levels of membrane PA. Thus, a depolarization-induced increase in PA should be small and transient.

In the bilayer model of the cell membrane, the phospholipids are oriented with their polar head groups near the membrane-water interface, and movement of phospholipids (flip-flop) is limited (*16*). This model does not allow for the rapid movement of complexes of calcium and PA through the membrane. However, calcium has been shown to convert acidic lipids from the bilayer configuration to a hexagonal phase (*17*). This conversion produces lipidic intramembranous particles that are not restricted to half of the bilayer (*17*), and these might function as ionophores.

An alternative possibility for the role of the PI effect in stimulus-secretion coupling is that the increased breakdown of PI results in the release of arachidonoyl residues (*18*). In the pancreas, this arachidonate is then converted to prostaglandins, which stimulate secretion (*18*). It is unlikely that arachidonate release is the basis of the PA effects observed in our study, because our results were obtained with dipalmitoylphosphatidate, which cannot be metabolized to arachidonate. This PA may be hydrolyzed to release palmitate, but we have found that palmitate does not alter synaptosomal calcium transport (*19*).

Our findings suggest an important role for PA in the mechanisms of psychoactive drug action. A variety of cationic drugs, including *d*-amphetamine and morphine, which increase neurotransmitter release, also inhibit PA phosphohydrolase (*2*). Thus, one of the mechanisms by which these drugs increase neurotransmitter release could involve a PA-related mechanism.

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Two-Hundred-Million-Year-Old Chromosomes: Deceleration of the Rate of Karyotypic Evolution in Turtles

Abstract. *Cladistic analyses of chromosomal banding patterns from 48 species of cryptodiran turtles, combined with a fossil-based method for estimating rates of karyotypic change, show that karyotypic evolution was twice as fast and involved different types of rearrangements in Mesozoic turtles when compared to more recent forms. The deceleration in rate of karyotypic change is correlated with decelerated morphological change and is indicative of adaptive evolution. Comparisons of banded karyotypes reveal that some chromosomes have remained unchanged for at least 200 million years.*

Rates of karyotypic evolution vary tremendously both within and among major taxa of animals and plants (*1–9*). Accurate determinations of such rates are desirable for testing alternative models of chromosomal evolution because most models attempt to explain why the rates of chromosomal rearrangement incorporation differ among taxa. For example, one model suggests that taxa characterized by slow rates of karyotypic evolution, morphological evolution, and speciation possess large effective population sizes (N_e). Conversely, taxa with small N_e due to low vagility, territoriality, or social structuring (factors that promote inbreeding and population subdivision) are expected to experience rapid karyotypic, morphological, and speciation rates (*1–5, 10*).

An alternative hypothesis (*11*) suggests that natural selection directly favors chromosomal rearrangements when their phenotypic effects confer an adaptive advantage. The early stages of major adaptive radiations are thought to be characterized by rapid rates of karyotypic evolution; later, the rates of karyotypic evolution decelerate after the incorporation of adaptive gene sequences. The types of rearrangements incorporated also are thought to change through time. Early stages of the canalization process incorporate rearrangements (such as inversions and translocations) that alter gene arrangement (and presumably regulation), while later stages are more likely to involve rearrangements less likely to have phenotypic effects (heterochromatin additions, centric fusions). The two models (termed the deme size and canalization models) are testable because they offer different predictions as to rates of karyotypic evolution. The deme size

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model predicts that rates of karyotypic evolution are correlated with effective population size. The canalization model predicts that rates are a function of the length of time a lineage has occupied its adaptive zone.

With the exception of the work of Baker and Bickham (*9*), all previous work on rates of karyotypic evolution have depended on either standard chromosome morphology or chromosome number (or both). Banding studies suggest that this approach may be accurate for some groups (*12, 13*) but the use of standard chromosome morphology greatly underestimates the true rates in other groups (*14*) and particularly the range of rates within groups (*9*). In none of the previous studies was the attempt made to document the deceleration of rates predicted by the canalization model (*11*).

As for this report, the minimum number of chromosomal rearrangements in 48 species from eight families and 29 genera of cryptodiran turtles was determined from a cladistic analysis (relationship based on shared derived characters) of the G-band patterns of each species. This represents roughly 28 percent of the species of the major radiation of turtles (*15*), and is the most complete analysis of any vertebrate group to date.

The primitive karyotype of the suborder, each family, and each genus was determined cladistically by methods described in detail elsewhere (*16*). Table 1 lists the number and types of rearrangements for each species relative to the primitive karyotype of each family. In determining rates, a rearrangement that characterized several species was counted as only a single event. For example, all emydine turtles have a translocation and an unknown rearrangement relative to the karyotype considered primitive to the family; and each was counted as only a single event (also shared with some batagurines). This procedure also was followed in determining rates of evolution from primitive familial (Table 1) and generic karyotypes. It is an underlying assumption that the primitive karyotype of each family and genus represents the karyotype of an ancestral species that occurred at a time estimated from the fossil record.

In order to test whether karyotypic evolution in turtles has decelerated, the rates of change for primitive family and genus karyotypes and for the species in polytypic genera were computed. Figure 1 is a cladogram showing the presumed branching sequence and the number of rearrangements in each step for the eight families. Twenty rearrangements (Table 1) were incorporated from the time of origin of the Cryptodira (estimated to be 200 million years ago) until the average time of origin in the fossil record of each family (92.5 million years ago) (*17*). The average number of rearrangements per family (2.50) divided by the average duration of time (107.5 million years) gives a rate (R_f) of 0.023 change per million years.

The average age of turtle genera was determined to be 45.2 million years (*17*). The number of chromosomal rearrangements from primitive family karyotype

Table 1. Species of turtles that have been C- and G-banded. The number and types of rearrangements required to derive the karyotype of each species from the karyotype proposed as primitive for its family, and the rearrangements required to derive the proposed primitive familial karyotypes from the proposed primitive karyotype of the suborder are shown. Abbreviations: 2*n*, diploid number; FU, fusion; FI, fission; PI, pericentric inversion; PA, paracentric inversion; TO, other type of translocation; H^+, heterochromatic addition; and UN, changes not identifiable from G-bands but a minimum estimate is given. Instances where more than one species in a genus were karyotypically identical are listed in (*26*).

Taxon	Number and types of rearrangements								Source
	2*n*	FU	FI	PI	PA	TO	H^+	UN	
Karyotype of species derived from primitive karyotype of its family									
Family Trionychidae									
Trionyx (three species)	66	0	0	0	0	0	0	0	(*20*)
Family Cheloniidae									
Chelonia mydas	56	0	0	0	0	0	0	0	(*22*)
Caretta caretta	56	0	0	0	0	0	0	0	(*25*)
Eretmochelys imbricata	56	0	0	0	0	0	3	0	(*25*)
Family Dermatemydidae									
Dermatemys mawii	56	0	0	0	0	0	0	0	(*24*)
Family Kinosternidae									
Kinosternon scorpioides	56	0	0	0	0	0	0	0	(*23*)
Kinosternon bauri	56	0	0	0	0	0	3	0	(*23*)
Kinosternon subrubrum	56	0	0	0	0	0	5	0	(*23*)
Sternotherus minor	56	0	0	0	0	0	5	0	(*23*)
Family Chelydridae									
Chelydra serpentina	52	0	0	0	0	0	0	0	(*21*)
Macroclemmys temminckii	52	0	0	0	0	0	0	2	(*21*)
Family Staurotypidae									
Staurotypus salvinii	54	0	0	0	0	0	0	0	(*23*)
Family Testudinidae									
Geochelone (two species)	52	0	0	0	0	0	0	0	(*25*)
Geochelone elongata	52	0	0	0	0	0	2	0	(*25*)
Family Emydidae									
Subfamily Batagurinae									
Sacalia bealei	52	0	0	0	0	0	0	0	(*12*)
Mauremys (two species)	52	0	0	0	0	0	0	0	(*12*)
Heosemys spinosa	52	0	0	0	0	0	0	0	(*25*)
Chinemys reevesii	52	0	0	0	0	0	0	0	(*21*)
Cyclemmys (two species)	52	0	0	0	0	0	0	0	(*21,25*)
Cuora amboinensis	52	0	0	0	0	0	0	0	(*25*)
Ocadia sinensis	52	0	0	0	0	0	0	0	(*25*)
Hieremys annandalei	52	0	0	0	0	0	0	0	(*25*)
Orlitia borneensis	50	0	0	0	0	1	1	1	(*25*)
Malayemys subtrijuga	50	0	0	0	0	1	1	1	(*25*)
Siebenrockiella crassicolis	50	0	0	1	0	1	1	1	(*12*)
Rhinoclemmys (three species)	52	0	0	0	0	1	1	1	(*12,25*)
Rhinoclemmys punctularia	56	0	0	0	0	1	1	3	(*12*)
Subfamily Emydinae									
Chrysemys (eight species)	50	0	0	0	0	1	0	1	(*12,25*)
Graptemys pseudogeographica	50	0	0	0	0	1	0	1	(*25*)
Terrapene (two species)	50	0	0	0	0	1	0	1	(*12,25*)
Deirochelys reticularia	50	0	0	0	0	1	0	1	(*25*)
Emydoidea blandingii	50	0	0	0	0	1	0	1	(*25*)
Clemmys guttata	50	0	0	0	0	1	0	1	(*25*)
Primitive family karyotypes derived from primitive karyotype of suborder									
Cheloniidae	56	0	0	0	0	0	0	0	(*22*)
Dermatemydidae	56	0	0	0	0	0	0	0	(*24*)
Kinosternidae	56	0	0	1	0	0	0	1	(*23*)
Chelydridae	52	0	0	0	0	1	0	2	(*21*)
Staurotypidae	54	1	0	0	0	1	0	0	(*23*)
Testudinidae	52	2	0	0	0	2	1	1	(*25*)
Emydidae	52	2	0	0	0	2	1	1	(*12*)
Trionychidae	66	4	0	2	0	0	1	1	(*20*)

to primitive genus karyotype for 29 genera (Table 1) was 14 for an average of 0.48. This average, divided by the duration of time from average age of family to genus (47.3 million years) gives a rate (R_g) of 0.01 change per million years.

The total number of rearrangements for 27 species in polytypic genera (Table 1) was 12 for an average of 0.444. This, divided by the duration of time from average age of genus to present (45.2 million years) gives a rate (R_s) of 0.009 change per million years.

The above analyses lead to the conclusion that the rate of karyotypic evolution in turtles has decelerated. Mesozoic turtles evolved karyotypically more than twice as fast as Tertiary and modern turtles ($R_f > 2R_g \cong 2R_s$). This supports the prediction of the canalization model. Alternatively, the eight Mesozoic species that gave rise to the modern families may have had a higher rate of karyotypic evolution than that characteristic of all Mesozoic turtles. This would suggest that taxa undergoing karyotypic evolution were, on the average, more successful. This could be the result of increased fitness caused by chromosomal rearrangements. Examination of Table 1 leads to a further corroboration of the canalization model. The types of identified rearrangements incorporated during the diversification of the families include centric fusions, pericentric inversions, and translocations. The types of rearrangements incorporated during the evolution of modern species include mostly heterochromatic additions. Thus, not only the rates of karyotypic evolution but also the kinds of incorporated rearrangements have changed through time as predicted by the canalization model (*11*).

Because turtles appear conservative in karyotype, banding comparisons are possible between distantly related species. Some chromosomes appear to have been conserved for 200 million years (Fig. 1). The maintenance of identical G-band patterns for such a vast amount of time has never before been documented and is suggestive of strong normalizing natural selection (*18*).

Many modern families of turtles originated in the Cretaceous, and their descendants have remained in the same family for as much as 150 million years. A similar set of circumstances characterizes salamanders, and has led Maxson and Wilson (*5*) to conclude that this group was morphologically conservative. While their conclusions also would pertain to turtles, it should be pointed out that from the time of origin of the suborder Cryptodira until the time of origin of the modern families this lineage produced taxa as diverse as sea turtles, tortoises, soft-shelled turtles, snapping turtles, and slider turtles. Obviously, cryptodires experienced rapid morphological and karyotypic evolution during the Mesozoic.

Turtles appear to be yet another group in which rates of karyotypic and morphological evolution are concordant. However, I do not believe this is because the same populational characteristics that promote rapid chromosomal evolution also promote rapid morphological evolution. Turtles are quite variable in terms of reproductive strategies, vagility, and, probably, effective population size. However, they are without exception extremely conservative karyotypically. It seems more likely that both morphological change and changes in gene arrangement are intimately related to adaptive evolution. Deceleration of the rate of evolution is a pattern that suggests effective adaptive evolution in multilevel situations (*19*).

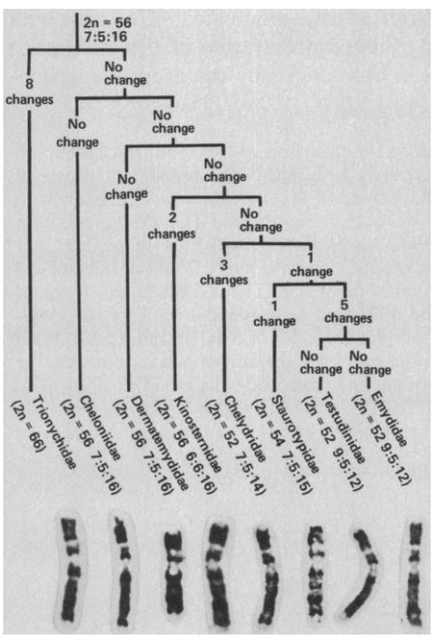

Fig. 1. Cladistic relationships of the eight families of Cryptodiran turtles. The primitive karyotype of the suborder is considered to be identical to the karyotype of species of the families Cheloniidae (*22*) and Dermatemydidae (*24*). The number of chromosomal rearrangements that are incorporated on each branch are shown. The diploid number ($2n$) and the A:B:C formula (*12*) for the primitive karyotype of each family is given. Because the Trionychidae is so divergent, the A:B:C formula is not given (*20*). The first group A chromosome of a species of each family is shown: left to right: *Trionyx spiniferus* (Trionychidae), *Chelonia mydas* (Cheloniidae), *Dermatemys mawii* (Dermatemydidae), *Kinosternon subrubrum* (Kinosternidae), *Macroclemmys temminckii* (Chelydridae), *Staurotypus salvinii* (Staurotypidae), *Geochelone elongata* (Testudinidae), *Graptemys pseudogeographica* (Emydidae). This chromosome (and some others) was found to be identical in all species of the eight families studied and has remained unchanged since the origin of the suborder Cryptodira, 200 million years ago.

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