J. theor. Biol. (1991) **149**, 339-360

Pigmentation Pattern Formation on Snakes

J. D. MURRAY† AND M. R. MYERSCOUGH‡

† *Applied Mathematics FS-20, University of Washington, Seattle, WA 98195, U.S.A. and ‡ Applied Mathematics FO7, University of Sydney, Sydney, N.S.W., Australia*

(*Received on* 20 *February* 1990, *Accepted in revised form on* 7 *August* 1990)

We consider a cell-chemotaxis model mechanism for generating some of the common, simple and complex, patterns found on the skin of snakes. By investigating the pattern generation potential of the model we show that many of the more complex patterns might result from growth of the integument during the pattern formation process. We suggest that many of the diverse elaborate patterns on snakes, and other species, can be generated by a single mechanism if the time scale of the pattern process is commensurate with the time scale associated with significant embryonic growth.

1. Introduction

Some of the most dramatic and beautiful biological patterns are the pigmentation patterns on the integument of animals. Mathematical models for the formation of such patterns have been proposed for mammalian coat markings (Murray, 1979, 1981*a*, 1988*b*; Bard, 1981; Young, 1984; Cocho *et al.*, 1987*a*, *b*), butterfly wing patterns (Nijhout, 1978, 1989; Murray, 1981*b*; Sibatani, 1981) and shells (Ermentrout *et al.*, 1986; Meinhardt & Klingler, 1987). In this paper we focus on pigment patterns on the snake (order: *squamata*) integument. Snakes—reptiles and amphibians in general in fact—are numerous and highly diverse in both morphology and physiology. Snakes and lizards exhibit a particularly rich variety of patterns many of which are specific to snakes. Even within the same species there is often extreme pattern polymorphism; the ubiquitous California king snake is a good example (Zweifel, 1981). Pattern anomalies frequently occur even on an individual snake as in Fig. 1(e) and (f). A cursory glance at any field guide (e.g. FitzSimons, 1970; Cogger, 1975; Arnold & Burton, 1978; Beller & King, 1979) shows not only straightforward pattern elements such as lateral and longitudinal stripes and simple spots but also a great range of patterns based on various complex pattern elements not found on other animals. A small selection of snake patterns, both regular and irregular, are shown in Fig. 1. In the case of butterfly wing patterns the seemingly complex patterns can in fact be generated by a relatively small number of pattern elements (Nijhout, 1978, 1990; Murray, 1981*b*, 1989). On the other hand, many of the common snake patterns (see section 4) do not seem to fall into any of the usual classes of patterns which can be generated by typical pattern generating mechanisms, such as the widely studied reaction-diffusion models [Turing, 1952; see, e.g. the

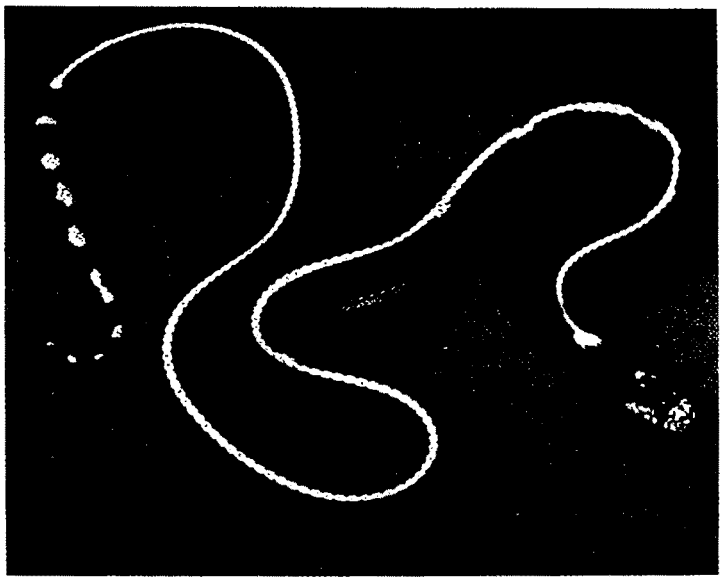
0022-5193/91/070339+22 $03.00/0

book by Murray (1989) for a full discussion] and the more recent mechano-chemical models (Murray *et al.*, 1983; Oster *et al.*, 1983; for a review, see Murray *et al.*, 1988 or Murray, 1989).

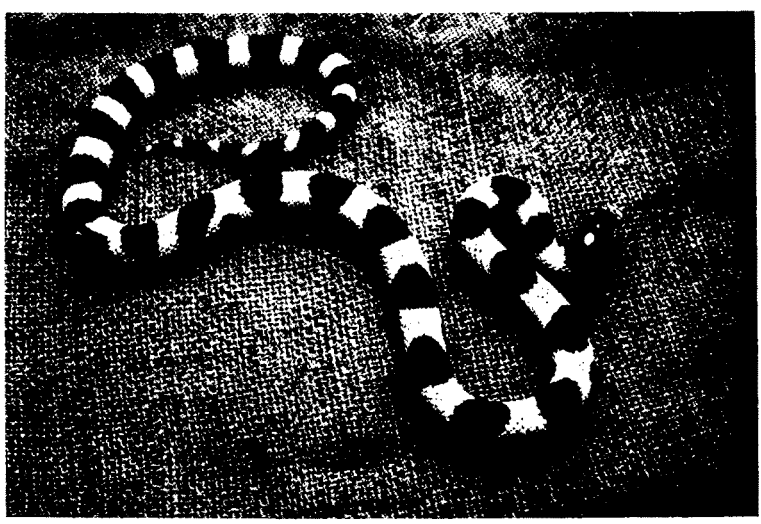
The skin of reptiles is the largest organ in their bodies and poses many interesting developmental problems (see the review by Maderson, 1985). The skin essentially consists of an external epidermis with a subadjacent dermis. Although the pattern formation mechanism is unknown we know that the pattern is fixed in the dermis. The basic skin pigment pattern remains on Lepidosaurs after the periodic replacement of the epidermis. This is the well-known skin-shedding exhibited by snakes and lizards. Pigment cell precursors, called chromatoblasts, migrate from the neural crest during development (Le Douarin, 1982) and more or less distribute themselves uniformly in the dermal skin. Whether or not the skin develops a pigmented patch depends on whether presumptive pigment cells produce pigment or remain quiescent. Chromatophore interactions may result in pigmented cells and unpigmented cells gathering in different regions to produce stripes or spots (Bagnara & Hadley, 1973). It is not known when chromatoblasts become committed to producing pigment. Evidence from mice (Mayer, 1980) shows that cells may be able to produce pigment long before they actually do so. Cells which are committed to pigmentation are also able to divide for some time, although they may lose this ability later on. Pigment cells in the epidermis probably come from the dermal region.

Experimental studies of pigment development and the migration of pigment cell precursors have been largely confined to amphibians, mammals and birds; see, for example, the recent work by Richardson *et al.* (1990). Although there is a large body of work on *crocodilia*, particularly on the alligator (*Alligator mississippiensis*), by Ferguson and his co-workers (for a definitive review see Ferguson, 1985) little has been done specifically on skin patterns on reptiles. An exception is the theoretical and experimental work on stripe patterning on alligators by Murray (1988*a*) and Murray *et al.* (1990). An underlying assumption in their work and ours is that the basic processes of migration, division and differentiation will be the same in snakes and other reptiles as in other animals. Relatively little research has been done on snake embryology [a partial list is given by Hubert (1985); see also the volume edited by Gans & Billett (1985) in the series on *reptilia*], ecology and evolutionary biology. Extensive embryological studies, however, have been done on alligators (Ferguson, 1985; Deeming & Ferguson, 1989, 1990: see also earlier references in these papers).

Hubert & Dufaure (1968) mapped the development of the asp viper (*Vipera aspis*). Pigmentation was first observed on the scales of the body, when the embryo was about 106 mm long, and extended to the head as development proceeded. The pattern, however, is almost certainly laid down earlier in development than when it first becomes visible. Zehr's (1962) observations of the development of the common garter snake (*Thamnophis sirtalis sirtalis*) suggest a similar developmental process. He noted that when the pigmentation pattern first appears it is not well-formed but becomes more defined as development proceeds. [A similar progressive development of final pattern occurs on many butterfly wing patterns (Nijhout, 1990)]. Treadwell (1962) reported that in embryos of the bullsnake (*Pituophis melanoleucus sayi*) three rows of spots appear on the sides of the embryo at 29 days and blotches appeared

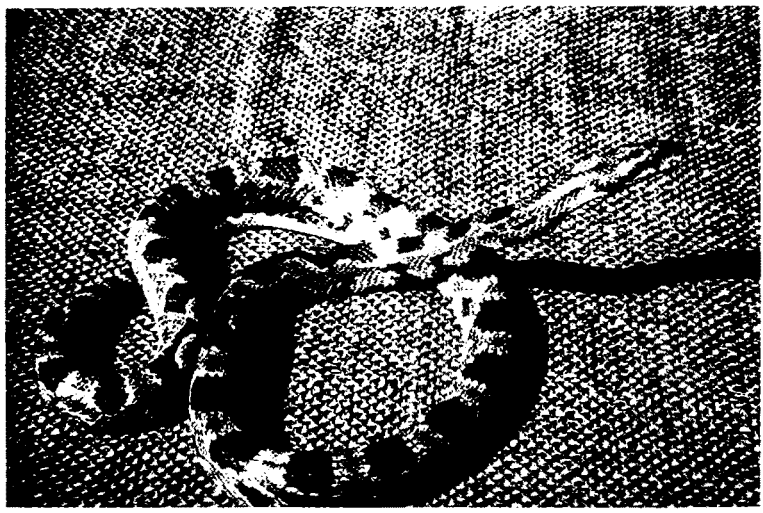


(a)

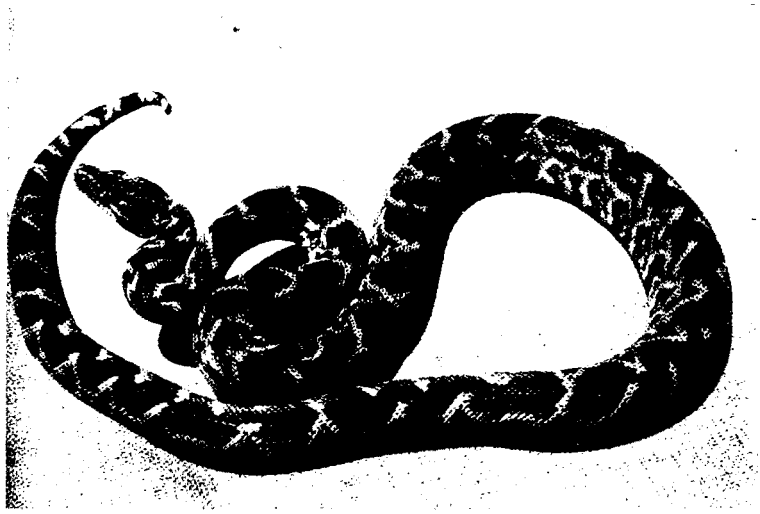


(b)

FIG. 1. Examples of snake skin patterns. Those in (a) and (b) are quite regular although in (a) even the simple stripe pattern exhibits aberration. In (e) and (f) there is a mixture of basic patterns on the same snake. (Photographs courtesy of Lloyd Lemke.)

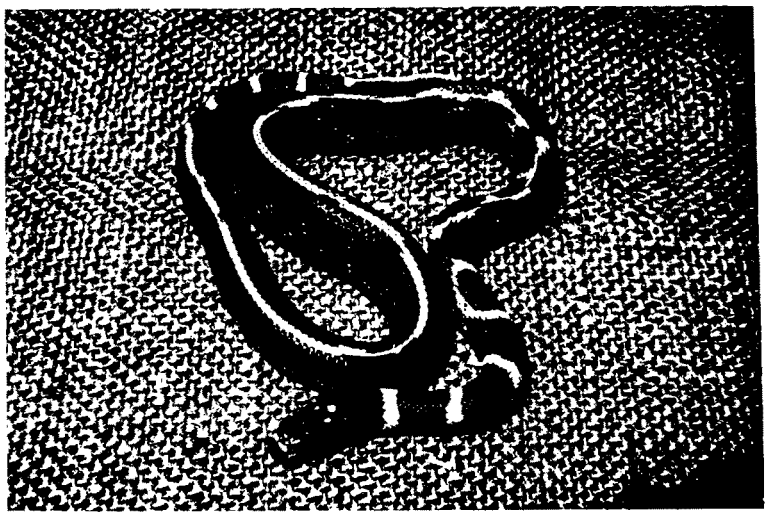


(c)

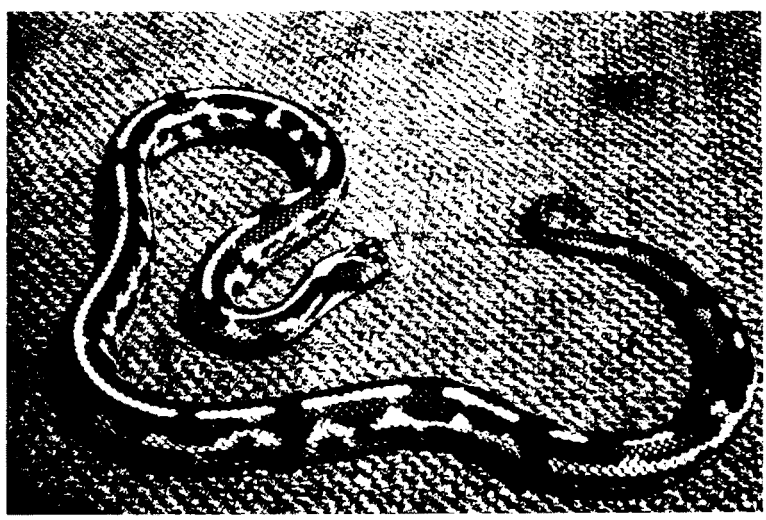


(d)

FIG. 1—continued



(e)



(f)

FIG. 1—continued

on the dorsal midline at 31 days. The timing of developmental events in snakes should be regarded with caution as the rate of development is significantly affected by the temperature of incubation of the eggs or the body temperature of the mother in live-bearing species (Saint Girons, 1985). In the case of the asp viper, for example, gestation periods from 90–110 days have been observed. In the case of the alligator (*A. mississippiensis*) Murray *et al.* (1990) specifically addressed the problem of when in development the stripe pattern is laid down. The model mechanism they considered is the same as the one we consider here. Murray *et al.* (1990) concluded that whatever mechanism is responsible for the pattern formation it would have to generate the spatial pattern around 35 days through the gestation period days for males and about 40 days for females; the gestation period is from 61–65 days. The pattern became visible shortly after it was formed. Histological sections of the skin showed that there were melanocytes present in the white regions between the dark stripes but these did not appear to produce melanin. One possible explanation for the lack of melanogenesis by these cells is that a threshold density of melanocytes could be necessary before melanogenesis can take place.

Murray (1988*a*) and Murray *et al.* (1990) discussed some of the implications of the alligator's embryonic growth on a time scale commensurate with the time required for the pattern generator to create the pattern. They showed that this could be responsible for the lighter shadow stripes observed on alligator bodies; these shadow stripes lie towards the ventral side of the body and lie between the distinct darker stripes on the dorsal side. There are thus two potentially important implications relevant to snake integumental patterns which arise from these alligator studies. One is that potentially quite different patterns can be generated on an embryo when significant growth occurs during the patterning process. The other is that presumptive pigment cell patterns may be generated some time before they start to produce pigment and the pattern becomes visually discernible.

Prior to the work on alligator stripe patterns mathematical models for pigment pattern formation divided roughly into continuous reaction-diffusion type models and discrete models which share some similarities with cellular automata. Murray (1979, 1981*a*, *b*) proposed a 2-morphogen reaction-diffusion model for mammalian coat patterns and the spatial patterns he obtained were in morphogen concentration. He then suggested that if the morphogen was above a certain threshold it stimulated the cells to produce pigment. Using this model and the concept of a chemical prepattern for pigment production he was able to generate patterns similar to those observed on the large cats, giraffes, zebras and many other mammals. He was also able to demonstrate the crucial effects of embryonic integument shape and size on the patterns obtained and discovered examples of developmental constraints, a subject of considerable current interest (Oster *et al.*, 1988; Oster & Murray, 1989).

Young (1984) presented a cellular automata model. Each cell was placed on a two-dimensional lattice and could be in one of two states, differentiated into a pigment bearing cell or dedifferentiated. The state of each cell was determined at each time interval by a summation of the states of neighbouring and nearby cells. He showed that such a mechanism tended to a steady-state and could produce both spots and stripes. Cocho *et al.* (1987*b*) also used a cellular automata where the state of the elements on the previous row of the lattice determined, by some arbitrary

rule, the state of the element in the current row. Thus, pattern spreads in a wave rather than evolving simultaneously over the whole domain. Using various combinations of automata rules they produced patterns similar to those on felines, fish and some snakes. The results are interesting but the automata rules are somewhat arbitrary and it is hard to relate them to specific cell biology. Cocho *et al.* (1987*a*) presented a model based on cellular interactions which minimize a discrete energy function for the system. They use this mathematical formulation as justification for a biological model for banding patterns on snakes and allophenic mice. This model, however, requires a spatially heterogeneous "field" to exist before the energy minimization commences and so gives no practical insight into the actual onset of spatial pattern.

Here we investigate in detail the cell-chemotaxis model mechanism pattern generator proposed by Oster & Murray (1989), based on the chemotaxis model for patterning in the slime mold *Dictyostelium discoideum* proposed by Keller & Segel (1970), and used by Murray (1988*a*) and Murray *et al.* (1990). The spatial pattern potential of chemotaxis (a kind of negative diffusion) based models have been exploited in variety of different contexts such as, interfacial patterns which appear during alloy solidification (Wollkind *et al.*, 1984), biocontrol interaction between goldenrod aphids and ladybird beetles (Kareiva & Odell, 1987) and mite predator-prey interaction on fruit trees (Wollkind & Collings, 1990)—in the latter two examples it is preytaxis rather than chemotaxis but the governing mathematical equations have similar aggregative terms.

In this paper we are particularly interested in the patterns which can be formed by the cell-chemotaxis mechanism when the integumental domain is growing during the patterning process. We find that the spatially heterogeneous solutions can be quite different to, and considerably more complex than all of those so far found in other studies. Although our motivation for this research was to try and generate some of the complex pigmentation patterns on snakes and lizards the study has implications for biological pattern formation in general since it is likely that many of the pattern forming mechanisms involved in embryogenesis are operative on a time scale commensurate with embryonic growth. The surprising novelty and complexity of new patterns which are generated by this model as a consequence of domain growth are likely to occur with other pattern forming mechanisms such as reaction-diffusion and mechano-chemical systems.

At this stage, because of the paucity of morphological data on snake embryology it is not possible to suggest when in development of the snake embryo the mechanism is operative. Our purpose here is to suggest how some of the diverse complex skin patterns on snakes could be generated. This is clearly a necessary first step in modeling any pattern formation process.

2. Cell-chemotaxis Model Mechanism

The model we consider here involves actual cell movement. Pattern formation models which directly involve cells are potentially more amenable to related experimental investigation. One possibility could be the continuum Oster-Murray mechano-chemical models for the dermis. However, in view of the experimental

work reported by Murray *et al.* (1990) we felt it was more appropriate at this stage to consider the simpler mechanism they used, namely a cell-chemotaxis mechanism for pattern formation where the cells are considered the chromatoblasts in the integument. Also, Le Douarin (1982) speculated that chemotaxis may be a factor in the migration of pigment cells into the skin. Heuristically we can see how chemotaxis could well be responsible for rounding up and sharpening of spots and stripes. In the model, we propose that chromatoblasts both respond to and produce their own chemoattractant. Such a mechanism can promote localization of differentiated cells in certain regions of the skin which we associate with the observed patterns on the snake integument. The cells, as well as responding chemotactically, are assumed to diffuse. Diffusion is a dispersive effect while chemotaxis is an aggregative effect. We also assume the cells undergo mitosis. We model the chemoattractant production by the cells by a simple Michaelis-Menten kinetics; the detailed form is not crucial here. The chemoattractant also diffuses and decays according to first-order kinetics.

We denote the density of cells by n and the concentration of chemoattractant by c. A relatively simple mathematical formulation of such a system as we have just described is

$$\begin{pmatrix}\text{rate of change}\\ \text{of cell density}\end{pmatrix} = \text{diffusion} - \text{chemotaxis} + \text{cell mitosis}$$

$$\partial n/\partial t = D_n \nabla^2 n - \alpha \nabla \cdot (n \nabla c) + rn(N - n), \tag{1}$$

$$\begin{pmatrix}\text{rate of change}\\ \text{of chemoattractant}\end{pmatrix} = \text{diffusion} + \begin{matrix}\text{chemoattractant}\\ \text{production}\end{matrix} - \text{degradation}$$

$$\partial c/\partial t = D_c \nabla^2 c + \frac{Sn}{\beta + n} - \gamma c, \tag{2}$$

where D_n and D_c are the diffusion coefficients of the cells and chemoattractant respectively. Here we have assumed a simple logistic growth form for the cell mitotic rate with parameters r and N, respectively the linear mitotic rate and initial uniform cell density. The chemotaxis parameter α is a measure of the strength of the chemotaxis effect. The parameters S and γ are measures, respectively, of the maximum secretion rate of the chemicals by the cells and how quickly the chemoattractant is naturally degraded; β is the equivalent Michaelis constant associated with the chemoattractant production. This is the specific model discussed by Oster & Murray (1989) in relation to developmental constraints. In spite of its relative simplicity it can display, particularly when growth is allowed to take place during the pattern formation process, remarkably complex spatial patterns evolution.

It is always convenient to cast the model equations in non-dimensional terms [see Murray (1989) for a general pedagogical discussion]. Among other mathematical advantages this reduces the number of relevant parameters by combining them in meaningful groups. We do this here by introducing the dimensionless quantities

$$\begin{aligned} &x^* = [\gamma/(D_c s)]^{1/2} x, \qquad t^* = \gamma t/s, \qquad n^* = n/\beta, \qquad c^* = \gamma c/S \\ &N^* = N/\beta, \qquad D^* = D_n/D_c, \qquad \alpha^* = \alpha S/(\gamma D_c), \qquad r^* = r\beta/\gamma, \end{aligned} \tag{3}$$

where s is a scale factor. We can think of $s=1$ as the basic integument size and carry out the simulations on a fixed domain size and increase s to simulate larger integuments. This procedure was introduced and used by Murray (1979, 1981*a*, *b*) in his study of animal coat patterns. With (3) the non-dimensional model equations become, on omitting the asterisks for notational simplicity,

$$\partial n/\partial t = D\nabla^2 n - \alpha\nabla\cdot(n\nabla c) + srn(N-n) \tag{4a}$$

$$\partial c/\partial t = \nabla^2 c + s[n/(1+n) - c]. \tag{4b}$$

The numerical simulations of these equations were carried out on a simple rectangular domain, whose length is considerably longer than the width, with zero flux of cells and chemoattractant on the boundaries. The detailed numerical simulations and bifurcating pattern sequences which can occur as the parameters vary are reported elsewhere (Maini *et al.*, 1991; Winters *et al.*, 1991).

The reason we consider a long rectangular domain is that we believe the skin patterns are laid down at a stage when the embryo is already distinctly snake-like; that is, it is already long and essentially cylindrical, even if it is in a coiled state [see, e.g. the details of the embryo of the asp viper (*Vipera aspis*) given by Hubert & Defaure (1968) and Hubert (1985)]. Although it would be more realistic to study the model mechanism on the surface of a coiled cylindrical domain the numerical simulation difficulties are already considerable even on a plane domain. At this stage we are primarily concerned with the variety of patterns that the mechanism can generate and we consider the rectangular domain to represent the cylindrical snake integument laid out on a plane. The main features of the patterns on an equivalent cylindrical surface will be similar.

Equation (4) has one positive homogeneous steady-state $n_0 = N$, $c_0 = N/(1+N)$. In the Appendix we show that this steady-state is unstable to spatial perturbations for a choice of parameters which satisfy certain conditions. Solutions of the equations with these parameters are spatially heterogeneous. The conditions which the parameters must satisfy and the subsequent results suggest certain experimental manipulations, particularly those which vary the uniform base cell density. How the bifurcation to spatially structured solutions can be influenced by varying a parameter of the system is discussed in detail by Oster & Murray (1989) and Murray (1989).

We now suggest that this cell-chemotaxis mechanism (1) and (2) is a candidate mechanism for generating the patterns found on snake integument. We further suggest that the observed patterns reflect an underlying spatial pattern in cell density. We do not know when in development the pattern generator operates nor how long it takes for the pattern to develop as compared with significant growth in the embryo integument, the relevant spatial domain for the model eqn (4). The size of the domain is thus a significant parameter in the model. Patterns can start to evolve as one of several parameters pass through bifurcation values which make the uniform steady-state unstable. In this paper we demonstrate some of the patterns eqn (4) can produce and relate them to specific snake patterns.

For our simulations we assumed that the rate of cell differentiation and the development of the snake embryo are such that the chemotactic system has come

to a steady-state, or nearly so, by the time the pigmentation patterns become fixed. Hence we solve eqn (4) at steady-state, that is

$$D\nabla^2 n - \alpha\nabla \cdot (n\nabla c) + srn(N-n) = 0 \tag{5a}$$

$$\nabla^2 c + s[n/(1+n) - c] = 0. \tag{5b}$$

These equations were solved on a long two-dimensional rectangular domain which reflects the geometry of the snake integument. We assume that cells are confined to this domain and so we impose zero flux boundary conditions on its edges. The package ENTWIFE used to solve eqn (5) numerically, as various parameters are varied, is described in some detail by Winters *et al.* (1991). Here we focus primarily on the results and biological implications and relevance of the solutions. Some analytical work on finite amplitude steady-state solutions of (5) in one space dimension has been done by Grindrod *et al.* (1989).

3. Simple Pattern Elements

By altering the values of the mitotic rate r and chemotactic parameters α we were able to generate a wide range of different stripe patterns and also regular spot patterns. Some examples of basic lateral striping are shown in Fig. 2(a). Lateral banding is a common pattern element in snakes. One example, the bandy-bandy (*Vermicella annulata*) is illustrated in Fig. 2(b). Other examples include the coral snakes, *Micrurus* species, the banded krait (*Bungarus fasciatus*) and the ringed version of *Lampropeltis getulus californiae* [Fig. 2(d)].

The model can also produce longitudinal stripes parallel to the long edges of the domain as in Fig. 3(a). This type of striping occurs, for example, in the ribbon snake (*Thamnophis sauritus sauritus*) as in Fig. 3(b) and in the garter snake (*T. sirtalis sirtalis*) and the four-lined snake (*Elaphe quatuorlineata*). For some parameter sets relatively small changes in domain size or parameter values may be sufficient to change the lateral stripes produced by this model to longitudinal stripes and vice versa. This implies that both lateral and longitudinal stripes can occur on different individuals of the same species. This is, in fact, the case with the California king snake (*L. getulus californiae*), which can be either laterally [Fig. 2(d)] or longitudinally striped as in Fig. 3(c): see also Fig. 1(a).

By an appropriate choice of parameter values we can also generate regular spot and blotch patterned solutions to eqn (5). Some of these are illustrated in Fig. 4(a). Regular spots form part of the pattern on many snakes. The Cape mountain adder (*Bitis atropos atropos*), for example, displays an alternating semicircular pattern similar to those generated by this model.

In an activator-inhibitor reaction-diffusion system stripes will form in preference to spots when the production of the activator is limited either by saturation of its production or by rapid diffusion or decay of the substrate as discussed by Meinhardt (1988, 1989) and Lacalli *et al.* (1988). Their work is primarily concerned with stripe formation in the fruit fly *Drosophila*. Different reaction-diffusion models specifically concerned with stripe patterning, and applied to the *Drosophila* embryo, have been

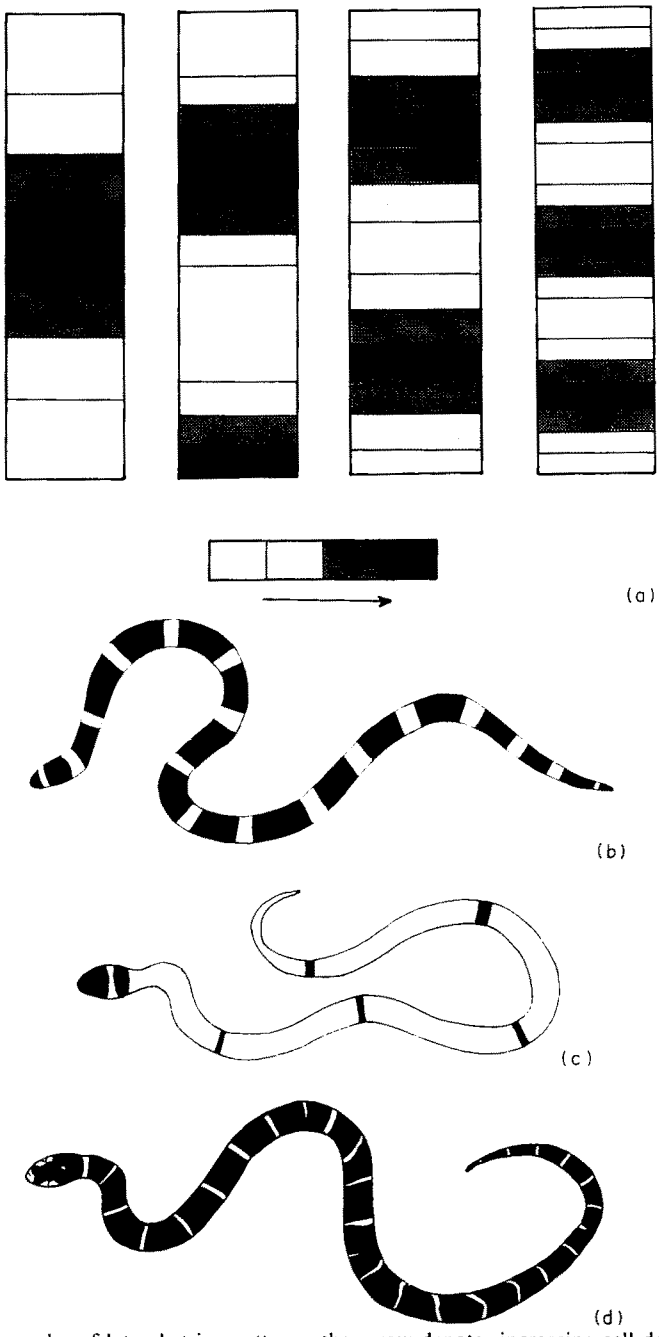


FIG. 2. (a) Examples of lateral stripe patterns: the arrow denotes increasing cell density. Parameter values in (5) vary for each example except for $D = 0{\cdot}25$, $N = s = 1$: for the first $r = 1{\cdot}52$, $\alpha = 12{\cdot}31$, the second $r = 1{\cdot}52$, $\alpha = 13{\cdot}4$, the third $r = 24{\cdot}4$, $\alpha = 118{\cdot}68$, the fourth $r = 1{\cdot}52$, $\alpha = 29{\cdot}61$. (b) Lateral stripes on the striped snake *Vermicella annulata*. (c) An example of sparse narrow stripes on the snake *Pseudonaja modesta*. (d) Laterally striped *Lampropeltis getulus californiae*.

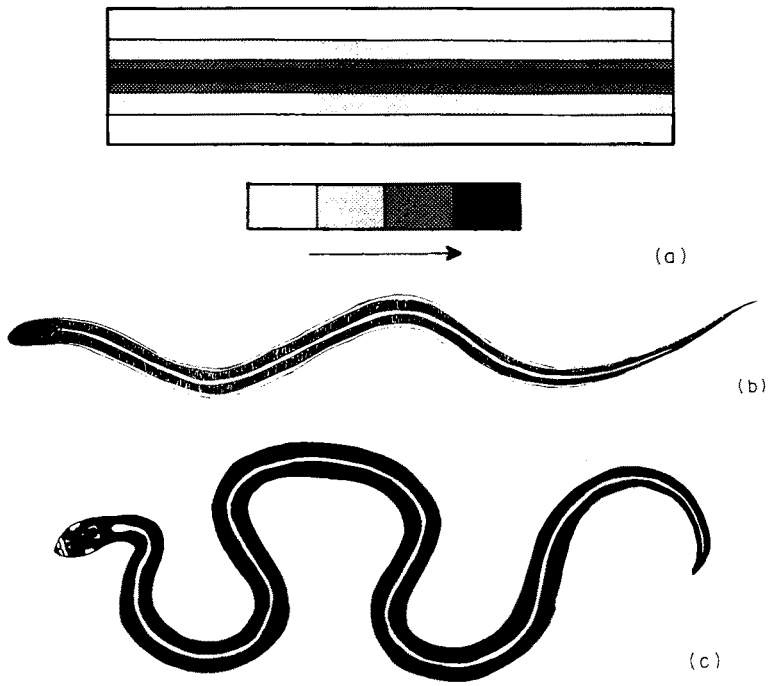


FIG. 3. (a) A computed solution giving a longitudinal strip: the arrow denotes increasing cell density. Parameter values for eqn (5) are $D = 0{\cdot}25$, $N = s = 1$, $r = 389{\cdot}6$, $\alpha = 1782$. (b) The snake *Thamnophis sauritus sauritus* generally exhibits longitudinal striping. (c) Longitudinally striped *Lamelpropelsis getulus californiae.*

proposed by Nagorcka (1988) and Lacalli (1990). An interesting and practical analytical procedure which indicates which pattern—stripes or spots—will form has recently been developed by Ermentrout (1991).

Whether spots or stripes form in this chemotactic model will depend on initial conditions, domain shape and size and on the values of the parameters α, D, r and N. We consider only changes in α but other parameters may also be used to illustrate the argument. For any particular initial conditions and domain size, stripes are most likely to form when the chemotactic response α is low (see, e.g. the bifurcation diagrams in Maini *et al.*, 1990). This also corresponds to slow production or rapid diffusion or decay of chemoattractant in the dimensional problem. When the chemotactic response is weak cells must be in large regions of high cell density to produce enough chemoattractant to form a sufficiently steep gradient in chemoattractant concentration. This steep gradient is needed to recruit enough cells to the cluster to balance the logistic loss. For steep gradients in chemoattractant concentration to exist a region of low cell density, where chemoattractant production is low, must be near the region of high cell density. Thus stripes, comprised of many cells but with regions with few cells nearby, will be preferred to spots when α is low. When the cells' chemotactic response is faster, gradients in chemoattractant concentration do not need to be so steep. Hence, fewer cells can produce enough chemoattractant

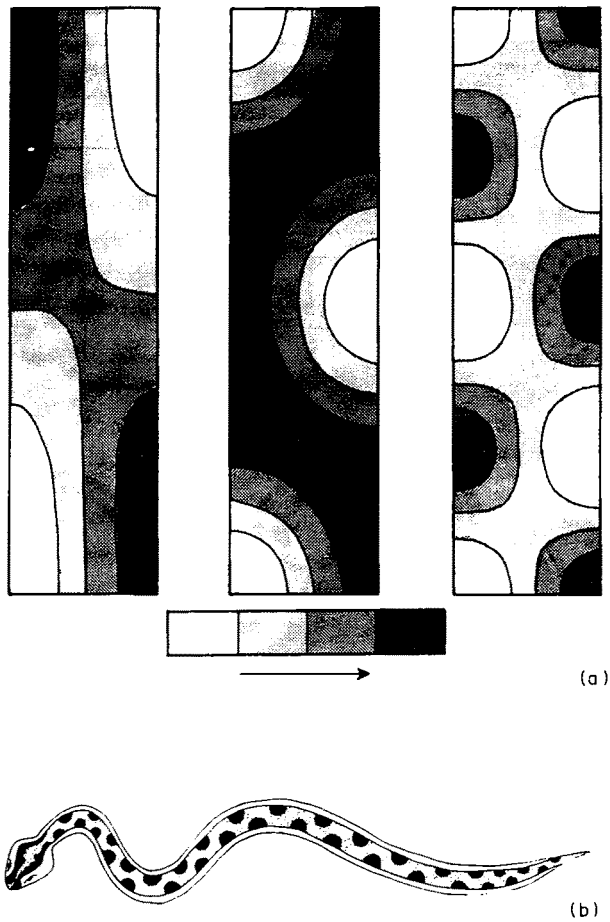


FIG. 4. (a) Computed solutions of the model system (5) giving regular spot patterns: the arrow denotes increasing cell density. Parameter values $D = 0{\cdot}25$, $N = s = 1$ with the following for the three patterns respectively: $r = 28{\cdot}22$, $\alpha = 135{\cdot}16$; $r = 28{\cdot}05$, $\alpha = 135$; $r = 1{\cdot}52$, $\alpha = 27{\cdot}06$. (b) The regular spot pattern on the snake the leopard snake *Bitis atropos atropos.*

to recruit other cells to the resulting cluster and these clusters are more likely to be spots.

Once steady-state patterning has been formed, increasing α may either define the existing pattern more sharply or, in some cases, lead to qualitative changes in the pattern, as described in the following section. We did not observe any case where stripes split into spots although we did observe instances where stripes split into two (see also Maini *et al.*, 1990). This happened in the absence of any mitosis. For an established stripe pattern, an increase in chemotactic response, that is α, gave very sharp, well-defined bands. Such isolated narrow bands of pigment cells are regularly observed in the ringed brown snake (*Pseudonaja modesta*) as illustrated in Fig. 2(c). For a reaction-diffusion mechanism to produce such sharp banding

the threshold for pigment production would have to be very finely tuned. In contrast, with a chemotaxis mechanism all that is required is that α/D be large. In the case of a spot pattern increasing α usually leads to sharper focussing of the cluster although qualitative changes were observed in one case. We discuss this in the next section.

4. Complex Patterns

This chemotactic model is highly non-linear and the pattern potential is not restricted to simple elements such as stripes and regular spots. It is not easy, however, to predict the type of complex patterns which can be obtained. For example, if we take a regular spot pattern and then solve the equations as the chemotactic component of the cells' motion, α, increases but without changing domain shape, the pattern shifts and changes its type. For sufficiently large α we get a pattern of pairs of spots as shown in Fig. 5(a). Such patterns occur, for example, in the leopard snake (*Elaphe situla*) as in Fig. 5(b). This species also exhibits a phase with single spots instead of paired spots.

A crucial aspect in the development of pattern could be the changing integument domain as a result of growth during the patterning process. We found, as expected, that changing the shape of the domain also produces complex patterns. Simple longitudinal growth of a laterally striped domain leads to the formation of additional stripes between established stripes as described by Murray *et al.* (1990) in the case of alligator skin patterns. Two examples of patterns obtained when we allowed lateral growth of the domain are given in Figs 6 and 7. In the first case, Fig. 6(a), lateral growth causes the asymmetric spot pattern to become symmetric as the aggregates of pigmented cells move into the centre of the domain. This type of centred spot pattern is very common. Examples include the corn snake (*E. guttata*) illustrated in Fig. 6(b) and various *Vipera* species. Starting from a slightly different spot pattern lateral growth can produce diamond patterns as in Fig. 7(a)(i). If the domain then becomes slightly narrower a wavy stripe pattern is generated as illustrated in Fig. 7(a)(ii). Diamond patterns are a characteristic feature of many rattlesnakes, such as the eastern diamondback rattlesnake (*Crotalus adamanteus*) illustrated in Fig. 7(b). The horseshoe snake (*Coluber hippocrepis*) also shows this diamond patterning. Near the tail where the body is narrower the diamond pattern may change to a wavy stripe as expected from the mathematical analysis, a feature pointed out by Murray (1981*b*) as an example of a developmental constraint. These results suggest that growth of the domain during the laying down of pigment patterns may have a very important role to play in the ultimate pattern that develops. This feature of course must apply to almost all pattern formation mechanisms.

5. Discussion

In this paper we have proposed and investigated a basic cell-chemotaxis model for pigment pattern formation on the snake integument. The model includes physical and chemical interactions between presumptive pigment cells and a chemoattractant

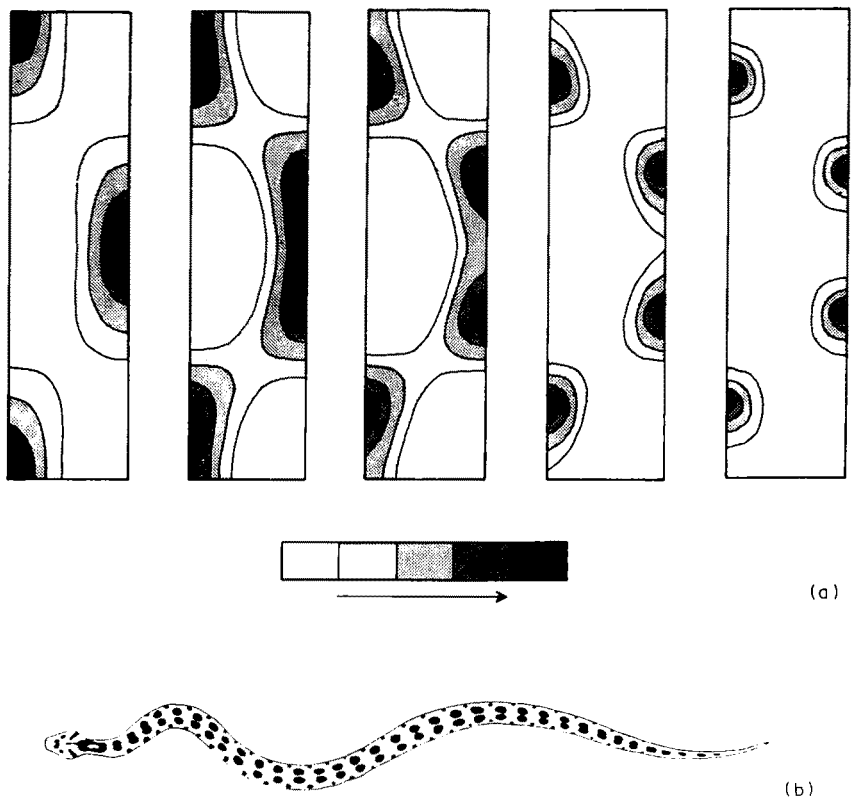


FIG. 5. (a) The changing pattern as the chemotactic parameter α is increased giving a paired spot pattern: the arrow denotes increasing cell density. Parameter values for (5): $D = 0{\cdot}25$, $N = s = 1$, $r = 1{\cdot}52$ with α increasing from $\alpha = 19{\cdot}92$–$63{\cdot}43$ (b) *Elaphe situla* showing paired spots.

produced by the cells. It differs from previous pigmentation pattern models which rely on creating chemical prepatterns. Chemotaxis was chosen as a plausible mechanism as it is known to be important in other developing systems and has been suggested as a mechanism for controlling pigment cell localization in the integument. Further evidence for a mechanism which directly involves cells is given by the experimental work reported by Murray *et al.* (1990).

We investigated numerically the steady-state patterns in two-dimensions which are solutions to the model eqn (5). The spatially heterogeneous solutions include such patterns as stripes and regular spots, namely patterns we might reasonably expect to obtain. However, we also found other patterns which we did not expect to find, such as the paired spot pattern and the wavy stripe and diamond patterns often observed on snakes.

In many snakes and lizards the body pattern continues to the end of the tail with little alteration, even where the tail is sharply tapered. One example is shown in Fig. 6(b). This contrasts with many mammalian patterns (Murray 1981*a*, 1988*b*) where if the tail pattern is mainly spots these almost always change to lateral stripes

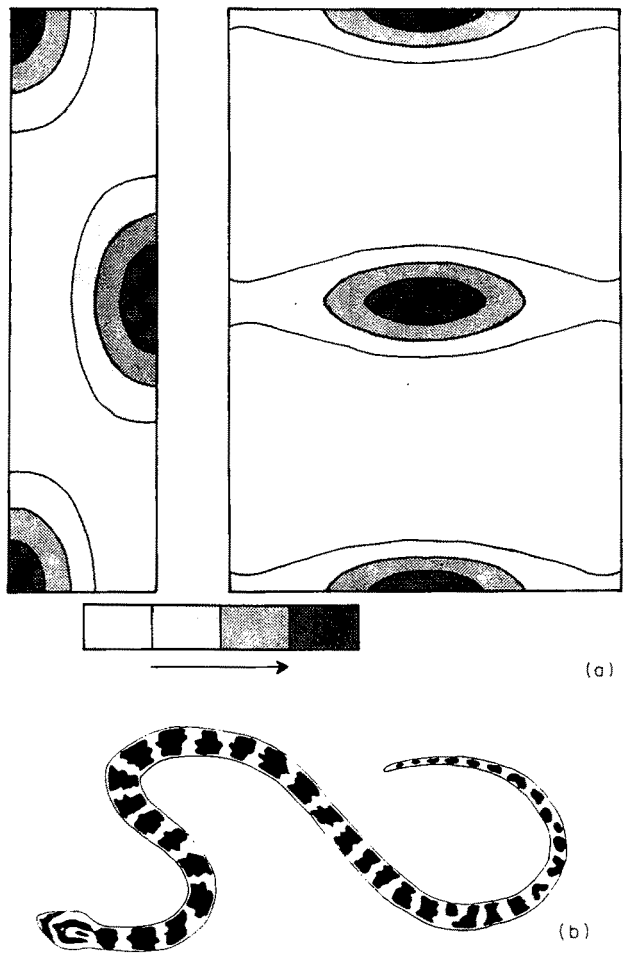


FIG. 6. (a) The effect of lateral growth of the domain can result in centred spots: the arrow denotes increasing cell density. Here the domain width is (i) 1 unit; (ii) 2·7 units. Parameter values for system (5) are $D = 0{\cdot}25$, $N = s = 1$, $r = 1{\cdot}52$, $\alpha = 20{\cdot}5$. (b) The snake *Elaphe guttata* typically has centred spot patterns.

as the domain tapers. It is possible that the aggregative effect of strong chemotaxis means that spots may be able to form even on tapering domains although further numerical calculations are required to confirm this. Ultimately, of course, we expect all spots to become stripes if the domain is thin enough during the pattern formation process. Another point, however, is that the taper in most snakes is considerably more gradual than is found on those animals, such as the cheetah and leopard, where spots degenerate into stripes just towards the tail tip.

The phenomenological similarities between actual snake patterns and the complex patterns produced by our chemotactic model is encouraging and provides motivation for further theoretical investigations and also for further experiments to investigate the possible role of chemotaxis in pigment pattern formation.

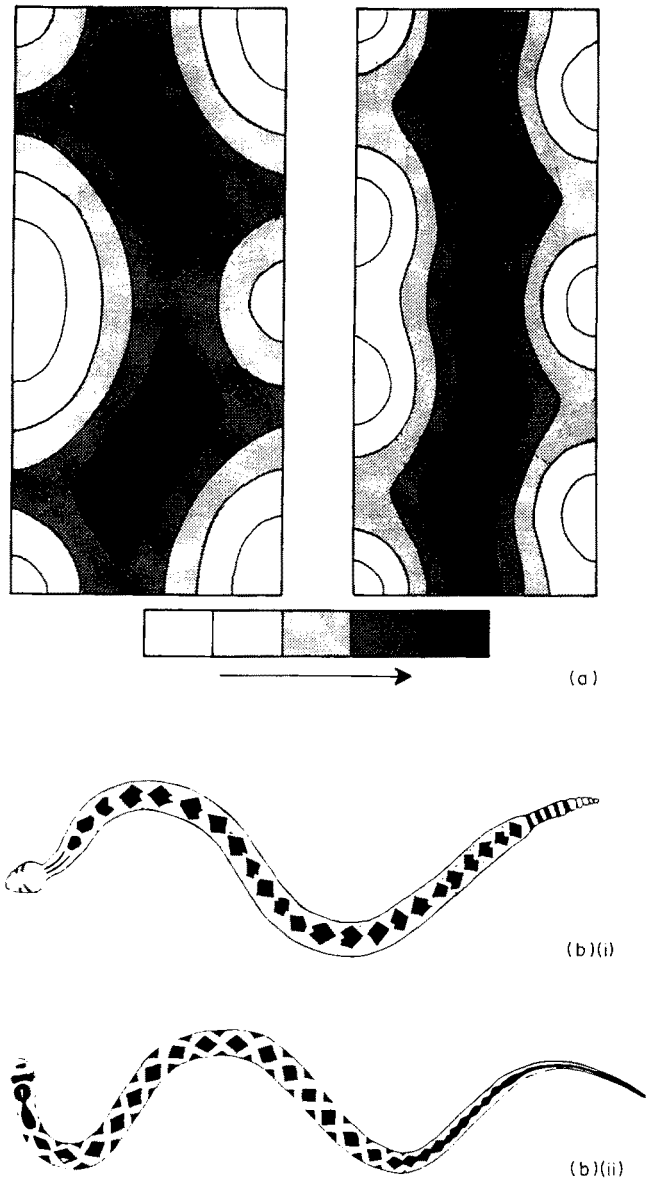


FIG. 7. (a) Here lateral growth of the domain gives diamond patterns and wavy stripes: the arrow denotes increasing cell density. Here the domain width is: (i) 1·84 units; (ii) 1·74 units. Parameter values for (5): $D = 0{\cdot}25$, $N = s = 1$, $r = 38{\cdot}05$, $\alpha = 177{\cdot}7$, (b) Examples of diamond patterns on snakes: (i) *Crotalus adamanteus*; (ii) *Coluber hippocrepis*. Note in (ii) the result of tapering on the pattern.

There is still much to be discovered in the numerical calculations of patterns in this model. In particular the effect of domain size on pattern merits further investigation and it would be interesting to investigate the effect on pattern by changing other parameters either singly or jointly during pattern formation. Since the initial uniform cell density (N) can be manipulated experimentally during development (see, e.g. the experimental results described by Oster *et al.*, 1988) this could be an interesting way to study such a model mechanism experimentally. The theory can make specific predictions.

This cell-chemotaxis model mechanism for pigment pattern formation is, of course, like previous models, speculative. Unlike previous models, however, it explicitly includes cell motility and cell–cell interaction through a chemical mediator for which there is some biological evidence or, at least, plausible justification. We have shown that this relatively simple model is capable of generating many of the observed simple and complex pigment markings found on a variety of snakes.

This work was in part supported by Grant DM-8902878 from the U.S. National Science Foundation. J.D.M. would like to thank Professor Richard Campbell (Biology, University of California, Irvine) for his encouragement to pursue snake patterning. M.R.M. wishes to acknowledge an Overseas Research Studentship (ORS) award. We would also like to thank Dr Hans Meinhardt for some constructive comments on the manuscript.

REFERENCES

ARNOLD, E. N. & BURTON, J. A. (1978). *A Field Guide to the Reptiles and Amphibians of Britain and Europe.* Collins: London.

BAGNARA, J. T. & HADLEY, M. E. (1973). *Chromatophores and Colour change: the Comparative Physiology of Animal Regeneration.* Prentice Hall: New Jersey.

BARD, J. B. L. (1981). A model for generating aspects of zebra and other mammalian coat patterns. *J. theor. Biol.* **93,** 363–385.

BELLER, J. L. & KING, F. W. (1979). *The Audubon Society Field Guide to North American Reptiles and Amphibians.* Alfred A. Knopf: New York.

BENTIL, D. E. & MURRAY, J. D. (1991). Pattern selection in biological pattern formation mechanisms. *Appl. math. Letts* (in press).

COCHO, G., PEREZ-PASCUAL, R. & RIUS, J. L. (1987*a*). Discrete systems, cell–cell interactions and color pattern of animals. I. Conflicting dynamics and pattern formation. *J. theor. Biol.* **125,** 419–435.

COCHO, G., PEREZ-PASCUAL, R., RIUS, J. L. & SOTO, F. (1987*b*). Discrete systems, cell–cell interactions and color pattern of animals. II. Clonal theory and cellular automata. *J. theor. Biol.* **125,** 437–447.

COGGER, H. G. (1975). *Reptiles and Amphibians of Australia.* Reed: Sydney.

DEEMING, D. C. & FERGUSON, M. W. J. (1989). Effects of incubation temperature on growth and development of embryos of *Alligator mississippiensis. J. comp. Physiol.* **B 159,** 183–193.

DEEMING, D. C. & FERGUSON, M. W. J. (1990). Morphogenetic analysis of embryonic development in *Alligator mississipiensis, Crocodylus johnstoni* and *Crocodylus porosus. J. Zool. Lond.* (in °press).

ERMENTROUT, B., CAMPBELL, J. & OSTER, G. (1986). A model for shell patterns based on neural activity. *The Veliger* **28,** 369–388.

FERGUSON, M. W. J. (1985). The reproductive biology and embryology of crocodilians. In: *Biology of the Reptilia* Vol. 14 *Development A* (Gans, C., Billet, F. & Maderson, P. F. A., eds) pp. 329–491. Wiley: New York.

FITZSIMONS, V. F. M. (1970). *A Field Guide to the Snakes of Southern Africa.* Collins: London.

GANS, C. & BILLETT, F. (1985). *Biology of the Reptilia* Vol. 15. John Wiley: New York

GRINDROD, P., MURRAY, J. D. & SINHA, S. (1989). Steady-state spatial patterns in a cell-chemotaxis model. *IMA J. math. Appl. Medic. Biol.* **6,** 69–80.

HUBERT, J. (1985). Embryology of the Squamata. In: *Biology of the Reptilia* Vol. 15 (Gans, C. & Billet, F., eds) pp. 1–34. John Wiley: New York.

HUBERT, J. & DUFAURE, J. P. (1968). Table de developpement de la vipere aspic: Vipera aspis L. *Bull. Soc. zool. France* **93,** 135-148.

KAREIVA, P. & ODELL, G. M. (1987). Swarms of predators exhibit "preytaxis" if individual predators use area search. *Am. Nat.* **130,** 233-270.

KELLER, E. F. & SEGEL, L. A. (1970). Initiation of slime mold aggregation viewed as an instability. *J. theor. Biol.* **26,** 399-415.

LACALLI, T. C. (1990). Modelling the *Drosophila pair-rule pattern* by reaction-diffusion: gap input and pattern control in a 4-morphogen system. *J. theor. Biol.* **144,** 171-194.

LACALLI, T., WILKINSON, D. A. & HARRISON, L. G. (1988). Theoretical aspects of stripe formation in relation to *Drosophila* segmentation. *Development* **103,** 105-113.

LE DOUARIN, N. M. (1982). *The Neural Crest.* Cambridge: Cambridge University Press.

MADERSON, P. F. A. (1985). Some developmental problems of the reptilian integument. In: *Biology of the Reptilia* Vol. 14 (Gans, C., Billet, F. & Maderson, P. F. A., eds) pp. 523-598. Wiley: New York.

MAINI, P. K., MYERSCOUGH, M. R., WINTERS, K. W. & MURRAY, J. D. (1991). Bifurcating spatially heterogeneous solutions in a chemotaxis model for biological pattern generation. *Bull. math. Biol.* (in press).

MAYER, T. C. (1980). The relationship between cell division and melanocyte differentiation in epidermal cultures from mouse embryos. *Dev. Biol.* **79,** 419-427.

MEINHARDT, H. (1988). Models for maternally supplied positional information and the activation of segmentation genes in *Drosophila* embryogenesis. *Development* **104** (Suppl.), 95-110.

MEINHARDT, H. (1989). Models for positional signalling with application to the dorsoventral patterning in insects and segregation into different cell types. *Development* **1989** (Suppl.), 169-180.

MEINHARDT, H. & KLINGLER, M. (1987). A model for pattern formation on the shells of molluscs. *J. theor. Biol.* **126,** 63-69.

MURRAY, J. D. (1979). A pattern formation mechanism and its application to mammalian coat markings. "Vito Volterra" Symposium on mathematical models in biology. Accademia dei Lincei, Rome Dec. 1979. In: *Lecture Notes in Biomathematics* **39,** 360-399. Springer-Verlag: Heidelberg 1980.

MURRAY, J. D. (1981*a*). A prepattern formation mechanism for animal coat markings. *J. theor. Biol.* **88,** 161-199.

MURRAY, J. D. (1981*b*). On pattern formation mechanisms for Lepidopteran wing patterns and mammalian coat markings. *Phil. Trans. R. Soc. Lond. B* **295,** 473-496.

MURRAY, J. D. (1988*a*). Modelling the pattern formation mechanism in the formation of stripes on alligators. In: *IXth International Congress on Mathematical Physics. Swansea* 1988, pp. 208-213. Adam Hilger: Bristol 1989.

MURRAY, J. D. (1988*b*). How the leopard gets its spots. *Sci. Am.* **256**(3), 80-87.

MURRAY, J. D. (1989). *Mathematical Biology.* Springer-Verlag: Heidelberg.

MURRAY, J. D., DEEMING, D. C. & FERGUSON, M. W. J. (1990). Size dependent pigmentation pattern formation in embryos of *Alligator mississippiensis*: time of initiation of pattern generation mechanism. *Proc. R. Soc. Lond. B* (in press).

MURRAY, J. D., MAINI, P. K. & TRANQUILLO, R. T. (1988). Mechanical models for generating biological pattern and form in development. *Phys. Rep.* **171,** 59-84.

MURRAY, J. D., OSTER, G. F. & HARRIS, A. K. (1983). A mechanical model for mesenchymal morphogenesis. *J. math. Biol.* **17,** 125-129.

NAGORCKA, B. N. (1988). A pattern formation mechanism to control spatial organization in the embryo of *Drosophila melanogaster. J. theor. Biol.* **132,** 277-306.

NIJHOUT, H. F. (1978). Wing pattern formation in Lepidoptera: a model. *J. expl. Zool.* **206,** 119-136.

NIJHOUT, H. F. (1990). A comprehensive model for colour pattern formation in butterflies. *Proc. R. Soc. Lond. B* **239,** 81-113.

OSTER, G. F. & MURRAY, J. D. (1989). Pattern formation models and developmental constraints. *J. expl. Zool.* **251,** 186-202.

OSTER, G. F., MURRAY, J. D. & HARRIS, A. K. (1983). Mechanical aspects of mesenchymal morphogenesis. *J. Embryol. exp. Morph.* **78,** 83-125.

OSTER, G. F., SHUBIN, N., MURRAY, J. D. & ALBERCH, P. (1988). Evolution and morphogenetic rules. The shape of the vertebrate limb in ontogeny and phylogeny. *Evolution* **42,** 862-884.

RICHARDSON, M. K., HORNBRUCH, A. & WOLPERT, L. (1990). Mechanisms of pigment pattern formation in the quail embryo. *Development* **109,** 81-89.

SAINT GIRONS, H. (1985). Comparative data on Lepidosaurian reproduction and some time tables. In: *Biology of the Reptilia* Vol. 15 (Gans, C. & Billet, F., eds) pp. 35-58. John Wiley: New York.

SIBATANI, A. (1981). Wing homeosis in Lepidoptera: a survey. *Dev. Biol.* **79,** 1-18.

TREADWELL, R. W. (1962). Time and sequence of appearance of certain gross structures in *Pituophis melanoleucus sayi* embryos. *Herpetologica* **18,** 120-124.

TURING, A. M. (1952). The chemical basis for morphogenesis. *Phil. Trans. Roty. Soc. Lond. B* **237**, 37–72.
WINTERS, K. H., MYERSCOUGH, M. R., MAINI, P. K. & MURRAY, J. D. (1991). Tracking bifurcating solutions of a model biological pattern generator. *IMPACT of Computing in Sci. and Eng.* (in press).
WOLLKIND, D. J. & COLLINGS, J. B. (1990). Diffusive instabilities in a one-dimensional temperature dependent model system for a mite predator-prey interaction on fruit trees: dispersal motility and aggregative preytaxis effects. *J. math. Biol.* (in press).
WOLLKIND, D. J., SPIRANGANATHAN, R. & OULTON, D. B. (1984). Interfacial patterns during plane front alloy solidification. *Physica* **12D**, 215–240.
YOUNG, D. A. (1984). A local activator-inhibitor model of vertebrate skin patterns. *Math. Biosc.* **72**, 51–58.
ZEHR, D. R. (1962). Stages in the development of the common garter snake *Thamnophis sirtalis sirtalis*. *Copeia* **1962**, 322–329.
ZWEIFEL, R. G. (1981). Genetics of color pattern polymorphism in the California king snake. *J. Heredity* **72**, 238–244.

APPENDIX

Here we present the linear analysis of eqn (4) for which there are two uniform steady-states for (n, c) given by $(0, 0)$ and $[N, N/(1+N)]$. The steady-state $(0, 0)$ is always unstable so we consider only the non-zero steady-state $[N, N/(1+N)]$.

In the standard way we set $n = N + u$, $c = N/(1+N) + v$ where $|u|$, $|v|$ are small, substitute into (4) and retain only linear terms. This gives the linear equations, which govern behaviour near the steady-state, as

$$\partial u/\partial t = D\nabla^2 u - \alpha N \nabla^2 v - srNu \tag{A.1a}$$

$$\partial v/\partial t = \nabla^2 v + s[u/(1+N)^2 - v] \tag{A.1b}$$

$$\boldsymbol{n} \cdot \nabla u = \boldsymbol{n} \cdot \nabla v = 0, \qquad \boldsymbol{x} \in \partial D, \tag{A.1c}$$

where $\boldsymbol{n}$ is the outward unit normal to the boundary of D: eqn (A.1c) is zero flux conditions. We look for solutions to (A.1) in the form

$$\begin{bmatrix} u \\ v \end{bmatrix} \propto \exp[i\boldsymbol{k} \cdot \boldsymbol{x} + \lambda t], \tag{A.2}$$

where $\lambda(k)$ determines the temporal growth rate of the disturbance with wavevector $\boldsymbol{k}$. Non-trivial solutions exist only if $\lambda(k^2)$, the dispersion relation, satisfies the characteristic polynomial

$$\lambda^2 + [(D+1)k^2 + rsN + s]\lambda + [Dk^4 + \{rsN + Ds - (sN\alpha)/(1+N)^2)\}k^2 + rNs^2] = 0, \tag{A.3}$$

and the wave with wavenumber $|\boldsymbol{k}|$ satisfies the boundary conditions (A.1c). If $\lambda(k^2) < 0$ for any k^2 then a disturbance of wavevector $\boldsymbol{k}$ will decay with time. If $\lambda(k^2) > 0$ for some k^2 then any disturbance with these wavenumbers grows and, because of the non-linearity of the system, the exponentially growing solution evolves to a non-uniform spatially structured solution.

On a two-dimensional domain with sides L_x and L_y, we consider the wavevector $\boldsymbol{k} = (k_x, k_y)$ where $k_x = m\pi/L_x$, $k_y = l\pi/L_y$, with m and l integers. These derive from the zero flux boundary conditions and the linear eigenfunctions $\cos(m\pi x/L_x)\cos(l\pi y/L_y)$. Therefore, on this rectangular domain the values of k^2 which produce a pattern are those where $\lambda(k^2) > 0$ where

$$\boldsymbol{k} \cdot \boldsymbol{k} = k^2 = \pi^2(m^2/L_x^2 + l^2/L_y^2). \tag{A.4}$$

We can choose parameters D, α, s, r and N to isolate only one unstable wavevector. This mode selection is simply a way of forcing a particular pattern to grow although choosing actual parameter values is not trivial: this problem has been addressed by Bentil & Murray (1991). The wavevector for the isolated mode occurs when $\lambda(k^2) = 0$, that is when k satisfies

$$Dk^4 + [srN + Ds - \{sN\alpha/(1+N)^2)\}]k^2 + s^2rN = 0. \tag{A.5a}$$

We require (A.5a) to have only one solution for k^2, so we further impose the condition for equal roots, namely

$$[srN + Ds - \{sN\alpha/1 + N^2\}]^2 - 4Ds^2rN = 0. \tag{A.5b}$$

The modulus of the critical wavevector is then given by

$$k_c^2 = [s^2rN/D]^{1/2}. \tag{A.5c}$$

By choosing D, s, r and N appropriately, we can find a k^2 from (A.4) which satisfies (A.5c), and then solve (A.5b) for α (we take the larger root for α, so that k_c^2 is positive). This determines the point in (N, D, r, s, α) parameter space where mode (A.5c) is isolated.

Note that if we decrease r or N, the critical wavenumber decreases, thus the spacing of the pattern increases, or if decreased enough, the pattern disappears altogether. This is a prediction of the model which could be tested experimentally and which was mentioned in section 5.