How the Leopard Gets Its Spots

A single pattern-formation mechanism could underlie the wide variety of animal coat markings found in nature. Results from the mathematical model open lines of inquiry for the biologist

by James D. Murray

Mammals exhibit a remarkable variety of coat patterns; the variety has elicited a comparable variety of explanations—many of them at the level of cogency that prevails in Rudyard Kipling's delightful "How the Leopard Got Its Spots." Although genes control the processes involved in coat pattern formation, the actual mechanisms that create the patterns are still not known. It would be attractive from the viewpoint of both evolutionary and developmental biology if a single mechanism were found to produce the enormous assortment of coat patterns found in nature.

I should like to suggest that a single pattern-formation mechanism could in fact be responsible for most if not all of the observed coat markings. In this article I shall briefly describe a simple mathematical model for how these patterns may be generated in the course of embryonic development. An important feature of the model is that the patterns it generates bear a striking resemblance to the patterns found on a wide variety of animals such as the leopard, the cheetah, the jaguar, the zebra and the giraffe. The simple model is also consistent with the observation that although the distribution of spots on members of the cat family and of stripes on zebras varies widely and is unique to an individual, each kind of distribution adheres to a general theme. Moreover, the model also predicts that the patterns can take only certain forms, which in turn implies the existence of developmental constraints and begins to suggest how coat patterns may have evolved.

It is not clear as to precisely what happens during embryonic development to cause the patterns. There are now several possible mechanisms that are capable of generating such patterns. The appeal of the simple model comes from its mathematical richness and its astonishing ability to create patterns that correspond to what is seen. I hope the model will stimulate experimenters to pose relevant questions that ultimately will help to unravel the nature of the biological mechanism itself.

Some facts, of course, are known about coat patterns. Physically, spots correspond to regions of differently colored hair. Hair color is determined by specialized pigment cells called melanocytes, which are found in the basal, or innermost, layer of the epidermis. The melanocytes generate a pigment called melanin that then passes into the hair. In mammals there are essentially only two kinds of melanin: eumelanin, from the Greek words *eu* (good) and *melas* (black), which results in black or brown hairs, and phaeomelanin, from *phaeos* (dusty), which makes hairs yellow or reddish orange.

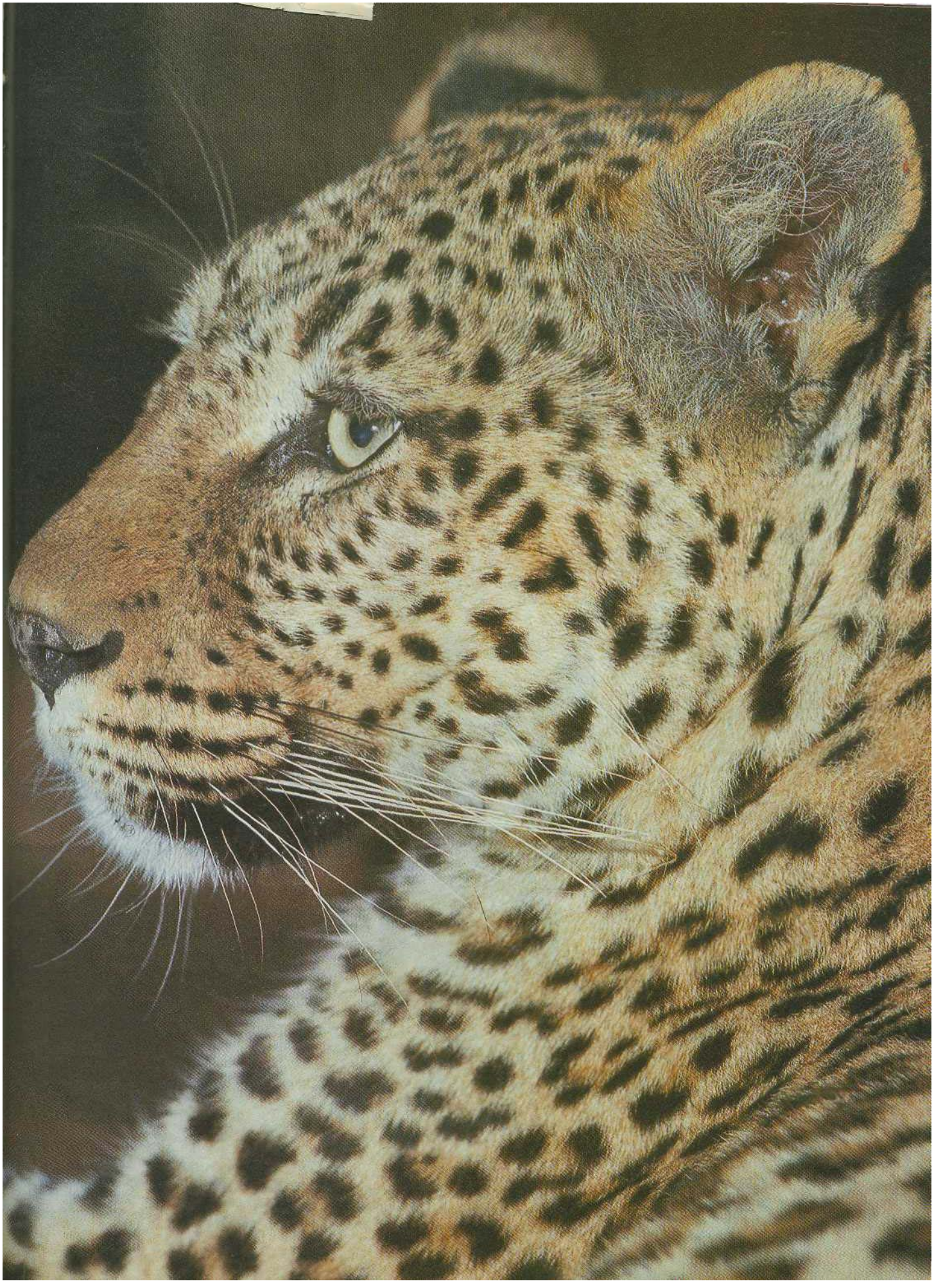
It is believed that whether or not melanocytes produce melanin depends on the presence or absence of chemical activators and inhibitors. Although it is not yet known what those chemicals are, each observed coat pattern is thought to reflect an underlying chemical prepattern. The prepattern, if it exists, should reside somewhere in or just under the epidermis. The melanocytes are thought to have the role of "reading out" the pattern. The model I shall describe could generate such a prepattern.

My work is based on a model developed by Alan M. Turing (the inventor of the Turing machine and the founder of modern computing science). In 1952, in one of the most important papers in theoretical biology, Turing postulated a chemical mechanism for generating coat patterns. He suggested that biological form follows a prepattern in the concentration of chemicals he called morpho-gens. The existence of morphogens is still largely speculative, except for circumstantial evidence, but Turing's model remains attractive because it appears to explain a large number of experimental results with one or two simple ideas.

Turing began with the assumption that morphogens can react with one another and diffuse through cells. He then employed a mathematical model to show that if morphogens react and diffuse in an appropriate way, spatial patterns of morphogen concentrations can arise from an initial uniform distribution in an assemblage of cells. Turing's model has spawned an entire class of models that are now referred to as reaction-diffusion models. These models are applicable if the scale of the pattern is large compared with the diameter of an individual cell. The models are applicable to the leopard's coat, for instance, because the number of cells in a leopard spot at the time the pattern is laid down is probably on the order of 100.

Turing's initial work has been developed by a number of investigators, including me, into a more complete mathematical theory. In a typical reaction-diffusion model one starts with two morphogens that can react with each other and diffuse at varying rates. In the absence of diffusion—in a well-stirred reaction, for example—the two morphogens would react and reach a steady uniform state. If the morphogens are now allowed to diffuse at equal rates, any spatial variation from that steady state will be smoothed out. If, however, the diffusion rates are not equal,

LEOPARD reposes. Do mathematical as well as genetic rules produce its spots?



diffusion can be destabilizing: the reaction rates at any given point may not be able to adjust quickly enough to reach equilibrium. If the conditions are right, a small spatial disturbance can become unstable and a pattern begins to grow. Such an instability is said to be diffusion driven.

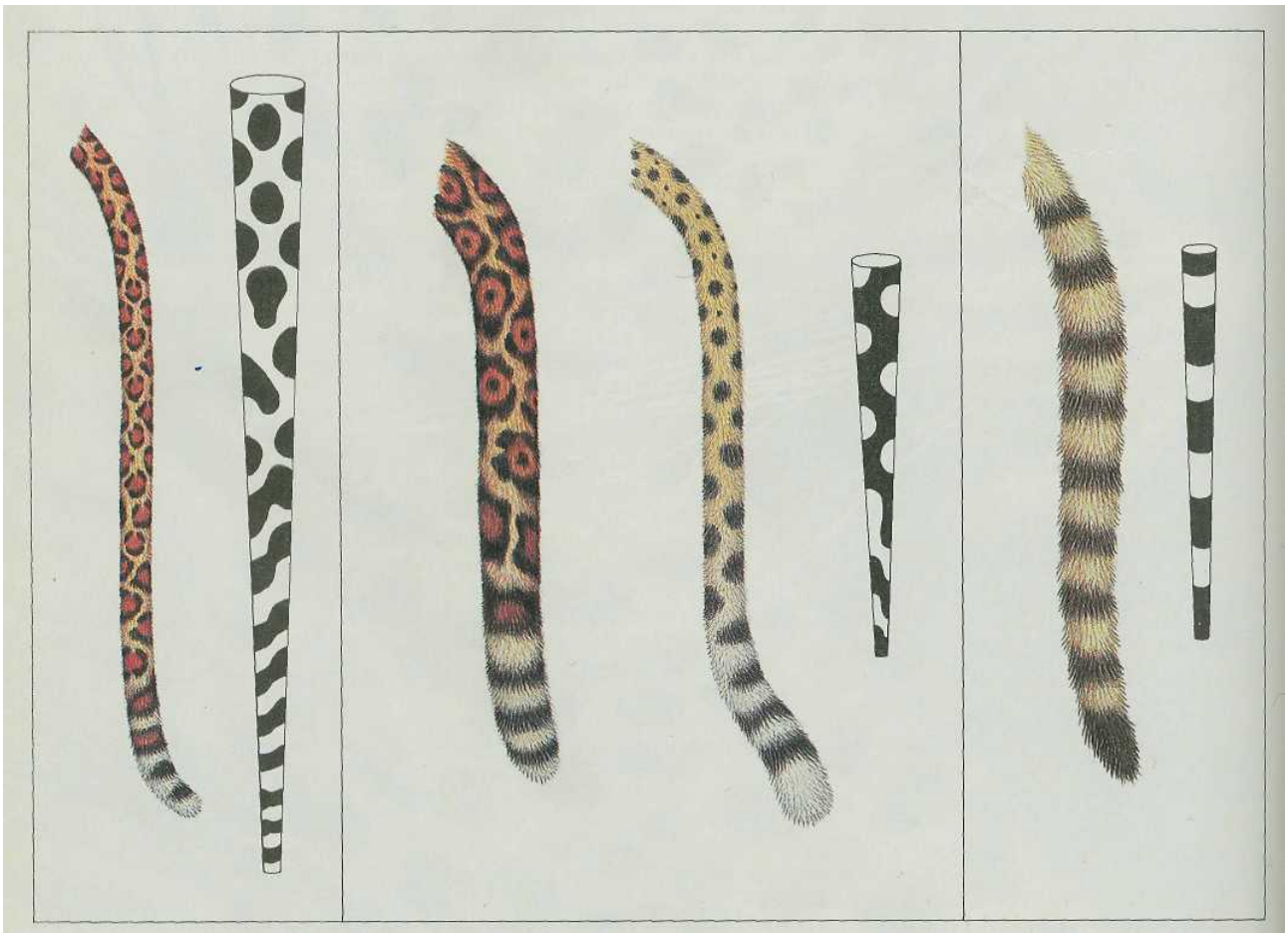
In reaction-diffusion models it is assumed that one of the morphogens is an activator that causes the mela-nocytes to produce one kind of melanin, say black, and the other is an inhibitor that results in the pigment cells' producing no melanin. Suppose the reactions are such that the activator increases its concentration locally and simultaneously generates the inhibitor. If the inhibitor diffuses faster than the activator, an island of high activator concentration will be created within a region of high inhibitor concentration.

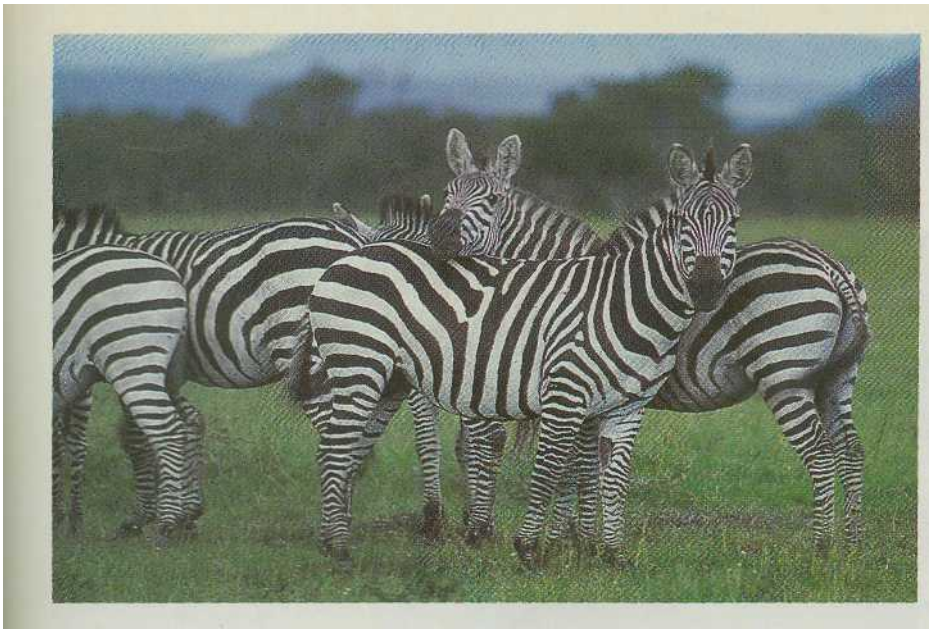
One can gain an intuitive notion of how such an activator-inhibitor mechanism can give rise to spatial patterns of morphogen concentrations from the following, albeit somewhat unrealistic, example. The analogy involves a very dry forest—a situation ripe for forest fires. In an attempt to minimize potential damage, a number of fire fighters with helicopters and fire-fighting equipment have been dispersed throughout the forest. Now imagine that a fire (the activator) breaks out. A fire front starts to propagate outward. Initially there are not enough fire fighters (the inhibitors) in the vicinity of the fire to put it out. Flying in their helicopters, however, the fire fighters can outrun the fire front and spray fire-resistant chemicals on trees; when the fire reaches the sprayed trees, it is extinguished. The front is stopped.

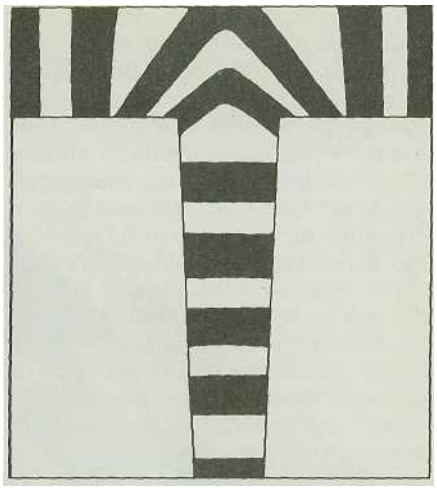
If fires break out spontaneously in random parts of the forest, over the course of time several fire fronts (activation waves) will propagate outward. Each front in turn causes the fire fighters in their helicopters (inhibition waves) to travel out faster and quench the front at some distance ahead of the fire. The final result of this scenario is a forest with blackened patches of burned trees interspersed with patches of green, un-burned trees. In effect, the outcome mimics the outcome of reaction-diffusion mechanisms that are diffusion driven. The type of pattern that results depends on the various parameters of the model and can be obtained from mathematical analysis.

Many specific reaction-diffusion models have been proposed, based on plausible or real biochemical reactions, and their pattern-formation properties have been examined. These mechanisms involve several parameters, including the rates at which the reactions proceed, the rates at which the chemicals diffuse and—of crucial importance—the geometry and scale of the tissue. A fascinating property of reaction-diffu-

MATHEMATICAL MODEL called a reaction-diffusion mechanism generates patterns that bear a striking resemblance to those found on certain animals. Here the patterns on the tail of the leopard *(left),* the jaguar and the cheetah *{middle)* and the genet *(right)* are shown, along with the patterns from the model for tapering cylinders of varying width *(right side of each panel).*







ZEBRA STRIPES at the junction of the foreleg and body *(left)* can be produced by a reaction-diffusion mechanism *(above).*

sion models concerns the outcome of beginning with a uniform steady state and holding all the parameters fixed except one, which is varied. To be specific, suppose the scale of the tissue is increased. Then eventually a critical point called a bifurcation value is reached at which the uniform steady state of the morphogens becomes unstable and spatial patterns begin to grow.

The most visually dramatic example of reaction-diffusion pattern formation is the colorful class of chemical reactions discovered by the Soviet investigators B. P. Belousov and A. M. Zhabotinsky in the late 1950's [see "Rotating Chemical Reactions," by Arthur T. Winfree; SCIENTIFIC AMERICAN, June, 1974]. The reactions visibly organize themselves in space and time, for example as spiral waves. Such reactions can oscillate with clocklike precision, changing from, say, blue to orange and back to blue again twice a minute.

Another example of reaction-diffusion patterns in nature was discovered and studied by the French chemist Daniel Thomas in 1975. The patterns are produced during reactions between uric acid and oxygen on a thin membrane within which the chemicals can diffuse. Although the membrane contains an immobilized enzyme that catalyzes the reaction, the empirical model for describing the mechanism involves only the two chemicals and ignores the enzyme. In addition, since the membrane is thin, one can assume correctly that the mechanism takes place in a two-dimensional space.

I should like to suggest that a good candidate for the universal mechanism that generates the prepattern for mammalian coat patterns is a reaction-diffusion system that exhibits diffusion-driven spatial patterns. Such patterns depend strongly on the geometry and scale of the domain where the chemical reaction takes place. Consequently the size and shape of the embryo at the time the reactions are activated should determine the ensuing spatial patterns. (Later growth may distort the initial pattern.)

Any reaction-diffusion mechanism capable of generating diffusion-driven spatial patterns would provide a plausible model for animal coat markings. The numerical and mathematical results I present in this article are based on the model that grew out of Thomas' work. Employing typical values for the parameters, the time to form coat patterns during embryogenesis would be on the order of a day or so.

Interestingly, the mathematical problem of describing the initial stages of spatial pattern formation by reaction-diffusion mechanisms (when departures from uniformity are minute) is similar to the mathematical problem of describing the vibration of thin plates or drum surfaces. The ways in which pattern growth depends on geometry and scale can therefore be seen by considering analogous vibrating drum surfaces.

If a surface is very small, it simply will not sustain vibrations; the disturbances die out quickly. A minimum size is therefore needed to drive any sustainable vibration. Suppose the drum surface, which corresponds to the reaction-diffusion domain, is a rectangle. As the size of the rectangle is increased, a set of increasingly complicated modes of possible vibration emerge.

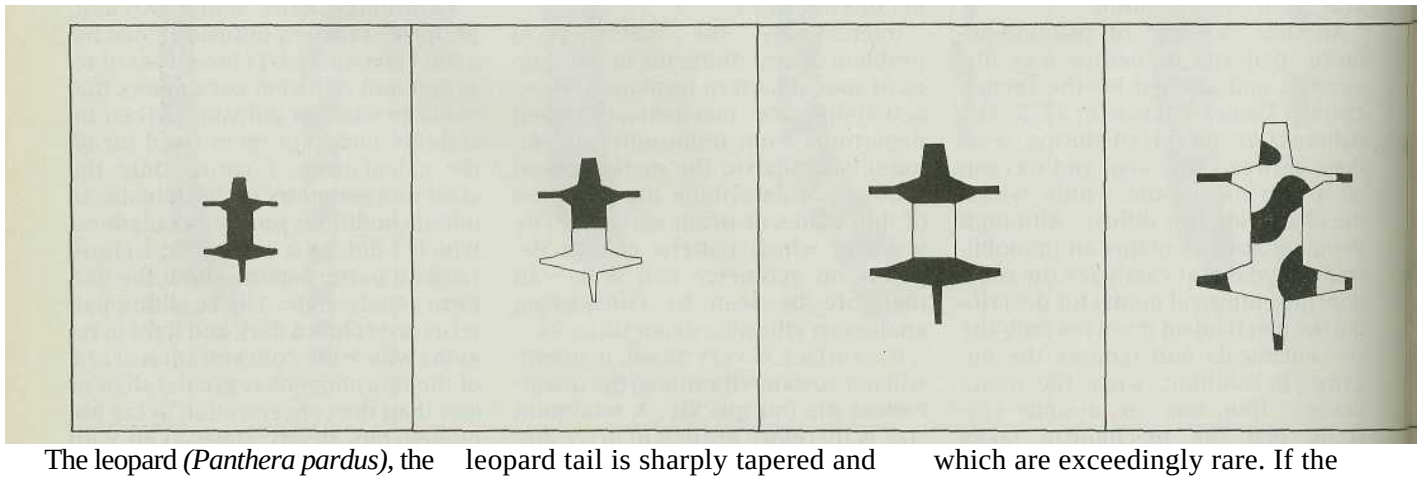
An important example of how the geometry constrains the possible modes of vibration is found when the domain is so narrow that only simple—essentially one-dimensional—modes can exist. Genuine two-dimensional patterns require the domain to have enough breadth as well as length. The analogous requirement for vibrations on the surface of a cylinder is that the radius cannot be too small, otherwise only quasi-one-dimensional modes can exist; only ringlike patterns can form, in other words. If the radius is large enough, however, two-dimensional patterns can exist on the surface. As a consequence, a tapering cylinder can exhibit a gradation from a two-dimensional pattern to simple stripes *[see illustration on opposite page].*

Returning to the actual two-mor-phogen reaction-diffusion mechanism I considered, I chose a set of reaction and diffusion parameters that could produce a diffusion-driven instability and kept them fixed for all the calculations. I varied only the scale and geometry of the domain. As initial conditions for my calculations, which I did on a computer, I chose random perturbations about the uniform steady state. The resulting patterns are colored dark and light in regions where the concentration of one of the morphogens is greater than or less than the concentration in the homogeneous steady state. Even with such limitations on the parameters and the initial conditions the wealth of possible patterns is remarkable.

EXAMPLES OF DRAMATIC PATTERNS occurring naturally are found in the anteater *(left)* and the Valais goat, *Capra aegagrus hircus (right).* Such patterns can be accounted for by the author's reaction-diffusion mechanism *(see bottom illustration on these*

How do the results of the model compare with typical coat markings and general features found on animals? I started by employing tapering cylinders to model the patterns on the tails and legs of animals. The results are mimicked by the results from the vibrating-plate analogue, namely, if a two-dimensional region marked by spots is made sufficiently thin, the spots will eventually change to stripes, cheetah *(Acinonyx jubatus),* the jaguar *(Panthem onca)* and the genet *{Genetta genetta)* provide good exampies of such pattern behavior. The spots of the leopard reach almost to the tip of the tail. The tails of the cheetah and the jaguar have distinctly striped parts, and the genet has a totally striped tail. These observations are consistent with what is known about the embryonic structure of the four animals. The prenatal relatively short, and so one would expect that it could support spots to the very tip. (The adult leopard tail is long but has the same number of vertebrae.)The tail of the genet embryo, at the other extreme, has a remarkably uniform diameter that is quite thin. The genet tail should therefore not be able to support spots,

The model also provides an instance of a developmental constraint, documented examples of



The leopard *(Panthera pardus),* the leopard tail is sharply tapered and which are exceedingly rare. If the

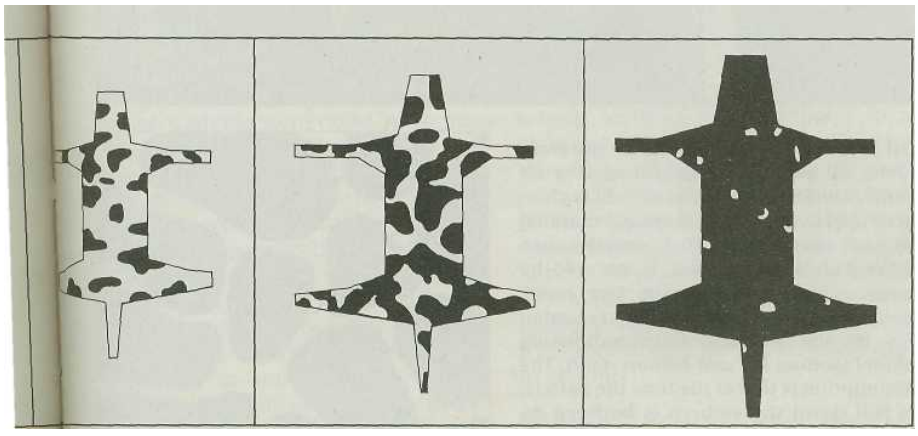
SCALE AFFECTS PATTERNS generated within the constraints of a generic animal shape in the author's model. Increasing the scale and holding all other parameters fixed produces a remarkable variety of patterns. The model agrees with the fact that

two pages). The drawing of the anteater was originally published by G. and W. B. Whit-taker in February, 1824, and the photograph was made by Avi Baron and Paul Munro.

prepattern-forming mechanism for animal coat markings is a reaction-diffusion process (or any process that is similarly dependent on scale and geometry), the constraint would develop from the effects of the scale and geometry of the embryos. Specifically, the mechanism shows that it is possible for a spotted animal to have a striped tail but impossible for a striped animal to have a spotted tail.

We have also met with success in our attempts to understand the markings of the zebra. It is not difficult to generate a series of stripes with our mechanism. The junction of the foreleg with the body is more complicated, but the mathematical model predicts the typical pattern of leg-body scapular stripes *[see illustration on page 83}.*

In order to study the effect of scale in a more complicated geometry, we computed the patterns for a generic animal shape consisting of a body, a head, four appendages and a tail *[see bottom illustration on these two pages}.* We started with a very small shape and gradually increased its size, keeping all the parts in proportion. We found several interesting results. If the domain is too small, no pattern can be generated. As the size of the domain is increased successive bifurcations occur: different patterns suddenly appear and disappear. The patterns show more structure and more spots as the size of the domain is increased. Slender extremities still retain their striped pattern, however, even for domains that are quite large. When the domain is very large, the pattern structure is so fine that it becomes almost uniform in color again.



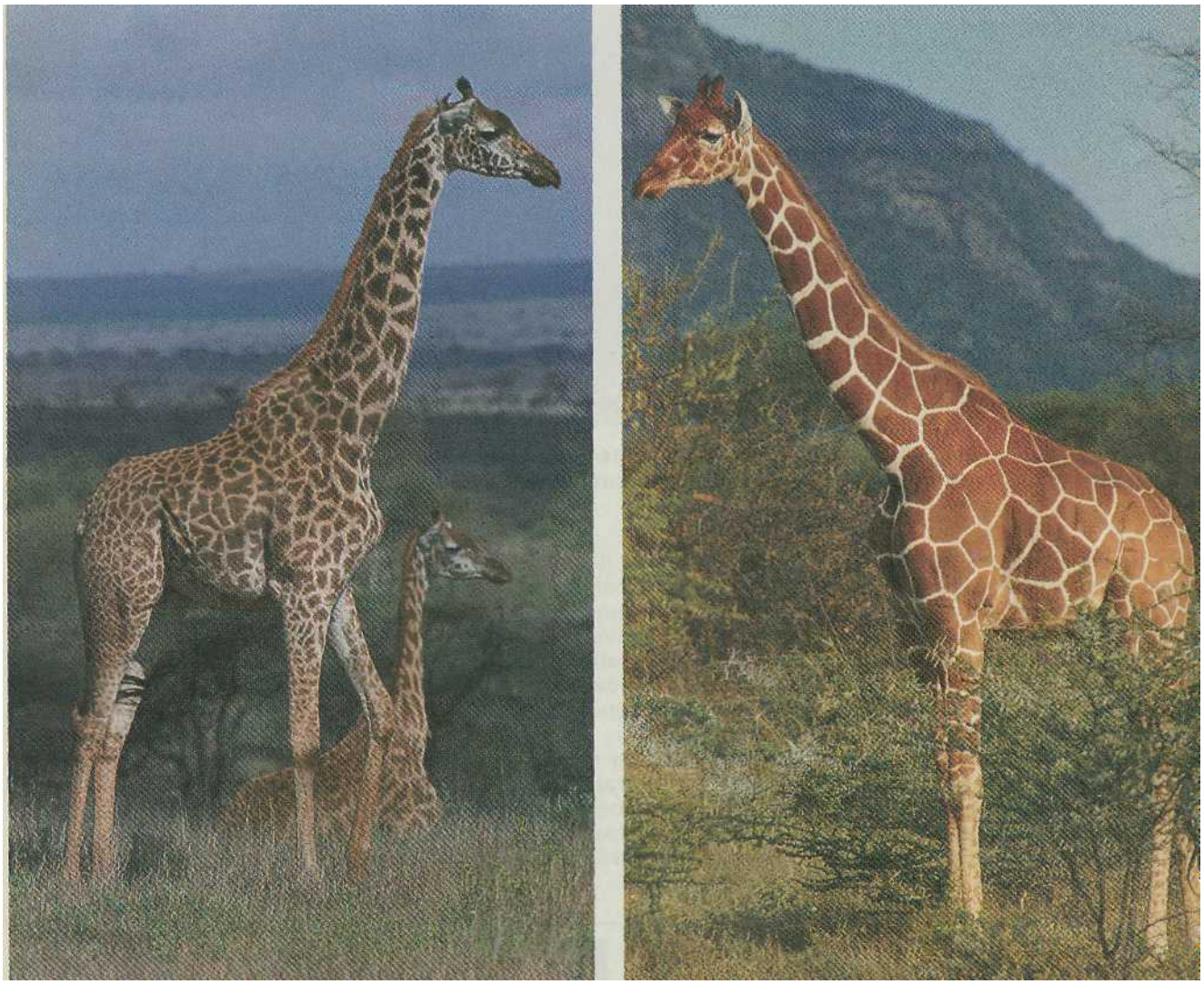
small animals such as the mouse have uniform coats, intermediate-size ones such as the leopard have patterned coats and large animals such as the elephant are uniform.

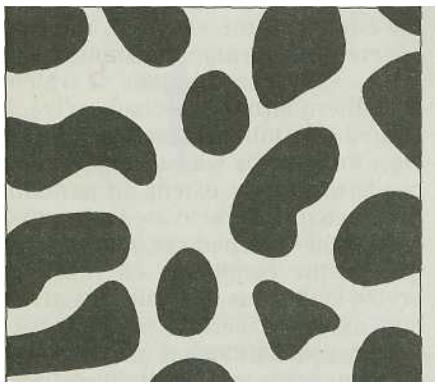
The effects of scale on pattern suggest that if the reaction-diffusion model is correct, the time at which the pattern-forming mechanism is activated during embryogenesis is of the utmost importance. There is an implicit assumption here, namely that the rate constants and diffusion coefficients in the mechanism are roughly similar in different animals. If the mechanism is activated early in development by a genetic switch, say, most small animals that have short periods of gestation should be uniform in color. This is generally the case. For larger surfaces, at the time of activation there is the possibility that animals will be half black and half white. The honey badger *(Mellivora capensis)* and the dramatically patterned Valais goat *(Capra ae-gagrus hircus)* are two examples *[see top illustration on these two pages}.* As the size of the domain increases, so should the extent of patterning. In fact, there is a progression in complexity from the Valais goat to certain anteaters, through the zebra and on to the leopard and the cheetah. At the upper end of the size scale the spots of giraffes are closely spaced. Finally, very large animals should be uniform in color again, which indeed is the case with the elephant, the rhinoceros and the hippopotamus.

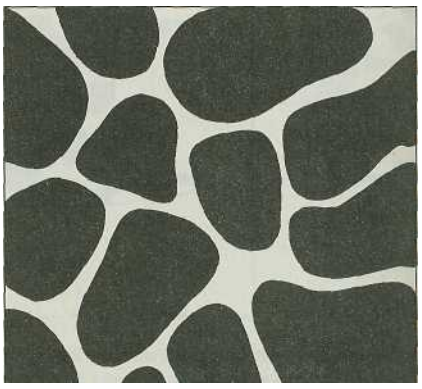
We expect that the time at which the pattern-forming mechanism is activated is an inherited trait, and so, at least for animals whose survival depends to a great extent on pattern, the mechanism is activated when the embryo has reached a certain size. Of course, the conditions on the embryo's surface at the time of activation exhibit a certain randomness. The reaction-diffusion model produces patterns that depend uniquely

on the initial conditions, the geometry and the scale. An important aspect of the mechanism is that, for a given geometry and scale, the patterns generated for a variety of random initial conditions are qualitatively similar. In the case of a spotted pattern, for example, only the distribution of spots varies. The finding is consistent with the individuality of an animal's markings within a species. Such individuality allows for kin recognition and also for general group recognition.

The patterns generated by the model mechanism are thought to correspond to spatial patterns of morphogen concentrations. If the concentration is high enough, mela-nocytes will produce the melanin pigments. For simplicity we assumed that the uniform steady state is the threshold concentration, and we reasoned that melanin will be generated if the value is equal to or greater than that concentration. The assumption is somewhat arbitrary, however. It is reasonable to expect that the threshold concentration may vary, even within species. To investigate such







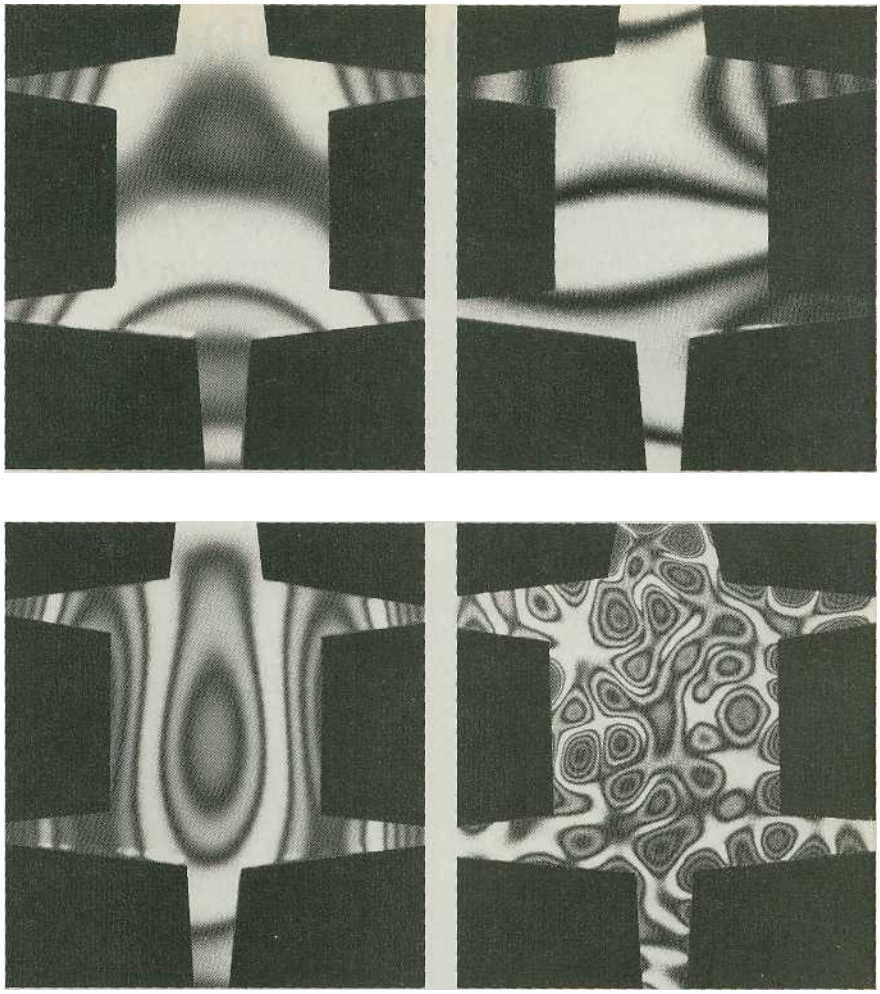
DIFFERENT GIRAFFES have different kinds of markings. The subspecies *Gi-raffa camelopardalis tippelskirchi* is characterized by rather small spots separated by wide spaces *(top left); G. camelopardalis reticulata,* in contrast, is covered by large, closely spaced spots *(top right).* Both kinds of pattern can be accounted for by the author's reaction-diffusion model *(bottom left and bottom right).* The assumption is that at the time the pattern is laid down the embryo is between 35 and 45 days old and has a length of roughly eight to 10 centimeters. (The gestation period of the giraffe is about 457 days.)

effects, we considered the various kinds of giraffe. For a given type of pattern, we varied the parameter that corresponds to the morphogen threshold concentration for melano-cyte activity. By varying the parameter, we found we could produce patterns that closely resemble those of two different kinds of giraffe *[see illustration on opposite page]*.

Recently the results of our model -have been corroborated dramatically by Charles M. Vest and You-ren Xu of the University of Michigan. They generated standing-wave patterns on a vibrating plate and changed the nature of the patterns by changing the frequency of vibration. The patterns were made visible by a holographic technique in which the plate was bathed in laser light. Light reflected from the plate interfered with a reference beam, so that crests of waves added to crests, troughs added to troughs, and crests and troughs canceled, and the resulting pattern was recorded on a piece of photographic emulsion *[see illustration at right]*.

Vest and Youren found that low frequencies of vibration produce simple patterns and high frequencies of vibration produce complex patterns. The observation is interesting, because it has been shown that if a pattern forms on a plate vibrating at a given frequency, the pattern formed on the same plate vibrated at a higher frequency is identical with the pattern formed on a proportionally larger plate vibrated at the original frequency. In other words, Vest and Youren's data support our conclusion that more complex patterns should be generated as the scale of the reaction-diffusion domain is increased. The resemblance between our patterns and the patterns subsequently produced by the Michigan workers is striking.

I should like to stress again that all the patterns generated were produced by varying only the scale and geometry of the reaction domain; all the other parameters were held fixed (with the exception of the different threshold concentrations in the case of the giraffe). Even so, the diversity of pattern is remarkable. The model also suggests a possible explanation for the various pattern anomalies seen in some animals. Under some circumstances a change in the value of one of the parameters can result in a marked change in the pattern obtained. The size of the effect depends on how close the value of the parameter is to a bifurcation value: the value at which a qualitative change in the pattern is generated.



STANDING-WAVE PATTERNS generated on a thin vibrating plate resemble coat patterns and confirm the author's work. More complex patterns correspond to higher frequencies of vibration. The experiments were done by Charles M. Vest and Youren Xu.

If one of the parameters, say a rate constant in the reaction kinetics, is varied continuously, the mechanism passes from a state in which no spatial pattern can be generated to a patterned state and finally back to a state containing no patterns. The fact that such small changes in a parameter near a bifurcation value can result in such large changes in pattern is consistent with the punctuated-equi-librium theory of evolution. This theory holds that long periods of little evolutionary change are punctuated by short bursts of sudden and rapid change.

Many factors, of course, affect animal coloration. Temperature, humidity, diet, hormones and metabolic rate are among some of them. Although the effects of such factors probably could be mimicked by manipulating various parameters, there is little point in doing so until more is known about how the patterns reflected in the melanin pigments are actually produced. In the meantime one cannot help but note the wide variety of patterns that can be generated with a reaction-diffusion model by varying only the scale and geometry. The considerable circumstantial evidence derived from comparison with specific animal-pattern features is encouraging. I am confident that most of the observed coat patterns can be generated by a reaction-diffusion mechanism. The fact that many general and specific features of mammalian coat patterns can be explained by this simple theory, however, does not make it right. Only experimental observation can confirm the theory.