Evolution of patterns on Conus shells

Zhenqiang Gong, Nicholos J. Matzke, Bard Ermentrout, Dawn Song, Jann E. Vendetti, Montgomery Slatkin, and George Oster

Departments of Electrical Engineering and Computer Science and Integrative Biology, University of California, Berkeley, CA 94720; Department of Mathematics, University of Pittsburgh, Pittsburgh, PA 15260; and Departments of Molecular and Cell Biology and Environmental Science, Policy and Management, University of California, Berkeley, CA 94720

Contributed by George Oster, December 12, 2011 (sent for review September 8, 2011)

The pigmentation patterns of shells in the genus Conus can be generated by a neural-network model of the mantle. We fit model parameters to the shell pigmentation patterns of 19 living Conus species for which a well resolved phylogeny is available. We infer the evolutionary history of these parameters and use these results to infer the pigmentation patterns of ancestral species. The methods we use allow us to characterize the evolutionary history of a neural network, an organ that cannot be preserved in the fossil record. These results are also notable because the inferred patterns of ancestral species sometimes lie outside the range of patterns of their living descendants, and illustrate how development imposes constraints on the evolution of complex phenotypes.

Pattern formation | developmental evolution | phylogenetics | ancestral inference

Pigmentation patterns on mollusk shells are typical complex phenotypes. They differ substantially among closely related species, but the complexity of the patterns makes it difficult to characterize their similarities and differences. Consequently, it has proven difficult to describe the evolution of pigmentation patterns or to draw inferences about how natural selection might affect them. In this report, we present an attempt to resolve this problem by combining phylogenetic methods with a realistic developmental model that can generate pigmentation patterns of shelled mollusks in the diverse cone snail genus Conus. The model is based on the interactions between pigment-secreting cells and a neuronal network whose parameters are measurable physiological quantities. The neural model used here is a generalization of models proposed earlier by Ermentrout et al. (1) and Boettiger et al. (2). Furthermore, the species have a well supported phylogeny that allows us to infer rates and patterns of parameter evolution.

We chose 19 species in the genus Conus for which Nam et al. have presented a resolved phylogeny (3). For each species, we found a model parameter set that matched the observed pigmentation pattern. Then we applied likelihood-based phylogenetic methods to measure phylogenetic signal in the model parameters, compare possible evolutionary models, estimate the model parameters of ancestral species, and then use these to infer the pigmentation patterns of ancestral species.

Neural Model

Fig. 1 shows a schematic of the mantle geometry and illustrates the basic principle of the neural model. The mathematical details are described in SI Appendix, Supplement A. The model is built on two general properties of neural networks: spatial lateral inhibition (also called center-surround), and “delayed temporal inhibition.” The latter can be viewed as “lateral inhibition in time” (4–6), as illustrated in Fig. 1C, Center.

The neural network equations describe the local pattern of neuron spiking. Local activity of excitatory neurons induces the activity of inhibitory interneurons in the surrounding tissue. The net spatial activity has “Mexican hat” shape, as shown in Fig. 1C (5–7). As shell material and pigment are laid down in periodic bouts of secretion, the surface pigment pattern is a space–time record of the animal’s secretory activity, in which distance from the shell aperture is proportional to the number of bouts of secretion. Excitation of a cell during a bout inhibits its excitation for some future number of bouts, so that an active neuron will eventually be inhibited and remain inactive for a “refractory” period. Thus, “delayed inhibition” is equivalent to “half a Mexican hat backward in time.” Finally, the secretory activity of pigment granule secre-
or small amounts of noise give rise to diversity among individuals while still maintaining the same qualitative pattern. Fig. 3A provides an example showing multiple instances of a simulation of *Conus croesus* such that there are small differences in initial data or the addition of a small amount of noise. The overall look of the pattern is the same, but there are clear individual differences.

Somewhat surprisingly, the regions of parameter space that correspond to cone shell patterns are fairly restricted and almost always require that the effective spatial interaction be lateral inhibition. When we chose parameters outside this range, we produced shell patterns that do not correspond to any known species (Fig. 3B).

Although our basic model is capable of producing many of the observed patterns, there are some species (e.g., *Conus textile*) in which the current secretion depends on sensing the history of previous pigment deposition, whereas space is the dimension along the growing edge of the cell. The resulting filtered activity is then passed through a space–time filter of neural activation and inhibition. Here, time represents the pigmentation pattern that was laid down in previous bouts, whereas space is the dimension along the growing edge of the cell. The resulting filtered activity is passed through nonlinearities for excitation and inhibition, and this net activity drives the secretory cells that lay down the new pigmented shell material. The spatial filter that implements delayed inhibition is half a Mexican hat. It generates a refractory period following a period of activity. The pigmentation pattern that implements delayed inhibition is half a Mexican hat. It generates a refractory period following a period of activity. The pigment secreting cells have a sigmoidal stimulus response curve. Feedback occurs as the current pigment deposition becomes part of the input to the sensory cells for the next secretion bout.

Shell patterns are a neurosecretory phenomenon rather than a diffusing morphogen phenomenon (2). However, from a theoretical viewpoint, morphogen models can be viewed as an approximation to the neural net model when the range of communication between neurons is short (9, 10). Therefore, in principle, morphogen models could have been used instead of the neural model (11). From a practical viewpoint, however, this would be considerably more difficult because a separate morphogen model is required for each shell pattern, whereas the neural model has a single set of parameters that are varied to match each pattern. Also, as the neural models are more general, they can generate a wider variety of patterns than can diffusible morphogen models. One other difference is fundamental. Morphogen models described by diffusion-reaction dynamics unfold with no “memory” of the system state other than the current state. The neural model, however, is a sensory feedback system in which the current secretion depends on sensing the history of the pattern before the current state.

**Phylogenetic Analyses**

**Inferred Parameter Values for Each Species.** We chose 19 species from the phylogeny published by Nam et al. (3) based on mitochondrial cytochrome C oxidase subunit I and rDNA sequences and on internal transcribed spacer 2 sequences from nuclear ribosomal DNA. There were sufficient data that the order of
branching events in the phylogeny could be completely determined with a high degree of statistical confidence.

The neural network model was fit to each living species in the phylogenetic tree. Nine species can be reproduced using the basic model (i.e., a single neural network). Six species (Conus tessulatus, Conus aurisiacus, Conus ammiralis, Conus orbignyi, Conus stercusmuscarum, and Conus laterculatus) require a spatial prepattern (generated by a “hidden” network), and four species (Conus dalli, C. textile, Conus aulicus, and Conus episcopatus) require spatio-temporal prepatterns (generated by one or two hidden networks).

In phylogenetic analyses of these shell parameters, we focus on the primary network, which can be compared across all species. The fitted parameters for each species are shown in SI Appendix, Supplement C. Images of real shells and their corresponding simulated ones are shown in Fig. 4.

Test for Phylogenetic Signal in Estimated Parameter Values. Pheno-type traits like body size and shape typically exhibit a substantial degree of “phylogenetic signal,” meaning that they are inherited, and the phenotypes of closely related species are strongly correlated (12). One purpose of the present study is to determine whether parameters of the neural-network model exhibit a phylogenetic signal. They will if the construction of the model accurately approximates the real developmental process of shell patterning. Therefore, we tested for a phylogenetic signal when the model parameters are fit to the observed pigmentation patterns. A basic test for phylogenetic signal in traits is to compare the observed data to a null model in which all phylogenetic signal are obliterated by randomly shuffling the species names or trait values at the tips of the phylogenetic tree (13). To test for a phylogenetic signal in the neural network parameters, we constructed a neighbor-

![Fig. 2](image-url)

**Fig. 2.** Definition of cell specific model parameters. (A) Gaussian excitation and inhibition kernels whose difference creates the Mexican-hat spatial field. (B) Temporal filter implementing delayed inhibition. \( \beta_1 \) \( \beta_2 \) is the strength of the temporal excitation (inhibition) and \( c_1 \) \( c_2 \) is the decay in “time” of the excitation (inhibition), wherein time is measured discretely in secretory bouts, denoted by \( n \) \( 0 < c_1 < c_2 < 1 \), so that the inhibition decays more slowly in time; thus, the most recent activity is excitatory and more distant activity is inhibitory. (C) Sigmoid response function of the secretory cells; \( \nu \) is the sharpness of the nonlinearity and \( \theta \) is the midpoint (there is one nonlinearity for excitation and one for inhibition).

![Fig. 3](image-url)

**Fig. 3.** (A) Both noise and chaos generate within-species pattern diversity. a, Three real C. crocatus shells. b, Three shells generated with 1% noise only. c, Three shells generated with slightly different initial conditions, but no noise. d, Three shells with both 1% noise and slightly different initial conditions. (B) Two examples of “unknown” patterns having too-wide inhibition fields.
joining phylogeny of the 19 species based on the parameter values alone and compared it with the DNA phylogeny of Nam et al. (3). The parameter-based phylogeny was obtained as described in SI Appendix, Supplement B.

For each method of measuring distances between trees, we constructed a null distribution on tree-to-tree distances by taking the parameter-based tree and randomly reshuffling the species names. The distances between the randomized null-parameter tree and the DNA tree were then calculated. This procedure was repeated 10,000 times to produce the null distribution.

The trees are compared in Fig. 5. Despite several dissimilarities between the DNA- and parameter-based trees, the observed distance between the trees is much less than expected under the null hypothesis of only random similarity between the trees (SI Appendix, Fig. S7). The differences are statistically significant—\( P = 0.0146 \) for the topology-based distance measure and \( P = 0.0001 \) the branch-length-based distance measure—indicating that the observed distance was smaller than all the 10,000 null distances generated. We conclude that there is a phylogenetic signal in the parameter values, despite the fact that they do not perfectly reflect the phylogenetic relationships of the group.

**Similarity of DNA- and Parameter-Based Trees.** Looking more closely at the parameter and DNA trees, we can see there is broad similarity but with notable exceptions. In both trees, there are two large clades, called arbitrarily clade 1 (\( C. stercusmuscarum \), \( C. aurisiacus \), \( Conus pulicarius \), \( Conus arenatus \), and \( C. laterculatus \)) and clade 2 (\( C. gloriamaris \), \( C. dalli \), \( C. textile \), \( Conus omaria \), \( C. episcopatus \), and \( C. aulicus \)), that are nearly the same in both trees, although the detailed branching order differs slightly. In addition, \( C. banadanus \) and \( C. marmoreus \) are sister groups in both trees. There are some conspicuous differences, however. Most notably, \( C. furvus \), \( C. tessulatus \), and \( C. orbignyi \) form a tight clade in the parameter tree yet are widely separated in the DNA tree. In fact, in the DNA tree, \( C. orbignyi \) is a well supported out-group to the other 18 species. \( C. ammiralis \) is part of clade 2 on the DNA tree.
but is quite separate on the parameter tree. *C. crocatus* is in clade 2 on the DNA tree and in clade 1 on the parameter tree (Fig. 5).

The overall similarity of the DNA-based and parameter-based trees is consistent with the hypothesis that the parameters of the developmental model evolved sufficiently slowly that sets of parameters in closely related species are similar. However, there are some exceptional lineages on which more rapid evolution of parameters seems to have occurred. The three species *C. furvus*, *C. tessulatus*, and *C. orbignyi* appear to have converged not only in pattern but in the developmental process that produces that pattern. *C. crocatus* appears to have shifted its pattern to become similar to species in clade 2, and both *C. ammiralis* and *C. consors* have undergone relatively rapid evolution that resulted in quite distinct patterns. The apparently higher rate of parameter evolution on these lineages is consistent with the action of natural selection either directly on pigmentation pattern or indirectly as a correlated response to selection on physiological processes that affect parameter values. In the absence of knowledge of the physiological basis of parameter values, we have no way to directly test for natural selection.

**Parametric and nonparametric tests of the Brownian motion model.** The estimation of parameter values for ancestral species in the phylogeny is most easily done if the Brownian motion model of continuous trait evolution can be used. Therefore, when we had established that detectable phylogenetic signal existed in the neural network parameters, we conducted a series of tests to assess the utility of Brownian motion versus other models for modeling the evolution of neural network parameters, as recommended by Blomberg et al. (14). We concluded that Brownian motion was an overall reasonable first approximation for the evolution of neural network parameters (SI Appendix, Supplement B).

**Discrete Characters. Hidden Networks Treated as Discrete Characters.** We can treat the presence or absence of a hidden neural network as a binary discrete character. Then, the presence or absence of this character can be mapped onto the phylogeny by using parsimony and maximum-likelihood reconstruction for discrete characters. The two methods give identical results. The presence of hidden networks was restricted to small subclades of the full clade. The presence/absence of hidden networks (Fig. 6 A and B show the presence of a space–time-dependent hidden network and space-dependent hidden network, respectively) showed strong phylogenetic clustering. Relatively few transitions from simple models (i.e., no hidden networks) to complex models (i.e., containing a hidden network) were needed for either character. For the space–time-dependent network, species in two small clades (*C. episcopatus/C. aulicus* and *C. textile/C. dalli*) are complex. For the space-dependent hidden network, a complex pattern is more dispersed in the phylogeny.

**Discrete phenotypic characters.** Other discrete characters were also mapped for comparison with the results for hidden networks. We mapped several discrete phenotypic characters on the phylogeny (SI Appendix, Supplement B). Cone shape is fairly scattered but shows some uniformity in small clades. Strikingly, prey preference shows extremely high conservation as was clear in the discussion of Nam et al. (3) compared with shell pattern characters. Each major clade is almost completely restricted to a certain prey, and the entire pattern is explained by the minimum possible number of transitions.

Fig. 6 shows the distributions of stripes and triangles in this group and the maximum-likelihood assignment of ancestral states. The presence and absence of stripes, in particular, is scattered throughout the phylogeny, indicating that they are evolutionarily labile, although triangle presence/absence shows some correlation with large clades. These observations are confirmed by standard parsimony statistics and their comparison with randomized-tip null models; presence/absence of stripes, despite these being visually striking patterns used in identification, appear to lack significant phylogenetic signal in that they do not show significantly more congruence with the phylogeny than is expected under the null model in which character states have been randomly shuffled among the phylogeny tips.

**Inference of Ancestral Shell Patterns.** We used a Brownian motion model to estimate parameter values in the species ancestral to the living species. We then ran the neural-network model with these estimated parameter values to predict the pigmentation patterns in the ancestral species. Those patterns are shown at the nodes in Fig. 4. Ancestral states for each parameter common to all species were estimated by using maximum-likelihood estimation on the tree inferred from DNA sequences, modeling the evolution of each parameter as an independent Brownian motion process (15, 16). Two other available methods—generalized least-squares and phylogenetically independent contrasts—gave similar estimates.

For the additional parameters used in the hidden networks, ancestral character estimation was performed as follows. Phylogenetically independent contrasts were applied to reconstruct the ancestral states of the hidden networks because it works from the tips downward, and so, unlike maximum likelihood, can be used when parameters for hidden networks are not available in the rest of the clade.

**Fig. 5.** Comparison of the DNA-based phylogeny of cone snails (Left, after Nam et al. (3), unrooted for display) and the parameter-based tree (Right, present study). Species labeled in blue exhibit major changes in topological position in the parameter-based tree. The observed tree-to-tree distances are significantly shorter than expected under a null hypothesis of random similarity (SI Appendix, Fig. 57).
The ancestral shell patterns are shown in Fig. 4. Each estimate has an associated variance and confidence intervals. To test the robustness of the ancestral patterns to uncertainty in parameter estimates, we randomly generated sets of parameters from the distribution of each parameter and generated ancestral patterns from each set. We found that some ancestral patterns are quite robust to uncertainty in estimated parameters whereas others are not. Fig. 7 shows two examples of each kind. The ancestral patterns for nodes 25 and 29 are quite similar for different sets of estimated parameters, whereas those for nodes 27 and 31 differ greatly among sets of estimated parameters, although various detailed similarities can still be detected even among these shells because of the underlying similarity of neural network parameters.

Discussion and Conclusions

We have taken a step in applying modern phylogenetic methods to understanding the development of complex phenotypic characters. The pigmentation patterns of Conus shells can be generated by a neural-network model that has a sound anatomical and physiological basis. The model parameters fitted to observed patterns show a substantial phylogenetic signal, indicating that the processes governing evolutionary change in shell patterns are, to some extent, gradual across the phylogeny. Our analyses have allowed us to estimate the shell pigmentation patterns of ancestral species, identify lineages in which one or more parameters have evolved rapidly, and measure the degree to which different parameters correlate with the phylogeny.

Our results are summarized in Fig. 4. This figure shows that pigmentation patterns in living species are well approximated by the neural-network model presented in this study. It also shows the inferred ancestral shell patterns. Often, recent ancestors of sister species show recognizable similarity to the pigmentation patterns in living species (e.g., nodes 26–27 and 31–32). Nodes more remote from the present often show ancestors that are generally similar to the living species (nodes 21, 22, 24, and 33). However, some ancestors are strikingly different from any of the living species in the group we analyzed. Interestingly, such patterns can be found in other living species. For example, the strong striping perpendicular to the axis of coiling of the shell found in node 37 is quite similar to that of Conus hirasei, Conus papuensis, or Conus mucronatus (17). Striping parallel to the axis of coiling of the shell, observed in other estimated ancestors, can also be found in living species, for example in some specimens of Conus hyaena and Conus generalis (ref. 17, pp. 354 and 392).

A unique feature of our results is that the inferred pigmentation patterns of ancestors may be quite different from the patterns of their descendants. The patterns generated by the neural-network model are not necessarily smooth functions of the parameter val-
number of parameters involved, a formal proof of uniqueness seems impossible; however, extensive experience with the numerical properties of the model suggests that each pattern is determined by a unique optimal (in the sense of a best fit to the observed pattern) set of parameters. A third assumption is that the parameters evolved independently of one another on the phylogeny. That assumption is largely supported by our analysis of phylogenetically independent contrasts. Correlation in parameters could be accounted for by using a model of correlated Brownian motion on the phylogeny, but such a model was not needed for our analysis.

In estimating parameters of ancestral species and predicting their pigmentation patterns, we have not taken into account the range of parameters consistent with estimated values for living species. Parameter values estimated by using maximum likelihood and a Brownian motion model have associated confidence intervals that could make more than one qualitatively different pigmentation pattern for each ancestral species consistent with patterns in living species. Application of our method to a group of cone snails with a detailed fossil record—for example, those in southeastern North America (21)—might allow a more rigorous assessment of the accuracy of these techniques, and of what degree of uncertainty should be assigned to them. Usefully and remarkably, shell pigmentation patterns in fossil Conus can be visualized under UV light (21). Application of this technique to Conus fossils could provide a partial validation of our predicted ancestral patterns.

Our analysis is somewhat similar to that of Allen et al. (11), who examined spotted patterns in felids by using a morphogen-diffusion model of pattern formation. Allen et al. showed that there is little phylogenetic signal in the model parameters, indicating that spotting patterns in felids evolve convergently under ecological influences. One difference between their study (11) and the present one is that we found phylogenetic signal in most of the neural network parameters that produce shell pigmentation patterns. This allowed us to infer ancestral patterns and to identify lineages in which relatively rapid evolution of some parameters have taken place.

We found phylogenetic signal in the continuous parameters of the primary neural network and in the presence/absence of a hidden network, suggesting that the model reasonably approximates the developmental processes underlying pigmentation patterns in the Conus species we considered. In contrast, various features of the pigmentation patterns, such as the presence of stripes and dots, do not have significant phylogenetic signal (*SI Appendix, Tables S2–S4*). This is in agreement with the conclusion of Hendricks.*

**ACKNOWLEDGMENTS.** The authors thank David Jablonski, John Huelsenbeck, Alan Kohn, Jonathan Hendricks, and Carole Hickman. We also acknowledge Hans Meinhardt for his encyclopedic treatment of morphogen-based lateral inhibition models, many of which provided the inspiration for our neural net models. N.J.M. was supported by National Science Foundation (NSF) Grant DEB-0919451, a Wang Fellowship, and a Tien Fellowship. J.E.V. and M.S. were supported in part by National Institutes of Health Grant R01-GM40282. G.O. was supported by NSF Grant DMS 0414039. B.E. was supported by NSF Grant DMS0581713.

---

*Hendricks JR, Geological Society of America Annual Meeting, November 2–5, 2003, Seattle, WA.

---


---

E240 | www.pnas.org/cgi/doi/10.1073/pnas.1119859109

Gong et al.