

where $C=C(x_1, y_1)=[y_1-\cosh x_1]/\sinh x_1$. $y(x, x_1, y_1)$ may be substituted into the integrand of I to give:

$$I(x_1, y_1)=[(1+y_1^2)\cosh x_1-2y_1]/\sinh x_1. \tag{2}$$

If this is differentiated with respect to x_1 and y_1 and the derivatives are set equal to zero then one can solve for x_1 and y_1. This will give:

$$\cosh(x_1)=(1+y_1^2)/(2y_1)$$

$$y_1=1/(\cosh x_1).$$

These are the same conditions as the transversality equations. In Fig. 4 we have plotted the second of these equations and the solution $y(x_1)=y_0 \exp-x_1$. The points of intersection of those two curves are those points for which we can simultaneously satisfy the Euler–Lagrange equations and the transversality condition.

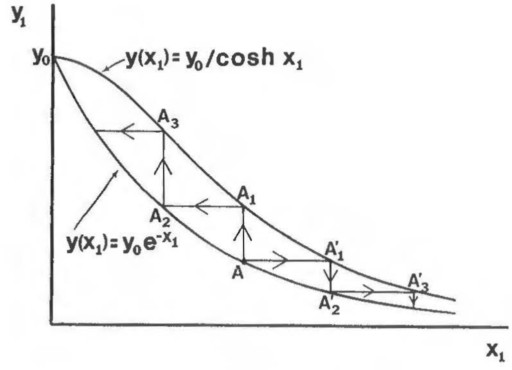

Figure 4. The exponential case in which x_1 and y_1 are chosen as extremals. The intersection of the extremal pattern function $y_0 \exp-x_1$ with the curve $y_0/\cosh x_1$ of candidate extremal values (x_1, y_1) gives the possible values of the extremal pair. The intersection consists of two points: the trivial case (x_0, y_0) and $(\infty, 0)$. The two piecewise linear curves each converging to one of the points of intersection represent paths by which extremalization might produce the points of intersection. Both curves start at A. If we take x_1 as given, then varying y_1 to seek an extremum (a minimum) takes us to the point A_1. Next, fixing the new y_1 and varying x_1 to seek an extremum (a maximum) gives A_2. Continuing in this fashion we see that the sequence of points A_n converges to (x_0, y_0). Alternatively, if we take y_1 as given, then varying x_1 to seek an extremum (a maximum) takes us to the point A'_1. Next, fixing the new x_1 and varying y_1 to seek an extremum (a minimum) takes us to the point A'_2. Continuing in this fashion we see that the sequence of points A'_n converges to $(\infty, 0)$. Since in both cases we can approach the limit point through alternately maximizing and minimizing we again see that the limit points are neither maxima nor minima for the functional.

The second partial derivatives of I with respect to x and y are

$$\frac{\partial^2}{\partial x_1^2} I = 2[(1+y_1^2)\cosh x_1 - y_1(1+\cosh^2 x_1)]/\sinh^3 x_1$$

$$\frac{\partial^2}{\partial y_1^2} I = 2\cosh x_1/\sinh x_1$$

$$\frac{\partial^2}{\partial x_1\,\partial y_1} I = 2[\cosh x_1 - y_1]/\sinh^2 x_1 .$$

The extremal value was found to be $(x_1, y_1) = (\infty, 0)$. Of course, (x_0, y_0) satisfies the necessary conditions for optimality as well, but, it is the trivial solution and does not interest us. At that point the limit of the determinant of the Jacobian matrix is zero because $I_{x_1x_1}$ and $I_{x_1y_1}$ tend to zero, so the Jacobian criterion gives no information. However, one can check that as $(x, y) \to (\infty, 0)$, $I(x, y) \to 1$. In this case we can see that I is neither maximized nor minimized by evaluating it on two curves which tend to $(\infty, 0)$ and observing that it increases to its limiting value on one and decreases to its limiting value on the other. For convenience we choose the curve $(x_1, 0)$ as $x_1 \to \infty$ and the curve $(-\ln y_1, y_1)$ as $y_1 \to 0$. On the first of these I increases to unity while on the second I decreases to unity. This is so because we have in a sense maximized over x_1 but minimized over y_1. For, $I_{x_1x_1}$ is negative and $I_{y_1y_1}$ is positive as $(x_1, y_1) \to (\infty, 0)$. In Fig. 4 we present a graphical argument for why this occurs by identifying two piecewise linear curves which converge to (x_0, y_0) and $(\infty, 0)$ by alternately maximizing and minimizing to obtain successive values of x_1, and y_1.

(ii) Exponential example, epimorphic case: fix y_1, find x_1. Now suppose $y_1 = y(x_1)$ is given but the value of x_1 is unknown. Only the second transversality condition is relevant since it is the result of varying x_1. Substitution of (1) into the transversality condition gives

$$[\alpha \exp(x_1) - (1-\alpha)\exp(-x_1)] = \pm[\alpha \exp(x_1) + (1+\alpha)\exp(-x_1)].$$

We restrict our attention to the case that x is a positive real number. That is, we assume x is not infinite. This forces α to be either 0 or 1. Now, if $y_1 > 1$, $y(x) = \exp x$ so $x_1 = \ln y_1$ and if $0 < y_1 < 1$, $y(x) = \exp -x$ so $x_1 = \ln y_1$. If $y_1 < 0$, the transversality conditions cannot be satisfied. If $x < 0$, and the exponential and negative exponential solutions are interchanged then the corresponding results still hold.

It can be shown that at the extremal value the sign of second derivative of I with respect to x_1 is positive when evaluated at $x_1 = \pm \ln y_1$. Thus, the method of vector calculus implies that the curves $\exp x$ and $\exp -x$ are both maxima.

The second variation for this problem is:

$$\int_{x_0}^{x_1} \delta y^2 + \delta y'^2 \, dx - y_1 y'(x_1)\, \delta x_1^2 .$$

This means that the curve $\exp(-x)$ minimizes, and $\exp(x)$ maximizes. So, if x_1 is positive then if $y_1 > 1$ exp x is a maximum and if $y_1 < 1$ $\exp - x$ is a minimum.

In this example we see that if minimality is determined by the second variation then any part of the curve $\exp(-x)$ minimizes the functional and any portion of the curve $\exp(x)$ maximizes the functional. If the method of vector calculus is used then $\exp(-x)$ and $\exp(x)$ both give minima to the right of zero and maxima to the left.

(iii) Exponential example, morphallactic case: fix x_1, find y_1. If x_1 is specified then only y_1 remains to be found. Since the first transversality equation is the result of varying y_1, only it should be applied. It gives $y'(x_1)=0$. Now, by using (1) to evaluate I and differentiating $I(x_1, y_1)$, one may show that the optimal y_1 satisfies $y_1 = 1/\cosh(x_1)$. So, the solution is:

$$y(x) = (\exp x + \exp(2x_1 - x))/(1 + \exp(2x_1)).$$

The function y is monotone decreasing on $[0, x_1]$ and assumes its minimal value at x_1.

We must check whether the solution minimizes I. From the first case we have the second derivative with respect to y_1 and it is positive for all $x_1 > 0$, thus indicating a minimum. The second variation formula has all terms zero except the one containing the integral so it gives no information.

Exponential example: summary. Here are the results obtained thus far in the exponential example. In the least constrained case, although we can satisfy the necessary conditions for an extremum the result is neither a maximum nor a minimum. In the epimorphic case, for $x_1 > 0$, we find three subcases. In one the transversality condition cannot be satisfied; in the other two we obtain either an exponential or a negative exponential. These latter two are both minima under the method of vector calculus but under the second variation the exponential is a maximum and the negative exponential is a minimum. This distinction is the result of the dependence on the sign of the first derivative which appears in the second variation. In the morphallactic case we obtain a minimum provided that $x_1 > 0$.

3.1. The stability of a minimum under a field dynamic. In this section we extend the epimorphic case to incorporate time evolution of the solution under a time dependent field dynamic. The field dynamic determines the geometry and pattern, x_1 and $y(x)$ as functions of time. We will produce these functions, denoting them by $x_1(t)$ and $y(x, t)$, and show that as t increases they tend to a limiting value $x_1(\infty)$ and a limiting function $y(x, \infty)$ which are the same as were determined in the epimorphic case of the exponential example.

To demonstrate asymptotic convergence we use an extension of the classical Liapunov stability theory from problems in which the solution to a differential equation takes values in a finite dimensional real space to problems in which the solution to a differential equation takes values in a Banach space. This extension is possible because there is a standard technique to convert any element of a large class of partial differential equations in any finite dimensional real space into an ordinary differential equation on a Banach space. The method uses a time-dependent Liapunov functional which decreases with time toward a limiting function $y(x, \infty)$ which are the same as were determined in asymptotic value. Its form approaches the time independent functional we extremized in the exponential example.

Figure 5 illustrates this idea. Imagine an infinitely long trough in the positive octant of R^3 which is sloped downwards and asymptotically gets closer to the time axis. The multivariate axis represents a collection of ordered pairs $(x_1, y(x))$ corresponding to admissible boundary values and pattern functions; the third axis represents the value of the time dependent functional on a particular ordered pair. The trough corresponds to the possible solutions to the field dynamic. Extremizing a time dependent functional at each instant of time isolates the path followed by a moving dot, which represents the state of the system as it develops from a particular initial condition.

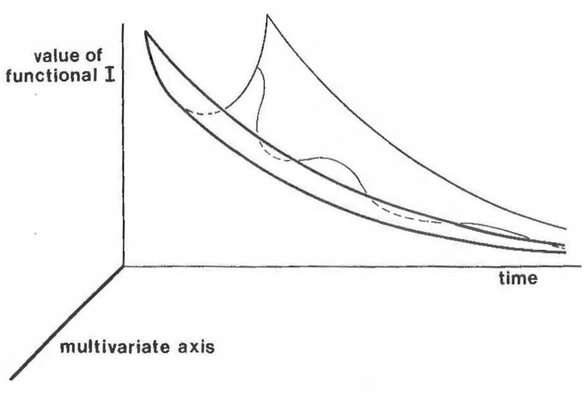

Figure 5. This diagram illustrates a way to imagine the relation between a time dependent functional and a field dynamic. The surface represents the collection of solutions to the time-dependent field dynamic. The multivariate axis corresponds to parameter values such as initial or boundary conditions and to solutions to the time-dependent field dynamic. The vertical axis represents the value of the functional, and the t axis represents time. When we prove stability results we are fixing an infinitely long, but possibly very narrow, subset of the surface and asking that the solution remain within it and approach a particular curve arbitrarily closely. In our example, the subset is in fact a curve such as that shown, and we verify that on it the functional is minimized.

Such a diagram illustrates the concept of an epigenetic landscape proposed by Waddington (1966). It also raises the problem that a field dynamic may admit more limiting functions than extremizing a time independent functional can produce. For, under mild conditions its Euler–Lagrange equation will have a unique solution, but a field dynamic may have many asymptotic states. We cannot guarantee that the trough will converge to a single point at infinity. In principle, it could expand, bifurcate, or curl, thus giving many local minima. Our present example leaves this problem unaddressed because it was too difficult. In fact, in the majority of cases, ensuring that a field dynamic will give a single asymptotic state is an unsolved problem in the theory of partial differential equations.

The form of Liapunov stability theorem we will use requires some definitions before we can state it. We will need a dynamical system, to be denoted by S, on a complete metric space, to be denoted by M, an equilibrium point, and a Liapunov function. Given these, and hypotheses to control how a solution approaches an equilibrium point, the theorem, (Henry, 1981) will guarantee that the equilibrium point is uniformly and asymptotically stable. This means that any solution near the equilibrium point tends toward the equilibrium point uniformly as time advances and that this convergence is uniform over a sufficiently small neighborhood of the equilibrium point.

Appropriate choices for the context in which we wish to apply the theorem are as follows. We extend the functional which was considered in the exponential example by allowing the parameter k to be dependent on time. Thus $k=k(t)$, where k is differentiable and strictly positive and has derivative strictly negative on R. Since k decreases to a value $k(\infty)>0$, $k'(t)$ increases to 0. The boundary value y_1 will be fixed as in the epimorphic case. We will take $x>0$ and assume that $y_1\in(0, 1)$ so that the asymptotic steady state is $\exp(-x)$ defined on $[0, x_1(\infty)]$. We present the calculations for this case only because $\exp(-x)$ is a minimum under both the vector calculus method and second variation method. If $y_1>1$ then the calculations using $\exp(x)$ are similar: replace the minus sign with a plus sign. Next, assume that the following partial differential equation is the growth dynamic for the morphogenetic field.

$$\frac{\partial}{\partial t} y(x, t)=y''(x, t)+k^2(t)y(x, t)+y(x, t)\frac{\mathrm{d}}{\mathrm{d}t} k(t). \tag{3}$$

This dynamic was chosen so that it would not be trivial over the M we shall soon define as it would have been if, for example, the $y(\mathrm{d}/\mathrm{d}t)k$ term were not included. This made the stability harder to prove but also made it genuinely non-trivial. The term $y''+k^2y$ was included because it is the Euler–Lagrange expression for the asymptotic exponential case. This indicates that the link between the field dynamic and the functional being minimized is very strong indeed. Had we known more about the solutions to the equation defining the field dynamic, with or without its last term, it is possible that we could have made stronger statements about stability properties.

Assume that at each instant the unknown pattern function minimizes the value of this time-dependent functional:

$$I(y(x, t), x_1) = \int_{x_0}^{x_1(t)} [y'(x, t)^2 + k^2(t)y(x, t)^2] \, dx.$$

To start verifying the hypotheses of the Liapunov stability theorem, we next define a dynamical system on a complete metric space. Fix a function $k(t)$ and a boundary value $y_1 \in (0, 1)$, and assume that $x_1(t)$ has been found. We will shortly find that solutions are of the form $\exp(-k(t)x)$, so we define the set M to be:

$$\{\exp k(t)x \text{ defined for } x \in [0, x_1(t)] | t > 0\}.$$

Several typical elements of M are shown in Fig. 6. Now, M is a complete metric space where the metric is defined for functions $f(x)$, $g(x)$ by:

$$\rho(f, g)^2 = \int_0^{x_1(\infty)} (f' - g')^2 + (f - g)^2 \, dx,$$

and the dynamical system S can be defined on M. It is the mapping $S(\tau)$: $M \to M$, defined for all $\tau > 0$ by:

$$S(\tau)\{\exp k(t)x|_{[0, x_1(t)]}\} = \exp k(t+\tau)x|_{[0, x_1(t+\tau)]}.$$

This mapping $S(\tau)$ is well defined, for we have assumed that $k(t)$ is strictly monotonic and will prove that $x_1(t)$ is strictly monotonic, too.

Because the set M is so small the form of stability we will prove is quite weak. Stability is usually defined with respect to a subset of the solution space which is closed under a map S. However the class used above is parameterized by t and so is a very small subset even within the solution space. As will be obvious from the calculations below stronger stability statements can be made for this example, but it was too difficult to identify a larger M. The last term of (3) was specifically included so that some stability result, however weak, could be proved. Had we not done so we would have had to examine a complete metric space of solutions to equation (3) with the last term removed; this is difficult. We accepted this deficiency because the objective of this analysis was only to demonstrate the plausibility of the approach.

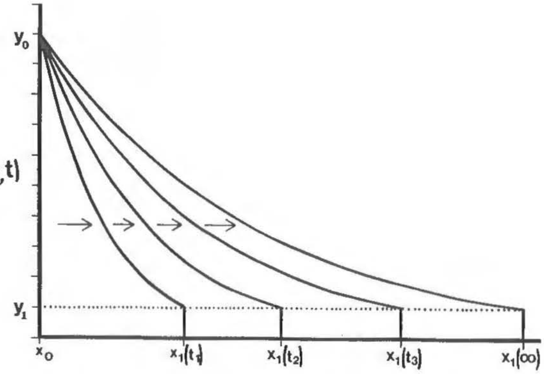

Figure 6. Development of the pattern function for the exponential example, epimorphic case under the time dependent dynamic assumed in the text, assuming $y_1 < y_0$; and times $t_1 < t_2 < t_2$. Arrows show increasing time and $x(\infty)$ is the asymptotic length of the leg.

Now we begin the actual mathematics of justifying our choices of M and S. To do so we first minimize the time dependent functional. The Euler–Lagrange and transversality equations become:

$$y''(x, t) - k^2(t)y(x, t) = 0,$$
$$y'(x, t)^2 - k^2(t)y(x, t)^2\big|_{x_1(t)} = 0.$$

We want to solve these equations given the boundary conditions that for all $t > 0$, $y(0, t) = 1$, and $y(x_1(t), t) = y_1$. For fixed t, solutions to the Euler–Lagrange equations are of the form:

$$y(x, t) = c_1(t)\exp k(t)x + c_2(t)\exp - k(t)x.$$

The transversality equation now becomes:

$$0 = k^2(t)c_1(t)c_2(t)\big|_{x_1(t)}.$$

This means that at least one of c_1, c_2 is zero for each t. Boundary conditions imply $c_1(t) = 1 - c_2(t)$ so that the only values the c_1 and c_2 can assume are 0 and 1. Since they must be continuous one of them is always zero and the other is always one. Now, because of our choice of y_1 the solution is $y(x) = \exp(-k(t)x)$, where for all $t > 0$,

$$y_1 = y(x_1(t), t) = \exp(-k(t)x_1(t)).$$

We see that:

$$x_1(t) = \ln(y_1)/k(t). \tag{4}$$

So, the sign of $x_1(t)$ is positive. Convergence of $x_1(t)$ to some limiting value $x_1(\infty)$ as $t \to \infty$ is guaranteed by the convergence of $k(t)$ to $k(\infty)$. For, we have assumed $k(t)$ is differentiable and strictly monotonic, in particular $k'(t) < 0$. Now, as $t \to \infty$, $k(t) \to k(\infty)$ and $k'(t)$ increases to zero. Since differentiating equation (4) gives:

$$\frac{\mathrm{d}}{\mathrm{d}t} x_1(t) = \left[\frac{\mathrm{d}}{\mathrm{d}t} k(t)\right] \ln(y_1)/k^2(t), \tag{5}$$

we see that $(\mathrm{d}/\mathrm{d}t)x_1(t)$ decreases to 0 and $x_1(t)$ increases to $x(\infty)$.

We now verify the key hypothesis of the Liapunov stability theorem so it will be seen that $y(x) = \exp - k(\infty)x$ defined on $[0, x_1(\infty)]$ is uniformly asymptotically stable. We must show that the sign of $(\mathrm{d}/\mathrm{d}t)I$ is always negative over M. Hence:

$$\frac{\mathrm{d}}{\mathrm{d}t} I = \int_0^{x_1(t)} \frac{\partial}{\partial t} F(t, x, y, y')\,\mathrm{d}x + F(t, x, y, y')\big|_{x_1(t)} \frac{\partial}{\partial t} x_1(t). \tag{6}$$

The integral term becomes:

$$2\int_0^{x_1(t)} y'(x, t)\frac{\partial}{\partial t} y'(x, t) + k(t)y(x, t)^2 \frac{\partial}{\partial t} k(t) + k^2(t)y(x, t)\frac{\partial}{\partial t} y(x, t)\,\mathrm{d}x.$$

Substitution from equations (3) and (4), integration by parts, rearrangement of terms and addition of the second term in the right hand side of (6) gives:

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t} I = {} & 2\frac{\partial}{\partial t} k(t) \int_0^{x_1(t)} y'(x, t)^2 + [(k(t)^2 + k(t)]y(x, t)^2\,\mathrm{d}x \\ & - \int_0^{x_1(t)} y''(x, t)^2 + 2k^2(t)y'(x, t)^2 + k^4(t)y(x, t)^2\,\mathrm{d}x \\ & + 2\{y'(x, t)[y''(x, t) + k^2(t)y(x, t)]\}\big|_0^x \\ & - \{y'(x, t)^2 + k^2(t)y(x, t)^2\big|_{x_1(t)} \left[\frac{\mathrm{d}}{\mathrm{d}t} k(t)\right] \ln(y_1)/k^2(t).\end{aligned} \tag{7}$$

Since $(\mathrm{d}/\mathrm{d}t)k < 0$ and they are sums of squares, the first two terms are negative. The candidate solution is $y(x, t) = \exp(-k(t)x)$ on $[0, x_1(t)]$. For any solution of that form the third term in equation (7) is negative and the last term in equation (7) is a positive function multiplied by equation (5) which we noted is negative. Thus each term in the last expression is negative so the time derivative of I is negative.

Now, by the generalized Liapunov theorem $\exp(-k(\infty)x)$ defined on $[0, x_1(\infty)]$ is uniformly asymptotically stable on M as t increases. A similar argument may be used to obtain the uniform asymptotic stability of $\exp(k(t)x)$ when $y_1 > 1$.

Uniform asymptotic stability can be proved for the morphallactic case in a similar way. Fix $x_1 > 0$. Assuming that, as before, we minimize at each instant of time the solution is:

$$y(x, t) = \{\exp(k(t)x) + \exp(k(t)[x_1 - x])\}/(1 + \exp 2k(t)x_1),$$

and the optimal boundary value as a function of time is:

$$y_1(t) = 1/\cosh k(t)x_1.$$

We can assume the same field dynamic and calculate dI/dt. After integration by parts and rearrangement the result is a sum of two terms, one of which is an integral of a sum each of whose terms is negative. The other term is:

$$y'(y'' + k(t)^2 y)\big|_{x_0}^{x_1}. \tag{8}$$

Since one can choose $x_0 = 0$ and x_1 is given, one can substitute the expression for $y(x, t)$ into equation (8) and prove that if for all t $k(t) < 1/2x_1$ then equation (8) is negative. Thus we have that $dI/dt < 0$. A complete metric space and a dynamical system can be defined so that:

$$\{\exp(k(\infty)x) + \exp(k(\infty)[x_1 - x])\}/(1 + \exp 2k(\infty)x_1),$$

taking value $1/\cosh k(\infty)x_1$ at x_1 is a uniformly asymptotically stable equilibrium point.

It is not possible to put these two stability results together to obtain uniform asymptotic stability for the solution to case (i) even under all hypotheses accumulated from cases (ii) and (iii), because, as we have seen, the solution in case (i) is neither a maximum nor a minimum.

Thus in the epimorphic and morphallactic cases we have used the transversality conditions to obtain a boundary location or boundary value. Then we examined for minimality by way of the method of vector calculus and the second variation. After guaranteeing minimality of these solutions they were taken as the candidates for equilibrium points. Then, we posited a field dynamic which would exhibit the stability properties we wanted, and concluded that the optimizing curves which we found were uniformly asymptotically stable limits for that dynamic.

Example 2. *Trigonometric functions*. Again let $k > 0$ and consider the functional:

$$I[x_1, y_1, y(x)] = \int_{x_0}^{x_1} [(y')^2 - k^2 y^2]\, dx.$$

The Euler–Lagrange equation is:

$$y''+k^2y=0.$$

It has general solution of the form:

$$y(x)=c_1 \sin kx+c_2 \cos kx. \tag{9}$$

This case is interesting because in a reaction diffusion system infinitesimal perturbations which grow can be represented as sinusoids and $y(x)$ corresponds to a mode in a Fourier decomposition.

If $(x_1, y_0)=(0, 1)$ and the factor k is absorbed into x, then an explicit form for the general solution is, if $x_1 \neq n\pi$:

$$y(x; x_1, y_1)=[(y_1-\cos x_1)/\sin x_1]\sin x+\cos x. \tag{10}$$

If $x_1=n\pi$ for some n then the constant c_1 cannot be determined, although $c_2=y_1=1$.

(i) Sinusoidal example, least constrained case: find x_1 and y_1. Application of the transversality conditions gives $y(x_1)=y'(x_1)=0$, so $y_1=0$. Now we will show that there is no extremal solution x_1, by way of contradiction. If there is an extremal value for x_1, then either $\sin x_1$ is zero or it is not zero. If $\sin x_1=0$ then because $0=y_1=\cos x_1$ we see that $\cos^2 x_1+\sin^2 x_2=0$, a contradiction. If $\sin x_1$ is not zero then the same contradiction can be derived as follows. Rescale equation (9) as before to see that:

$$c_1=-c_2 \cot x_1.$$

If this is used in $y'(x_1)=0$ it will give the same contradiction, when c_2 is not zero. If $c_2=0$ then $y_1=0$, also a contradiction for, if so, then because $y'(x_1)=0$, we have that either $c_1=0$, too or $\cos kx_1=\sin kx_1=0$, and both are impossible. Thus there is no simultaneous solution to the transversality conditions and the Euler–Lagrange equation. So, there is no extremal curve for this extremization problem. Exactly the same conclusions can be inferred from the method of vector calculus. It will also give equations equivalent to the transversality conditions.

(ii) Sinusoidal example, epimorphic case: fix y_1, find x_1. Only the second transversality condition should be applied. It gives:

$$k^2y(x_1)^2+y'(x_1)^2=0,$$

which means $y(x_1)=y'(x_1)=0$, if we assume solutions are real. This means y_1 was not arbitrary. If we try to solve the equations in spite of this one will find the same result as in case (i), namely, one can derive a contradiction.

(iii) Sinusoidal example, morphallactic case: fix x_1, find y_1. If $\sin x_1=0$ then $y_1=\cos x_1$. To find y_1 if $\sin(x_1)$ is not zero, equation (10) may be used. The first transversality condition is $y'(x_1)=0$, which gives $c_1=\tan x_1$. If $\cos x_1=0$, c_2 cannot be determined; if $\cos x_1$ is not zero, then $y_1=\cos x_1+c_1 \sin x_1$.

Now, we must check the solution curve to see whether or not it minimizes the functional. In the only case of interest, where neither $\sin x_1$ nor $\cos x_1$ is zero, equation (10) may be used. If it is substituted into the functional one finds:

$$I(x_1, y_1)=[(1+y_1^2)\cos x_1-2y_1]/\sin x_1.$$

The second derivative of I with respect to y_1 is $2 \cot x_1$, which is positive, negative or zero according to $x_1 \in (n\pi,(2n+1)\pi/2)$, $2x_1=(2n+1)\pi$, $x_1 \in ((2n-1)\pi/2, n\pi)$. For x_1 in the first interval the function $y(x)=\tan x_1 \sin x+\cos x$ minimizes the functional over boundary values at x_1. If x_1 is fixed the extremalizing curves only minimize the functional for x_1 less than π (Elsgolts, 1977). The second variation formula gives no information.

If $x_1=n\pi$ for some integer n, then $y(x)=\cos x+c_1 \sin x$, where c_1 is still undetermined. If this $y(x)$ is substituted into the functional, it becomes identically zero; the problem cannot be solved.

In summary, the sinusoidal example shows that the programme carried out in the first example cannot be followed here, at least not for the least constrained or epimorphic cases, since no minimum exists. In the morphallactic case, for certain ranges of x_1, we were able to minimize the functional and so, in principle, our programme could be carried out. We would then have to identify a field dynamic, perhaps the obvious analogue to (3). We did not examine stability for the morphallactic case, since our interest was primarily epimorphic regeneration.

Example 3. *Jacobi elliptic functions.* Let α, $\beta>0$ and consider the functional:

$$I[x_1, y_1, y(x)]=\int_{x_0}^{x_1} y'^2-\alpha y^2+\tfrac{1}{2}\beta y^4 \, dx.$$

The Euler–Lagrange equation is:

$$y''+\alpha y-\beta y^3=0.$$

This can be simplified by replacing x with $\sqrt{\alpha}x$ and setting $\varepsilon=\beta/\alpha$, assuming that α is not zero. Now, the Euler–Lagrange equation becomes:

$$y''+y-\varepsilon^3=0. \tag{11}$$

The third order term is a simple way to investigate the consequences of non-linearities which will certainly be present in biological systems. The same three cases as before will be examined here and it will be seen that identification of the analytic form of solution is non-trivial. Minimality will be examined in a separate section, and the phase portrait will be used as an aid to visualizing what the solutions look like when they can be identified.

Observe that equation (11) admits the first integral:

$$y'^2+y^2-(\varepsilon/2)y^4=C, \tag{12}$$

where C is a real constant. It is the same as the constant occuring in proposition 1 and its various possible values define all the energy levels the system may assume. These are given in the phase portrait.

3.2. Phase portrait analysis. Before we solve the three problems for this example we illustrate what the solutions look like by examining the phase portrait for the differential equation $y''+y-\varepsilon y^3=0$, where for the moment we assume that it can be regarded as an initial value problem in which $y(x_0)$ and $y'(x_0)$ are given, rather than as a boundary value problem in which $y(x_0)$ and $y(x_1)$ are given. (Conditions for the equivalence of these two classes of problem will be cited later.) The phase curves are in one-to-one correspondence with the solutions to the differential equation and we will, in particular be able to identify those solutions to the differential equation which correspond to minimizing the functional. Thus, examining the phase portrait will graphically show when solutions are possible and what they represent in terms of morphogenesis.

To justify the use of the phase portrait for our boundary value problems we note that they are closely related to initial value problems. These two classes of problems are equivalent under fairly mild hypotheses (Jordan and Smith, 1977). The equivalence is generated by a one-to-one correspondence between initial slopes and values assumed at some later fixed point. The correspondence is bijective because the solution curves tile a region of the plane. It was by using this equivalence that we could produce Fig. 7, the phase portrait associated with the elliptic functional. The regions are marked according to the value C assumes on them where C is the constant which indicates that the system is conservative.

To draw the phase portrait first pass to an equivalent system of differential equations:

$$y'=z,$$

$$z'=\varepsilon y^3-y,$$

where $y(0)=y_0$, $z(0)=z_0$ are given. The solutions $y(x)$ and $z(x)$ may be presented graphically, on a Cartesian plane with coordinates axes labeled y and z. This is done in Fig. 7. An analytic expression for the phase trajectories comes from equation (12):

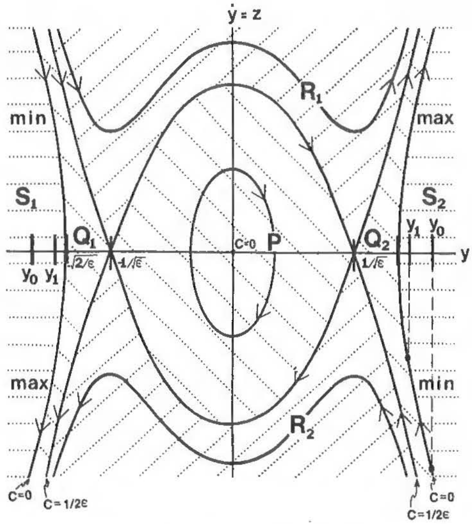

Figure 7. This is the phase portrait for equation (12), modified from Ross, (1964). Each solution to equation (12) corresponds to a curve in the phase portrait. The arrowheads on each of the phase curves indicate the direction in which it is traversed with increasing x. We assume that ε is given, here $\varepsilon=1$. We denote regions by the values which C assumes on them: S, $C<0$; P, Q, $0<C<1/2\varepsilon$; R, $C>1/2\varepsilon$. There are three equilibrium points: two saddle points at $\pm 1/\sqrt{\varepsilon}$ and one center at the origin. Within the region P, bounded by the separatrices the phase paths are closed Jordan curves. The values of (y, y') must lie on one of the two curves marked $C=0$ if the function y optimizes the functional. They meet the y axis at $\pm\sqrt{(2/\varepsilon)}$. The parts of the curve which are maxima and minima are marked on the figure as are the positions of y_0 and y_1 for which an extremal solution can be found. For the left branch of the $C=0$ curve these values must be to the left of the left branch; the corresponding values in the right half plane are mirror symmetric. For these values we can choose a maximum or a minimum depending on whether we choose the part of the $C=0$ curve going from y_0 to y_1 to be above or below the y-axis.

$$z(x)=\pm[C-y^2(x)+\varepsilon y^4/2]^{1/2},$$

in which the phase trajectories are indexed by the parameter C. We will be most interested in the case that $C=0$, for by proposition 1, requiring $C=0$ is equivalent to applying the second transverality condition. Note that although the independent variable x does not appear explicitly it parametrizes each of the phase curves. As x increases from 0, the solution moves along the phase curve on which (y_0, z_0) lies.

The points at which $(y', z')=(0,0)$ are called singular points. They correspond to constant solutions and the behavior of solutions through nearby points as x increases determines whether they are stable or unstable. As is indicated on the diagram, the singular points for this equation are $(1\sqrt{\varepsilon}, 0)$,

$(-1/\sqrt{\varepsilon}, 0)$, and $(0, 0)$. Linearization can be used to determine the local behavior of the vector field (y', z') and the local behavior about a singular point can prove analytically what the phase portrait presents graphically. It can be shown that the first two singular points are saddle points, i.e., they are stable along one line and unstable along another, and the third is a centre, i.e. the solution travels along a closed curve about it.

The qualitative properties of the phase curves depend on C as is indicated by Fig. 6. To examine these properties look at what happens when $z=0$. We obtain from equation (12) that

$$\varepsilon y^4 - y^2 + C = 0.$$

This quartic is examined in detail in the Appendix; we can choose $z=0$ and examine how solutions meet the y-axis as C varies. The existence of real roots to the quartic corresponds to the existence of y-intercepts. In particular, note that for $C=0$ the phase curve is disconnected and its y-intercepts are 0, $\pm\sqrt{(2/\varepsilon)}$. For y_0 or y_1 in $(-\sqrt{(2/\varepsilon)}, \sqrt{(2/\varepsilon)})$, because each solution to a differential equation lies in a single phase curve, it is clearly impossible to find a value of y' so that the initial and final point of y will lie on the phase curve defined by $C=0$. In general the constant solution $(0, 0)$ will fail to satisfy boundary conditions.

The reconciliation among the types of phase curves originating near singular points is achieved by special curves called separatrices, which carve the phase plane into the distinct regions. In our example one separatrix occurs for $C=1/2\varepsilon$. Further details on the phase plane are given in the appendix.

Now we can use the phase portrait to identify when there is a minimizing solution. It will shortly be shown that the coefficient of δx_1^2 in the second variation formula is $yy'(1-\varepsilon y^2)$, evaluated at x_1. We will see that solutions in the least constrained and epimorphic case require that $y^2 \geqslant 2/\varepsilon$. For a minimum the coefficient of δx_1^2 must be strictly positive, thus $y(x_1)$ and $y'(x_1)$ must be of opposite sign. So we know that if a minimum exists its endpoint must be on part of the $C=0$ curve in the lower right or upper left quadrant of the phase plane. The two curves obtained for $C=0$ have opposite orientation, that is, as x increases on the left hand curve y' decreases but on the right hand curve y' increases. Now assume $|y_0|, |y_1| > \sqrt{(2/\varepsilon)}$ are given. If both are positive then we must require $y_0 > y_1$ so that a solution which starts (y_0, y_0') on the part of the curve $C=0$ in the lower right quadrant can remain on that part of the $C=0$ curve where it will give a minimum. Then, there will exist a unique y_1', and hence a unique x_1, so that as x increases from x_0 to x_1 it parametrizes a minimizing curve for the functional. If $y_0 < y_1$ then we end up maximizing the functional.

If y_0, $y_1 < 0$, then analogous reasoning may be used to show that when $y_0 < y_1$ the resulting curve minimizes the functional, and when $y_0 > y_1$ the resulting curve maximizes the functional. These are the only cases in which the functional can be minimized.

(i) Elliptic function example, least constrained case: find x_1 and y_1. Choosing $C=0$ is equivalent to applying the second transversality condition. The first transversality condition requires that $y'(x_1)=0$. Using this with equation (12), and taking $C=0$, requires that $y_1=0, \pm 1/\sqrt{\varepsilon}$. First assume y_1 is not zero; later it will be shown that if $y_1=0$ then no solution exists. Solving equation (12) for x, (Jordan and Smith, 1977) and writing γ for $\varepsilon/2$ we may write:

$$x_1 = \int_{y_0}^{y_1} 1/\sqrt{(\gamma y^4 - y^2)}\, dy. \tag{13}$$

Using the substitution $\sqrt{\gamma}y = 1/\cos\phi$ one may easily show that:

$$x_1 = \arccos(1/\sqrt{\gamma}y_1) - \arccos(1/\sqrt{\gamma}y_0). \tag{14}$$

This may be rewritten to give:

$$y_1 = 1/\{\sqrt{\gamma}\cos[x_1 + (\arccos 1/\sqrt{\gamma}y_0)]\}. \tag{15}$$

Deriving equation (15) did not require that y_1 not be zero. But, since cosine is a bounded function, it does show that if y_1 is zero there is no extremal value for x_1.

Since the derivation of equations (14) and (15) was done formally it remains to set bounds on their validity. Note that as ϕ varies over $[0, \pi/2]\cup[\pi/2, \pi]$, $y = 1/\sqrt{\gamma}\cos\phi$ varies over $[1/\sqrt{\gamma}, \infty]\cup[-\infty, -1/\sqrt{\gamma}]$. If $|y| < 1/\sqrt{\gamma}$ then $\varepsilon y^4 - y^2 < 0$ so that (13) has a complex valued integrand. Thus, for a real solution to exist one must require that $|y_0| \geqslant 1/\sqrt{\gamma}$ and that the domain of integration in equation (13) excludes $(-1/\sqrt{\gamma}, 1/\sqrt{\gamma})$. This further explains why $y_1=0$ is impossible. It also shows that $y_1 = \pm 1/\sqrt{\gamma}$ accordingly as the solution is contained in $(-\infty, 1/\sqrt{\gamma})$ or $(1/\sqrt{\gamma}, \infty)$ and gives an end point suggesting the limb grows so that $|y|$ decreases to $1/\sqrt{\gamma}$.

(ii) Elliptic function example, epimorphic case: fix y_1, find x_1. The same calculations as in case (i) may be performed so equation (14) remains valid. Note that if we choose y_0 so that the second term in the argument of the RHS of equation (15) is zero and fix y_1, then $y_1 = 1/(\sqrt{\gamma}\cos x_1)$ which forces $|y_1| > 1/\sqrt{\gamma}$ so we see that no optimal value of x_1 can exist, in general, for curves inside region P in Fig. 7.

(iii) Elliptic function example, morphallactic case: fix x_1, find y_1. The transversality condition gives $y'(x_1)=0$. Also, equation (12) holds but C is not in general zero. Solutions must also satisfy:

$$x_1 = \int_{y_0}^{y_1} 1/\sqrt{(C-y^2+\gamma y^4)}\,dy. \tag{16}$$

The solution changes form depending on the size of C. Given C, y_1 can be determined. Given a solution and therefore y_1, one can impose $y'(x_1)=0$ to determine C. Thus finding C is equivalent to finding y_1. Examination of the forms of solution for all values of C will cover all possible values of y_1. There are four subcases: (1) $C<0$; (2) $C=0$; (3) $C\in(0, 1/2\varepsilon)$; (4) $C>1/2\varepsilon$. In the first subcase it can be shown that $y(x)=y_1/\text{cn}(x/g)$ where g is a constant determined by transforming equation (16) to an elliptic integral (Byrd and Friedman, 1954). Details are in the Appendix. Now the transversality condition gives:

$$0=[\text{sn}(x_1/g)\,\text{dn}(x_1/g)]/\text{cn}^2[x_1/g). \tag{17}$$

Since g depends on C and x_1 is known, equation (17) and the boundary condition will determine y_1.

For subcase 2 the result is the same as in case (i) of this example.

For subcase 3 one may show that the form of solution is:

$$y(x)=\sqrt{A}\,\text{sn}(\sqrt{(\varepsilon B)}\text{x}), \tag{18}$$

where A and B are functions of ε and C. Details are in the Appendix. So, the transversality condition gives that:

$$0=\sqrt{A}\text{cn}(\sqrt{(\varepsilon B)}x_1)\,\text{dn}(\sqrt{(\varepsilon B)}x_1).$$

Since the elliptic function dn is always strictly positive the last equation means that $\text{cn}\sqrt{(\varepsilon B)}x_1=0$ and therefore $x_1\in\{2n+1)\text{K}/\sqrt{(\varepsilon B)}|n\in N\}$, where K is half the period of dn (Bowman, 1961). This means one can find C, possibly many values for C. Now, from equation (10) one calculates $y_1=\sqrt{A}$.

For subcase 4, the calculations were too difficult to complete because the forms of solution involve a ratio of elliptic functions and a fractional linear term. The form of solution was found and is given in the Appendix.

3.3. Examining for Minimality. Since the method of vector calculus is quite difficult even for the simplest of these solutions—either one must differentiate something extremely complicated or one must know C as a function of y in these cases the second variation formula will be applied. The coefficients of δx_1^2, δy_1^2, and $\delta x_1\,\delta y_1$ are, respectively:

$$yy'(1-\varepsilon y^2),\ 0,\ 2y(\varepsilon y^2-1),$$

all evaluated at x_1. The integral term appearing in the second variation is present in all three cases. It is:

$$\int_{x_0}^{x_1} (3\varepsilon y^2 - 1)\,\delta y^2 + \delta y'^2\,dx.$$

In the least constrained and epimorphic cases after imposing the transversality conditions one finds that $y_1 = 0, \pm\sqrt{(2/\varepsilon)}$. If $y_1 = 0$ then all coefficients are zero so we can make no conclusion. So, assume y is not zero. In the least constrained case the coefficient of $\delta y_1\,\delta x_1$ is not zero which means that the extremal solution is neither a maximum nor a minimum. In the epimorphic case we have the integral term and the coefficient of δx_1^2 which is:

$$y'(x_1)y_1(1 - \varepsilon y_1^2).$$

A solution can only exist if $y_1^2 \geqslant 2/\varepsilon$ so $(1 - \varepsilon y_1^2) < -1$. This means that a minimum exists if we choose $y'(x_1)$ and y_1 to be of opposite sign and force the integral term to be positive, for example by choosing x_0, y_0 and y_1 so that $y^2 > 1/(3\varepsilon)$ on $[x_0, x_1]$. This can be done by using equation (12) with $C = 0$ to express $y'(x_1)$ in terms of y_1. The ranges will depend on ε. We did not carry this out because it was complicated and all we wanted was the conclusion that a minimizing solution exists.

In the morphallactic case we only have the integral term. Therefore we know that on the interval the solution is a minimum but we have no information as to whether or not it is a minimum with respect to variation of the boundary value.

We wanted to examine the stability of time dependent solutions which obeyed a field dynamic. However the candidates for limiting solutions we found were quite complicated. This meant it would be exceedingly difficult to find a simple enough field dynamic that we could, on the basis of theoretical calculations, determine uniform asymptotic stability. The simplest field dynamic we could study would be essentially one which forced the Euler–Lagrange equation to be time dependent, thus:

$$\frac{\partial}{\partial t}\,y = y'' + y - \varepsilon y^3$$

could be chosen. In fact, the stability properties of this equation have been determined (Chafee and Infante, 1974). There are several points which are uniformly asymptotically stable, so no unique optimizing solution exists which is stable for that choice of growth dynamic.

Now we summarize the results of this third example. The least constrained and epimorphic cases both gave solutions. In the first of these cases the solution was neither a maximum nor a minimum. In the second case the solution is a minimum under complicated conditions on the initial conditions and the fixed boundary value. In the morphallactic case we found four different forms of solution, corresponding to different values of a parameter. We could not guarantee any of these were ever minimal. Whether or not a solution gives a minimum depends on the boundary values in a complex way. We can only note a necessary condition for an extremal in the least constrained and epimorphic cases is that either $y_0 > y_1 > \sqrt{(2/\varepsilon)}$ or $y_0 < y_1 < -\sqrt{(2/\varepsilon)}$. The solutions corresponding to phase curves in the region P of Fig. 7, a region not admitting extremal solutions, were one of the two forms of solution to equation (11) that were considered by Totafurno (1985).

This example was sufficiently complicated that we introduced the phase portrait to enable us to picture what the solution curves looked like and to know when to expect a solution. Because of the non-linearities we were unable to make any statement about stability. However, many possible field dynamics would be compatible with the solutions we found. Again, our purpose was only to demonstrate the plausibility of a technique. So, we did not pursue a stability result although we conjecture it could be done.

4. Discussion. We have assumed that developmental mechanisms generate an axially symmetric cylindrical leg in which the length and the pattern of a state variable are unique extremals of a time-independent functional. The functional is an integral over proximo-distal positions, x, of a function of the state variable $y(x)$ and its gradient $y'(x)$. These assumptions imply that if an axially symmetric region of the leg is displaced to an abnormal proximo-distal level, regeneration will restore the normal local pattern of y values. If a section of the leg is deleted, it will regenerate (Fig. 1). If a grafting operation produces a discontinuity of pattern, regeneration will eliminate the discontinuity (e.g. Fig. 2). Thus the principle of continuity follows from the assumption that pattern and form extremize an integral for which the integrand does not depend directly on x.

As examples we have studied functionals which give three patterns of $y(x)$ that have been used to model regeneration—an exponential gradient, a sinusoid, and a Jacobi elliptic function. Each of these patterns is obtained as a solution of the Euler–Lagrange equation which results from setting the first variation of a functional equal to zero. A transversality condition must also be satisfied to provide extremals of unconstrained boundary values. When such extremal values exist, a solution is obtained which is continuous and is constructed of pieces of the pattern function. In each example we assumed that the boundary values (x_0, y_0) at the base of the leg are given, and we sought extremal values (x_1, y_1) at the distal end for three conditions.

(i) Find x_1 and y_1. This might represent ontogenetic growth without specification of a distal boundary condition. This is the *least constrained case.*

(ii) Find x_1 given y_1. This corresponds to ontogenetic growth or epimorphic regeneration with the distal boundary condition specified. This is the *epimorphic case.*

(iii) Find y_1 given x_1. In this case the size of the domain for regeneration is fixed, but the distal boundary value is not specified. This might represent morphallactic regeneration; we call it the *morphallactic case.*

In each case where an extremum was obtained, we inquired whether the extremum was a minimum.

For the exponential, in the least constrained case we found an extremal solution but it was neither a maximum nor a minimum. The epimorphic case can be resolved into three subcases. An extremal solution exists for two of these but not for the third. For one of the former two subcases the solution is a minimum according to two criteria, but for the other subcase the two criteria disagree about the character of the extremum. In the morphallactic case a minimum of the functional was obtained when x_1 and y_1 were positive. Furthermore, for the epimorphic and morphallactic cases we found a time-dependent Liapunov functional which converged to the steady state functional, and a time-dependent dynamic which asymptotically produced the time-independent pattern.

For the sinuosid an extremal could only be found in the morphallactic case, and even then only under fairly stringent hypotheses. For the Jacobi elliptic function, in the least constrained case there is an extremal solution which is not a minimum or a maximum. In the epimorphic case we were unable to determine the character of the extremal solution. In the morphallactic case solutions to the extremal problem could assume four distinct forms. Under complicated conditions on the ranges of parameters and on x_1 and y_1 we were able to argue that a minimizing curve existed. One of the four forms of solution for the morphallactic case was the same form as Totafurno (1985; see also Totafurno and Trainor, 1987) used to model epimorphic regeneration of supernumerary legs in salamanders. One might object to the use of this morphallactic solution on two grounds. The solution is periodic in space; the number of periods to be intercalated is not uniquely defined. Also, although the solution restores the continuity of pattern by the production of supernumerary legs, the spatial scale of the pattern does not extremize the functional.

Totafurno (1985) considered a second form of solution to the Euler–Lagrange equation. This form does not correspond to solutions for the least constrained or epimorphic cases; it is unclear whether it corresponds to a solution for the morphallactic case.

4.1. Generalizations. There are three obvious ways to generalize the procedure for obtaining a time-independent pattern and form for a leg.

(i) Allow more state variables $y_1, \ldots y_n$ in the integrand. In an axisymmetric leg, one of these might represent the distance from the surface of the leg to the symmetry axis, so that non-cylindrical limbs could be treated. Other variables might be concentrations of interacting morphogens in a reaction–diffusion system.

(ii) Allow derivatives of higher order in the integrand. The dynamic for some physical processes, including elasticity, requires such derivatives (Shames and Dym, 1985).

(iii) Treat a surface that is not axisymmetric by using two spatial parameters (u, v) rather than the length x as the independent variables. The proximal boundary of the leg is a closed Jordan curve delimiting a region in the u–v plane. Several state variables could be used, one to represent the shape of the surface and others to represent the patterns of morphogens. For such cases the Euler–Lagrange equations and a generalization of the transversality conditions have been obtained (Courant and Hilbert, 1965). The theory of minimal surfaces (Spivak, 1975) can be used to analyze this problem.

An issue which remains ambiguous is the choice of a functional to extremize, given a dynamic expressed as one or more differential equations. Our examples involve a class of reaction–diffusion equations in a single dependent variable. We showed that for this class, the differential equation corresponds to a unique functional. In general a given differential equation may be obtained as the Euler–Lagrange equation from any functional in a large class (Rosen, 1967, Chapter 5.3). Do all of these functionals, when extremized with variation of boundary conditions, give the same boundary conditions? Is there a physical principle which selects a particular functional as the appropriate one for morphogenesis? Diverse extremal principles have been used to analyze problems in structural mechanics (see discussion of Reissner's principle by Shames and Dym, 1985); the physical principles governing morphogenesis might also admit diverse functionals for extremization. These issues seem interesting and important, but they are beyond the scope of this work.

The use of a Liapunov functional to characterize asymptotic stability of the time-independent state suggests a further line of inquiry. It may be possible to obtain the time-dependent dynamic for regeneration as Euler–Lagrange equations from a time-dependent functional. If so, the same functional may be a Liapunov functional for the dynamic, so that asymptotic convergence to a time-independent state would be assured. However, it is noteworthy that there are reaction–diffusion dynamics not known to be derivable from any stationary principle, and without a known Lyapunov functional (Ben-Jacob *et al.*, 1985, Section 5.2).

4.2. Remarks on modelling surfaces which bear patterns of state variables. To compare various models for the shaping of surfaces which bear positional information, such as our model and the model of Cummings (1985), one must note the distinction between the extrinsic geometry and the intrinsic geometry of a surface, as made in differential geometry (Do Carmo, 1976; Lipschutz, 1969). An abstract surface can be placed, or embedded, in Euclidean 3-space (R^3) in many different ways; these embeddings are diffeomorphic to each other. Intrinsic geometry is the study of those properties which are not affected by changes of coordinates during the deformation of a surface without stretching it or contracting it. Such a deformation is isometric; it preserves the distances between points in the surface. Intrinsic parameters are invariant under isometric diffeomorphisms. The first fundamental coefficients and the Gaussian curvature are intrinsic parameters. Since distances are measured by means of the first fundamental form, its preservation under isometries is not surprising.

Extrinsic geometry characterizes the embedding which situates an abstract surface in an ambient space. Among the extrinsic parameters which characterize a local region of the surface are the principal curvatures and the second fundamental coefficients. Clearly, the principal curvatures can be affected by deformations which preserve the distances between points in the surface. Since the second fundamental coefficients arise from the way a surface is embedded in R^3 it is not surprising that they are extrinsic. It is remarkable that the product of two extrinsic parameters, the principal curvatures, gives an intrinsic parameter, the Gaussian curvature.

The class of possible embeddings includes many more realizations of an abstract surface than are meant by "orientation in space" or "rigid motion". Some examples will show this (possibly unexpected) diversity of embeddings. Consider a square in the Euclidean plane, with the Euclidean metric. Not only can we translate and rotate the square without changing its intrinsic parameters; we can deform it in many other ways. For instance, we might form it into a wavy surface. So long as each ripple extends across the breadth of the square, the pattern of waviness does not change the intrinsic parameters; but the embedding of the abstract surface in R^3 has changed. Similarly, suppose a cap of a sphere is cut off at a colatitude less than 90°. Its intrinsic parameters are unchanged by pressing along its rim and so deforming its boundary from a circle to an ovoid; but its embedding has changed.

To characterize the changes in shape which a surface undergoes during development, knowledge of extrinsic parameters as well as intrinsic parameters is necessary in general. Models such as ours, which try to predict the extrinsic geometry of a limb, use both types of parameters. Limited progress is possible from information about intrinsic parameters: if one assumes that growth is isotropic and that a condition on the smooth spatial variation of the local growth rate is valid, this rate can be inferred from the relation between the Gaussian curvature and the first fundamental coefficients (Todd, 1985a,b). However, efforts to model the shape of a surface and the pattern of state variables on it without using extrinsic parameters are likely to encounter difficulties. Cummings (1985) has made such a model. He assumed that growth is isotropic and that the Gaussian curvature is a scalar function of a state variable which obeys the Helmholtz equation (the spatial part of the linear wave equation). He used the Helmholtz equation, and the relation between the Gaussian curvature and the first fundamental coefficients, as two equations to solve for the geometry of the surface and the pattern of the state variable.

It is not clear under what circumstances this model will generate a unique surface with a unique pattern of the state variable. The eigenvalue used in the Helmholtz equation might correspond to more than one eigenfunction; if so, which eigenfunction should be used? If the surface is not complete one must assume boundary values and show that they are compatible with the coupled partial differential equations. The fundamental theorem of surfaces (Lipschutz, 1969) provides such a compatibility condition. The geometry of the boundary curve, together with the intrinsic geometry of the surface determined from the model, may define its extrinsic geometry uniquely. This is true for the square and for the cap of a sphere discussed above. However, it is not true in general; there may be many ways to embed the abstract surface in R^3, or there may be no embedding at all, as for a Klein bottle. As they devise models for the morphogenesis of surfaces in ontogeny and regeneration, theoreticians must deal with these issues in order to provide experimenters with unequivocal inferences.

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APPENDIX

On the Forms of Solutions in Example Three. To develop the forms of solution some preliminary calculations are required.

LEMMA 1. *$\varepsilon C \leqslant 1/4$ implies there exist A, B such that* $C-x^2+\varepsilon x^4=\varepsilon(A-x^2)(B-x^2)$.

Proof. It is sufficient to show $C=\varepsilon AB$ and $1=\varepsilon(A+B)$ admit simultaneous solutions. If $\varepsilon C \leqslant 1/4$ then $0=\varepsilon B^2-B+C$ has two real roots. Let B be one of them. Now $1=\varepsilon[B+C/(\varepsilon B)]$, so choose $A=C/(\varepsilon B)$. ■

Note that if γ is a root of $C-x^2+\varepsilon x^4$ so is $-\gamma$. The roots of $C-x^2+\varepsilon x^4$ are either purely imaginary or real, for $\varepsilon C \leqslant 1/4$. If $\varepsilon C \leqslant 1/4$ the sign of C determines the nature of the roots: if $C>0$, then A and B have the same sign and in particular positive, in which case the roots are all real. If $C<0$, then A and B have opposite signs and there are two real roots and two imaginary roots.

LEMMA 2. *$\varepsilon C > 1/4$ implies $C - x^2 + \varepsilon x^4$ has no real roots.*

Proof. Note that for $F_C(x) = C - x^2 + \varepsilon x^4$ C represents a shift up or down. If $\varepsilon C = 1/4$ then $F_C(x) = \varepsilon[x^2 - 1/(2\varepsilon)]^2$. $F_C' = 0$ forces $x = 0, \pm 1/\sqrt{(2\varepsilon)}$. The graph of F_C is above the horizontal axis and as C increases it ceases to touch the horizontal axis so there can be no real solutions. ■

These two calculations were used to identify which values of the parameter correspond to which regions of the phase portrait. It can be shown that the two saddle points lie on the phase curve corresponding to $C = 1/(2\varepsilon)$. If $C > 1/(2\varepsilon)$ then by lemma 2 the phase curves must be in region R. If $C < 0$, then by lemma 1 the phase curves must lie in region S for they admit only two y-intercepts. If $C \in (0, 1/(2\varepsilon))$ then by lemma 1 and Fig. 7 there are four y-intercepts, so these curves have one component in each of the regions Q1, P, and Q2.

LEMMA 3. *$\varepsilon C > 1/4$ implies F_C has no purely imaginary roots.*

Proof. By way of contradiction if $i\gamma$ is a root of F_C then γ is a root of $\varepsilon x^4 + x^2 + C$. But $\varepsilon C > 1/4$ implies $C > 0$ which means $\varepsilon x^4 + x^2 = C < 0$ which has no real roots. ■

Since, any integral which has the square root of a quartic in the denominator of its integrand can be expressed in terms of elliptic functions we apply the above observations to the integral which appears in equation (16). We see that it will assume one of the following forms:

(1) If $\varepsilon C \leqslant 1/4$ and $C > 0$, then it is:

$$\int 1/\sqrt{(a^2 - x^2)(b^2 - x^2)]}\, \mathrm{d}x.$$

(2) If $C = 0$ we obtain case (i) of example 1.

(3) If $\varepsilon C \leqslant 1/4$ and $C < 0$ then it is:

$$\int 1/\sqrt{[(a^2 + x^2)(x^2 - b^2)]}\, \mathrm{d}x.$$

(4) If $\varepsilon C > 1/4$ then it is:

$$\int 1/\sqrt{[(x-a)(x-a^*)(x-b)(x-b^*)]}\, \mathrm{d}x,$$

where * indicates the complex conjugate and the limits of integration must be allowed to vary.

These forms can be found in integral tables for certain domains of integration, those which exclude points for which the integrand becomes infinite.

For the first case three forms of solutions can be found by using 216, 219, and 220 (Byrd and Friedman, 1954). They are:

(1) For $y_0 = a$ and $y_1 > a > b > 0$, and $g = 1/a$ one can show that:

$$y_1(x_1) = \pm[b^2 + (b^2 - a^2)/(\mathrm{sn}^2(x_1/g) - 1)].$$

(2) For $a > b \geqslant y_1 > 0$, and $g = 1/a$ one can show that:

$$y_1(x_1) = b\,\mathrm{sn}\,x_1/g.$$

(3) For $a > b = y_1 > y_0 \geqslant 0$, and $g = 1/a$ one can find $y_0(x_1)$ from:

$$\mathrm{sn}^2 x_1/g = [a^2(b^2 - y_0^2)]/[b^2(a^2 - y_0^2)].$$

For the third case entry 211 gives that for $y_1 > b = y_0 > 0$ and $g^2 = 1/(a^2 + b^2)$ that:

$$y_1(x_1) = b/\mathrm{cn}(x_1/g).$$

For case four, entry 267 gives, for parameters g, g_1, a_1, and b_1 defined in terms of a and b that:

$$\mathrm{sn}(x_1/g)/\mathrm{cn}(x_1/g) = (y_1 - b + a_1 g_1)/(a_1 + g_1 b_1 - g_1 y_1),$$

from which one can solve for $y_1(x_1)$.

These formulae are the analytic forms of solutions in the phase portrait. It remains to show that they satisfy the differential equation. This hasn't been done yet but if the derivation is correct then they must be solutions.

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