Cellular Migration and Morphological Complexity in the Caecilian Brain

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ABSTRACT The morphology of the tectum mesencephali and the medial pallium is studied in species representing the six families of caecilians (Amphibia: Gymnophiona) in order to determine whether differences in brain morphology are related to function, phylogenetic history, or life history strategies. In general, the caecilian tectum is characterized by simplification in having little to no lamination and few migrated cells. The degree of morphological complexity differs between species and between brain regions. Our data suggest that changes in brain morphology are due to a mosaic of different influences. We did not find a strict correlation between visual system reduction and tectal morphologies. However, phylogenetic effects exist. The greatest degree of morphological complexity is found in members of the Rhinatrematidae, a family that is considered basal to the lineage. Thus, simplification of brain morphology in caecilians must be considered a secondary or derived rather than a primitive feature. Direct development and miniaturization are correlated with the greatest simplification in the tectum mesencephali and medial pallium. There is a relationship between differences in brain morphology and heterochrony in caecilians, as in other amphibians. J. Morphol. 231:11–27, 1997. © 1997 Wiley-Liss, Inc.

The morphology of the brain of members of the three modern orders of amphibians—frogs (Anura), salamanders (Caudata) and caecilians (Gymnophiona)—differs not only in the relative sizes of different brain regions but also in the degree of morphological complexity of the parts. The caecilian brain is characterized by a large telencephalon, and a very small tectum mesencephali, an important area for sensory integration that receives primary visual projections. Small size is in concordance with the reduction of the visual system in these animals (Mantuffel and Himstedt, ’85; Wake, ’85). The tectum mesencephali also differs between frogs and salamanders. The tectum of anurans is large, expanded, and multiply laminated (Fig. 2a). It consists of nine alternating cellular and fiber layers (Pottet, ’89). The superficial fiber layers (layers 7–9) contain a substantial number of migrated cells (up to 30%). These layers resemble the tectum of most other groups of vertebrates (Vanegas et al., ’84). In contrast, the tectum of salamanders and caecilians (Fig. 2b,c) is very simple. It has only a periventricular cellular layer and a superficial fiber layer containing very few migrated cells (Roth et al., ’90b; Schmidt and Wake, ’91). In salamanders, not only the tectum mesencephali but also other regions are affected by the reduction of cell migration (Roth et al., ’93; Wake et al., ’88). Only the medial pallium always shows cell migration (Roth et al., ’93). In the past, simple morphology was interpreted to be a primitive (Herrick, ’48; Leghissa, ’62) and unspecialized state. However, a recent phylogenetic analysis suggests that simple morphology is an apomorphic trait and due to secondary, derived simplification (Roth et al., ’92, ’93).

Major factors that trigger brain morphogenesis are 1) stimulation by afferents and 2) changes in the time course of development (Finlay et al., ’87; Roth et al., ’93; Schmidt and Roth, ’93). Caecilians are characterized by a reduction of the entire visual system. The degree of reduction differs interspecifically (Wake, ’85). It is currently not known

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whether or how the reduction of the visual periphery affects the morphological complexity of the tectum and what secondary effects on the development of other brain regions might arise. Is, for example, the expansion of the telencephalon and the elaborated olfactory system (Schmidt and Wake, '90) in these animals indirectly related to the reduction of the visual system?

In urodeles, simplified brain morphology is considered to be due to a change in the time course of development, in the sense that late ontogenetic stages are retarded or lost (Roth et al., '93; Schmidt and Roth, '93). In consequence adult animals retain juvenile characters in the adult stage and are thus considered paedomorphic. The retardation of development in these animals is thought to be due to a decrease in rates of cell metabolism and cell proliferation that is correlated with an increase in genome and cell size (Sessions and Larson, '87; Roth et al., '90a; Szarski, '76, '83; Cavalier-Smith, '78). The genome sizes of caecilians (Sessions and Wake, unpublished) lie between those of salamanders, which are characterized by large genomes and a very simple tectal morphology (Roth et al., '90a, '93), and those of frogs, which have small genomes and a multiply laminated tectum. Changes in the time course of development also occur in the context of differences in life history strategies. For example, some frogs and salamanders that have direct development (fertilized eggs laid on land, development through metamorphosis before hatching so that a free-living larval period does not occur) are characterized by an acceleration or condensation of early ontogenetic stages. The more common and presumably plesiomorphic life history mode is shown by species that lay eggs that develop into larvae, characterized by a suite of characters involving a free-living aquatic period before a relatively profound metamorphosis into the juvenile/adult form. Among salamanders the most
simplified brains in terms of degree of lamination and cellular migration occur in miniaturized, direct developing, derived species (Roth et al., '90a, '94).

The gymnophione amphibians show a diversity of structure-function modifications and life history patterns that is not seen in other amphibians, at least to the same extent. These features include limblessness, visual reduction, and viviparity (retention of embryos with maternal nutrition following resorption of yolk, and birth of fully metamorphosed young). In order to determine whether differences in brain morphology are related to function and/or phylogenetic history and/or life history strategies, we investigated the morphology of the tectum mesencephali and the medial pallium of representatives of the six families of caecilians, which differ in both the degree of reduction of the visual system and in life history strategies.

MATERIALS AND METHODS

Species examined

Tectal morphology was studied in representatives of all six families of the Order Gymnophiona (Fig. 1) (see Duellman and Trueb, '86; Nussbaum and Wilkinson, '89; but also see Hedges et al., '93). Families and species studied include Rhinatrematidae (northern South America), Epicrionops petensi and Epicrionops bicolor (both with an aquatic larval stage); Ichthyophiidae (southeast Asia), Ichthyophis kohtaensis (aquatic larval stage); Uraeotyphlidae (India), Uraeotyphlus narayani (aquatic larval stage) inferred from condition of congener [Wilkinson, '92]; Caeciliidae (Central and South America, Africa, Seychelles, India), Caecilia occidentalis (Peru, life history not known), Dermophis mexicanus (Central America, viviparous), Gymnopus multiplica (Central America, viviparous), Hypogeophis rostratus (Seychelles, direct development), Geotrypetes seraphini (Ghana, viviparous), Sylva-caecilia grandisonae (Ethiopia, semiaquatic larval stage [Largent et al., '72]), Boulengerula taitana (Kenya, direct development) [Nussbaum and Hinkel, '94]), B. boulengeri (Tanzania, life history unknown, perhaps direct development [Nussbaum and Hinkel, '94]), Idiocranium russelli (Cameroon, direct development); Typhlonectidae (South America), Typhlonectes natans and Chthoerpeton indistinctum (both viviparous); Scolecomorphidae (East and West Africa), Scolecomorphus kirkii and Scolecomorphus ulugurensis (West Africa, both viviparous) (see Table 1 for reproductive modes).

Methods

The morphology of the tectum was examined in heads of adult specimens, obtained from numerous individuals, museums, and collections (see Wake, '86, '92). Material was formalin-fixed and preserved in 70% ethanol. Heads were removed, decalcified, and embedded in paraffin and serially sectioned (transversely or horizontally) at 7–10 µm. Every third slide was stained with picr-ponceau, hematoxylin-eosin, or Mallory’s azan according to standard procedures (Humanson, '79). Since an exact identification of tectal borders can be made only from transverse sections, only those species for which We had such sections were studied quantitatively (E. bicolor (N = 2), D. mexicanus (N = 3), T. natans (N = 1), B. boulengeri (N = 1), and S. ulugurensis (N = 1)). Every third section was drawn with the aid of a camera lucida. From these drawings we determined total cell number and number of migrated cells in the superficial fiber layer and calculated the percentage of white matter. Areas (i.e., the periventricular cellular layer and the white matter) were measured using a graphic tablet (Summagraphics, Austin, TX) and its program. Each migrated cell was counted. In order to approximate the genome size, we determined the nuclear size by calculating the mean diameter of nuclei of eight to twelve cells/section, ten sections per specimen (genome size is highly correlated with nuclear and cell size; see Discussion and Table 1). Tables 2 and 3 present analyses of comparative organization of the tectum, telencephalon, and the visual system, emphasizing degrees of differentiation, complexity, and cell migration for the former two components and components present (complexity) in the latter (see Tables and Discussion). Because we found large differences in the numbers of migrated cells in different tectal regions (medial zone and lateral zones), we calculated for the whole tectum as well as separately for the medial and lateral tectum. Cell number within the periventricular cellular layer was determined by counting cells in different tectal regions of the same size (four counts for each cross-section). Using the cell numbers of these volumes, we extrapolated the cell numbers of the total volume of the periventricular cellular layer. Brains that were sectioned...
horizontally were studied from photomicrographs at specific levels of the tectum and by comparing the degree of cellular migration and morphological differentiation in these sections. The degree of morphological differentiation was evaluated by counting the number of fiber layers within the periventricular gray. No measurements were made from

Fig. 2. Transverse sections of tectal hemispheres in the anuran *Bufo bufo* (a), the urodele *Salamandra salamandra* (b), and the caecilian *Typhlonectes natans* (c). The frog tectum is multilayered; those of the salamander and the caecilian are bilayered. Note the differences in the number of migrated cells (open arrows) within the superficial fiber layer (SF). The thick line transecting the tectal layers indicates the thickness of the periventricular gray. Bars = 100 µm.
brains that were sectioned horizontally (E. petersi, U. narayani, C. occidentalis, G. multipli-
cata, H. rostratus, G. seraphini, S. grandisonae, B. taitana, and S. kirkii), because of non-
comparability with other data, so our results are more qualitativa-
hen as well as in the number of migrated cells that occur in the superficia-
fiber layer. In some species the periventricular gray is homogeneous (Fig.
3a,b,c), whereas in other species it is traversed by fibers that, however, in most cases do not constitute continuous layers (Fig.
3d,f). Those species that have the greatest degree of morphological complexity show different layers (Fig. 3e,g,h). The caudal tectum has a greater degree of lamina-
tion than the rostral tectum. We distinguish an ependymal layer, an adjacent fiber layer, an inner cellular layer, an adjacent fiber layer, an outer cellular layer, and a superficial fiber layer containing migrated cells (Fig. 3e,g,h).

Since nothing is known about the cytoarchi-
tecture of the caecilian tectum and because different layers in the caecilian tectum cannot easily be homologized with those in the tectum of frogs and salamanders, we do not adopt the descriptive numbering used for the tectal layers of frogs and salamanders (Potter, ’69; Roth, ’87).

Species-specific variation

Descriptions are organized phylogeneti-
cally, from basal families to the more derived (see Fig. 1). Figures 3 and 4 compare morpho-
logical differentiation in the tectum, Figures 5–7 neuronal migration in tectum (Fig. 5) and telencephalon (Figs. 6, 7). Consequently, the reader should note the figure order as it applies to these species-level descriptions and then refer to the figures again relative to the discussion of each region.

Family Rhinatrematidae

Epicrionops bicolor (Fig. 3e,g,h). E. bi-
color possesses a morphologically well-orga-
nized tectum. The morphologically most com-
plex region of the tectum mesencephali is the lateral zone of the tectum; here, several tectal layers are distinguishable. The periventricular gray is divided into three cellular layers and two fiber layers. We distin-

**RESULTS**

Descriptive morphology of the tectum

mesencephali

In general, the caecilian tectum appears rather simple in that it consists primarily of

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Life history</th>
<th>Nuclear diameter in micrometer</th>
<th>Standard deviation</th>
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<td></td>
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<tr>
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<td>o</td>
<td>6.8 1.27</td>
<td></td>
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<td>o</td>
<td>7.1 0.82</td>
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</tr>
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<td>6.8 0.54</td>
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<td>Scolecomorphidae</td>
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<td></td>
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<td>6.2 0.81</td>
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<td>7.7 1.16</td>
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1d, direct development; i, inferred; o, oviparous, larvae; v, vivipar-
ity; ?, unknown.
inner fiber layer, a cellular layer (thickness of about five cell diameters), an outer fiber layer, an outer cellular layer (thickness of about seven cells), and the superficial fiber layer that contains migrated cells, which are far more numerous in the lateral than in the medial zone. In contrast to the lateral zone, there is no clear distinction of different cellular layers in the medial zone. There is a better distinction of different cellular layers in the caudal than in the rostral tectum.

Epicrionops petersi (Fig. 4c). As in E. bicolor, the caudal part of the tectum mesencephali in E. petersi is morphologically more complex than the rostral part. In the caudal part we distinguish an ependymal layer, an adjacent fiber layer, an inner cellular layer that is separated from an outer cellular layer by a fiber layer, and a superficial fiber layer containing migrated cells.

Table 2. Morphological organization of the tectum mesencephali and the visual system, and cellular migration in the tectum mesencephali and the telencephalon in caecilians

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Tectal organization class</th>
<th>Visual organization class</th>
<th>Tectal migration class</th>
<th>Telencephalic migration class</th>
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<td>Uraeotyphlidae</td>
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<td>Scolecomorphus uluguruensis</td>
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<td>1</td>
<td>2</td>
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*Categories 1-3 indicate simple, moderate, and complex classes of organization, migration, and differentiation; simple is often secondary rather than primary. See text for discussion.

Table 3. Tectal migration relative to the amount of white matter in caecilian brains

<table>
<thead>
<tr>
<th>Taxon</th>
<th>% Migrated cells</th>
<th>% White matter</th>
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<tr>
<td></td>
<td>TT MT LT</td>
<td>TT MT LT²</td>
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<tr>
<td>Rhinatrematidae</td>
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<tr>
<td>Epicrionops bicolor</td>
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<td>65.5 57.4 65.5</td>
</tr>
<tr>
<td>Scolecomorphidae</td>
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<td>55.8 50.4 59.1</td>
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<tr>
<td>Caeciliidae</td>
<td></td>
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</tr>
<tr>
<td>Darmophis mexicanus</td>
<td>9.6 3.3 15.5</td>
<td>67.3 50.2 76.1</td>
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<tr>
<td>Boulengerula boulengeri</td>
<td>3.8 0.5 7.3</td>
<td>63.1 48.5 72.2</td>
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<td>Typhlonectidae</td>
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</tr>
<tr>
<td>Typhlonectes natans</td>
<td>5.0 2.0 7.8</td>
<td>60.3 53.2 65.8</td>
</tr>
</tbody>
</table>

*Compare tectal cell migration percentages to nuclear/cell sizes and life history modes in Table 1.

²LT = lateral zone of the tectum; MT = medial zone of the tectum; TT = total tectum.
Fig. 3. Morphological differentiation in the tectum mesencephali. Transverse sections. Boulengerula boulengeri (a–c) has a homogeneous periventricular gray in the rostral (a) as well as in the caudal (b) tectum. In T. natans (d,f) there are subdivisions within the periventricular gray without definition of layers. Epicrionops bicolor (e,g,h) has the greatest degree of morphological differentiation. Starting from the ventricle, we distinguish an ependymal layer, a subsequent fiber layer, an inner cellular layer, an outer fiber layer, an outer cellular layer, and the superficial fiber layer containing migrated cells. There is a greater degree of morphological complexity in the caudal (h) than in the rostral tectum (g). Photomicrographs for panels c, d, e show a higher magnification of the periventricular gray in B. boulengeri (c). T. natans (d), and E. bicolor (e). a, b, f, g, h: Bars = 100 μm. c, d, e: Bars = 50 μm.
U. naryani is the lateral zone of the caudal tectum. Here we distinguish an ependymal layer, an adjacent inner fiber layer, a thick inner cellular layer, a subsequent outer fiber layer, a thin outer cellular layer, and the superficial fiber layer containing migrated cells. In the rostral tectum, we observe only an ependymal layer, a fiber layer, a thick homogeneous...
cellular layer, and the superficial fiber layer that contains a moderate number of migrated cells.

Family Scolecomorphidae

Scolecomorphus kirkii (Fig. 5b). The tectum consists of one homogeneous periventricular cellular layer and a superficial fiber layer that contains a few migrated cells.

Scolecomorphus uluguruensis. As in S. kirkii, there are no subdivisions of the loosely packed homogeneous periventricular cellular layer. Moderate numbers of migrated cells are more concentrated in the lateral zones but are also present in the medial zones.

Family Caeciliidae

Boulengerula boulengeri (Fig. 3a,b,c). B. boulengeri has a very simple tectal morphology in that we find only an ependymal layer, a well-differentiated inner fiber layer, a thick homogeneous periventricular gray, and few migrated cells in the lateral zone.

Fig. 5. Degree of neuronal migration within the tectum mesencephali. Horizontal sections. (a) Idiocranium russelsii. (a) has limited neuronal migration; Scolecomorphus kirkii (b) has moderate neuronal migration; T. natans (c) has extensive neuronal migration. Illustrations for panels d and e are the dorsal (d) and ventral (e) tectum of S. grandisonae conjoined to show that the greatest degree of neuronal migration is in the lateral part of the ventral tectum. An exception to that general condition for caecilians is C. occidentalis (f), in which migration is greater in the dorsal tectum. Bars = 100 μm.
Boulengerula taitana. The tectum mesencephali of B. taitana is rather simple. In general, we distinguish an ependymal layer, a fiber layer that is better expressed in the rostral than in the caudal tectum, a homogeneous periventricular cellular layer, and a superficial fiber layer containing migrated cells that are most numerous in the central tectum.

Gymnopis multiplicata (Fig. 4a). The morphology of the tectum mesencephali in G. multiplicata is rather simple in that we distinguish only an ependymal layer, a subsequent fiber layer that is more elaborated in the rostral than in the caudal tectum, a thick periventricular gray matter that does not have any further lamination, and a superficial fiber layer containing many migrated cells.

Dermophis mexicanus. The tectum mesencephali in Dermophis has a moderate to great degree of morphological complexity. We observe an ependymal layer, an adjacent fiber layer, a periventricular gray matter that is subdivided into two cellular layers with a fiber layer lying between them, and a superficial fiber layer that contains many migrated cells in the lateral zone of all parts of the tectum. There are few migrated cells in the medial zone. Although Dermophis possesses a subdivision of the periventricular gray similar to that of Epicrionops, this subdivision is not as elaborated as in Epicrionops.

Geotrypetes seraphini (Fig. 4b,d). Tectal morphology in this species is similar to that in H. rostratus. The caudal tectum is morphologically more complex than the rostral tectum. Within the caudal tectum, we distinguish an ependymal layer, a very thin fiber layer, a thick inner cellular layer with a low cell density, a thin outer fiber layer and an outer cellular layer where the cell density is higher than in the inner layer, and a superficial fiber layer containing migrated cells. The number of migrated cells is highest in the central tectum. In the rostral tectum, we distinguish an ependymal layer, a well-differentiated fiber layer, a thick homogeneous cellular layer, and a superficial fiber layer with migrated cells.

Idiocranium russelli (Fig. 5a). I. russelli possesses the greatest reduction of the tectum; it is very small and very poorly laminated. There is only a thin fiber layer that divides the periventricular gray into two thin layers in the ventralmost part of the tectum. The superficial fiber layer contains only very few migrated cells.

Sylvacaecilia grandisonae (Fig. 5d,e). Morphological complexity is greatest in the caudal tectum. In this part, we distinguish an ependymal layer, a thin fiber layer, two cellular layers that are separated by an outer fiber layer, and a superficial fiber layer. More rostrally, there is no distinction of different layers within the periventricular gray matter. S. grandisonae possesses few migrated cells. Most migrated cells are found in the lateral part of the central tectum.

Caecilia occidentalis (Fig. 5f). In this species, a great degree of morphological complexity is found in the dorsal tectum. We distinguish an ependymal layer, a few fibers between the ependymal layer and a subsequent thin cellular layer (which is not continuous), an outer fiber layer, a thick cellular layer, and the superficial fiber layer that contains many migrated cells.

Hypogeophis rostratus. The tectum mesencephali is morphologically more complex in the caudal than in the rostral part and in the lateral than in the medial part. In the caudal tectum we distinguish an ependymal layer, a well-differentiated fiber layer, a thick inner cellular layer where cells are loosely packed, an outer cellular layer where cells are more densely packed, and a superficial fiber layer containing migrated cells. There are very few fibers between the inner and outer parts of the cellular layer. In the rostral tectum there is no distinction of different parts of the thick periventricular cellular layer, and there are fewer migrated cells within the superficial fiber layer.

Family Typhlonectidae

Typhlonectes natans (Figs. 3d,f, 5c). T. natans has a moderate degree of morphological complexity. We distinguish an ependymal layer, a well-differentiated fiber layer, a cellular layer, and a superficial fiber layer that contains a moderate number of migrated cells. Migrated cells occur mainly in the lateral zone of the tectum; only few are found in the medial zone. There is a slight distinction of two different cellular layers within the periventricular cellular layer, separated by few fibers. However, there is no clear distinction of an outer fiber layer within the periventricular gray.

Chthonerpeton indistinctum. The morphology of the tectum in C. indistinctum is similar to that of T. natans in that there is a
slight but not well-expressed distinction of subdivisions of the periventricular gray matter. Moderate numbers of migrated cells are present in the superficial fiber layer.

Neuronal migration in the tectum mesencephali and telencephalon

The degree of cellular migration differs in the caecilians examined (Figs. 3–5; Table 2). Our quantitative studies reveal that there are species that have substantial cell migration (i.e., *E. bicolor* and *D. mexicanus*) (~9%), *T. natans* possesses a moderate number of migrated cells (5%), whereas limited cell migration (~4%) exists in *S. uluguruensis* and *B. boulengeri*. The lowest number of migrated cells occurs in *I. russelli*, which has very few or even no migrated cells (Fig. 5a). *I. kohtaoensis*, *S. kirkii*, *B. taitana*, *B. boulengeri*, and *S. grandisonae* have more migrated cells than does *I. russelli* (Fig. 5a) but still few migrated cells (Fig. 5). *E. petersi*, *G. multiplicata*, *G. seraphini* (Fig. 4b,d), and *C. occidentalis* (Fig. 5f) possess a substantial number of migrated cells comparable to their abundance in *E. bicolor* and *D. mexicanus*. The degree of cellular migration in *H. rostratus* and *C. indistinctum* is comparable to that of *T. natans* (Figs. 3d,f, 5c). In general, migrated cells occur primarily in the lateral zone of the tectum. There are few migrated cells in the medial zone of the tectum (Fig. 5).

Quantitative measurements indicate that the degree of neuronal migration is correlated with the relative size of the white matter (Table 3). Species in which the percentage of the white matter is relatively high (67.3% in *D. mexicanus* and 65.5% in *E. bicolor*) have the greatest degree of neuronal migration.
migration (9.6% in D. mexicanus and 9.5% in E. bicolor). T. natans has a moderate percentage of white matter (60.3%) and is characterized by a moderate degree of cellular migration (5%). Further, a lower percentage of white matter is correlated with a lower degree of neuronal migration (i.e., S. uluguruensis with 55.8% and 3.7%, respectively). An exception is B. boulengeri, which possesses a relatively large amount of white matter (63.1%) but only little neuronal migration (3.8%). The correlation between the degree of neuronal migration and the relative size of the white matter not only fits interspecifically but also for different tectal regions. In all species examined quantitatively, the medial zone of the tectum has a low percentage of white matter (48.5–57.4%) and limited neuronal migration (0.5–3.3%). The lateral zone of the tectum is characterized by a higher percentage of white matter (59.1–76.1%) and more migrated cells (5.1–15.5%).

Telencephalon (Figs. 6, 7)

There are great differences in the degree of cellular migration in the telencephalon of the species examined (Tables 2, 3; Figs. 6, 7). The least cellular migration in the telencephalon was found in E. bicolor (Fig. 6a) and E. petersi. Boulengerula taitana, B. boulengeri (Fig. 6b), I. russelli (Fig. 7a,d), Sylvacaecilia grandisonae (Fig. 7b), C. occidentalis, S. kirkii, H. rostratus, and I. kohtaoensis have more, but still not many, migrated cells. T. natans (Fig. 7e), C. indis-
tinctum, S. uluguruensis (Fig. 6c), U. narayani (Fig. 7c), G. seraphini, D. mexicanus, and G. multiplicata have a substantial number of migrated cells.

Nuclear/cell size (Table 1)

Nuclear sizes range from 5.6 µm (S. uluguruensis) to 8.1 µm diameter (G. multiplicata) (Table 1). The correlation of nuclear size with genome size and cell size has been discussed extensively (Cavalier-Smith, '78, '82; Horner and Macgregor, '83; Sessions, '84; Sessions and Larson, '87; Szarski, '76, '83). Because of the very limited cytoplasm surrounding the nuclei of the brain cells measured, we use nuclear diameters as a referent to cell size in this discussion. In general, animals that have a free-living aquatic larval stage in their life histories (E. bicolor, E. petersi, I. kohtaoensis, S. grandisonae, and (larval stage presumed) C. occidentalis and U. narayani) are characterized by large cells (6.8–7.9 µm). Viviparous species are divided into two groups, with the New World taxa G. multiplicata, D. mexicanus, T. natans, and C. indistinctum possessing large cells (7.1–8.1 µm) and the African S. kirkii, S. uluguruensis, and G. seraphini possessing small cells (5.6–6.2 µm). Species that have direct development (B. taitana, B. boulengeri (presumably), H. rostratus, and I. russelli) are characterized by small cells (5.6–5.9 µm).

Cell sizes correlate with body sizes to some degree only for the small species examined. However, the cells of I. russelli, a truly miniaturized species (Wake, '86), are no smaller than those of small but less dramatically miniaturized species. Similarly, there is considerable variation in body size and proportions among the “large-celled” taxa with aquatic larvae and among the viviparous African “small-celled” forms. Given the small number but wide range of taxa examined, it appears that reproductive mode (see Table 1 and Discussion) and biogeography are better correlated with cell size than is body size.

DISCUSSION

Simplification of the tectum mesencephali in caecilians—a secondarily derived process?

Our studies indicate that the tectum mesencephali in caecilians not only is small compared to other brain regions but is characterized by a low degree of morphological complexity. It more closely resembles the tectum mesencephali of salamanders, even though the degree of lamination and the number of migrated cells in the most developed caecilian tectum (E. bicolor) are greater than in most salamanders. In salamanders, only ambystomatids (e.g., Ambystoma mexicanum [personal observation]) possess a degree of lamination and cellular migration that is similar to that of E. bicolor. In the past, the complexity of brain morphology often was used as a parameter for evaluating brain evolution. A complex brain was regarded as representing a derived condition, while a simple morphology was thought to be the plesiomorphic condition (Herrick, '48; Bullock, '84). Following these arguments, the anuran tectum would be the most derived, while the salamander tectum would represent the plesiomorphic condition; the caecilian tectum would be morphologically intermediate between that of those two orders. However, a phylogenetic analysis of tectal morphology among vertebrates (Roth et al., '93) revealed that the simple morphology is an apomorphic trait that must be considered a secondary simplification rather than a primitive feature. Thus, in contradiction to the above-mentioned hypothesis, the laminated tectum represents the primitive condition, whereas the simplified tectum is derived. A comparison of tectal morphology in different caecilian species clearly supports the latter hypothesis. E. bicolor and E. petersi, members of the Rhinatrematidae, the basal family of the lineage (Duellman and Trueb, '86; Nussbaum and Wilkinson, '89; Hedges et al., '93) (Fig. 1), have the highest degree of morphological complexity and migration. In these species all tectal layers that have been described in anurans (Potter, '69; Székely and Lázár, '76) can be distinguished, even though they are not as elaborated as in anurans. The tectal morphology of many derived species (i.e., B. boulengeri, S. kirkii, and S. uluguruensis) is rather simple, in the sense that only one thick periventricular cellular layer and a superficial fiber layer can be distinguished. There are only a few migrated cells. These results are in concordance with comparative studies on the tectum mesencephali of salamanders. Roth et al. ('93) demonstrated that in salamanders the simplest tectal morphology is found in those species that are highly derived (i.e., the bolitoglossines) (see also Roth and Schmidt, '93).
Simplification of the caecilian tectum mesencephali—a consequence of heterochrony?

Comparative studies of tectal morphology in vertebrates in general, as well as in salamanders and frogs in particular (Roth et al., '92, '93, '94), suggest that the simplification of the nervous system is a consequence of paedomorphosis (late developing features of outgroups are retarded or even missing in members of the lineage in question). A widespread influence of paedomorphosis on salamander evolution has been described for many morphological traits (Wake, '66) and also exists for the brain (Roth et al., '92, '93; Schmidt and Roth, '93); for example, although the general pattern of tectal development in the anuran Rana temporaria and the salamander Pleurodeles waltl is similar, certain developmental traits, especially those that appear at later ontogenetic stages of Rana (e.g., the migration of cells to the superficial tectal layers), are reduced in Pleurodeles (Schmidt and Roth, '93). The tectum of frogs and salamanders develops along a gradient from rostral to caudal as well as from lateral to medial; that is, the lateral part of the rostral tectum is the region where cellular proliferation, lamination, and cellular migration occur earliest, and the medial part of the caudal tectum is the part where these processes occur latest. In adult salamanders in general, there are very few migrated cells. There are no differences concerning the distribution of migrated cells in the medial and lateral tectum. This suggests that some early as well as later developmental processes are suppressed in these animals. In caecilians, a substantial number of migrated cells occurs in the lateral part of the tectum, which suggests that only the phase of cell proliferation within the medial proliferative zone that corresponds to the last proliferative phase in frogs is not expressed. In caecilians, the lateral and the medial parts of the tectum not only differ in number of migrated cells, but also in the degree of lamination. For example, in E. bicolor, the lateral part of the tectum has a clear distinction of different cellular and fiber layers, while the medial part does not. Thus, retardation of late ontogenetic processes in caecilians is not as extreme as it is in salamanders. In contrast to the situation in salamanders, in some caecilian species (G. seraphini, H. rostratus, U. narayani, E. bicolor, E. petersi, S. grandisonae, B. taitana) there is a greater degree of lamination in the caudal than in the rostral tectum (Fig. 3g,h). This cannot be explained by paedomorphosis, because the caudal tectum is the last part to develop. There are two possible routes by which a greater degree of lamination in the caudal tectum could occur. First, a condensation of early ontogenetic processes and an extension of late processes might have occurred in these animals. Second, in caecilians most aspects of the reduction of the visual system appear to occur in later ontogenetic stages (Wake, '85). In larvae the visual system is better developed than in adults. Owing to the fact that the caudal tectum is that part of the tectum which develops latest, it might have a greater potential for plasticity at late developmental stages than does the rostral tectum that develops earlier. Thus, as a consequence of reduction of the visual system at late developmental stages, tectal afferents representing other than visual modalities might have been expanded in the caudal tectum in some caecilian species.

In frogs and salamanders, a negative correlation exists between morphological complexity of the brain and genome and cell size (Roth et al., '92, '94). This correlation may be explained by the fact that increases in genome sizes generally lead to decreases in rates of cell metabolism, cell proliferation, and differentiation (Sessions and Larson, '87; Szarfski, '76, '83; Cavalier-Smith, '78, '82; Horner and Mcgregor, '83). Substantial differences in genome sizes exist among amphibians. Frogs have small genomes (0.9–19 pg DNA/haploid nucleus [Mahony, '86; Moriscalchi, '90; Olmo, '83]), while salamanders are characterized by large genomes (13.7–83 pg [Hally et al., '86; Olmo, '83; Sessions and Kezer, '91]); the genome sizes of caecilians lie between those of frogs and salamanders (from 8–26 pg [Sessions and Wake, unpublished data]). In general, the degree of lamination and cellular migration in caecilians is in concordance with the concept that the degree of paedomorphosis is correlated with cell size. However, this correlation does not fit a comparison among caecilian species. In contradistinction to frogs and salamanders, which show a negative correlation between genome size and cell size and the degree of morphological complexity, there is a positive correlation between cell size and morphological complexity in caecilians. Those species that have the smallest cell sizes have brains that are relatively simple, whereas species
that have large cells are characterized by a greater degree of morphological complexity. This suggests that there are additional factors that influence brain morphology. Studies in frogs and salamanders (Roth et al., '94) revealed that in salamanders one important parameter related to the degree of lamination and cell migration is brain size. Brain size is determined by the rate of cellular proliferation, the rate of neurite outgrowth, and the duration of development. Thus, the degree of morphological complexity might simply be a function of the time course of these processes. In addition to changes in genome size, factors that are involved in these processes are changes in the synthesis of growth factors and the stimulation by affereents.

Changes in rates of development of those processes necessarily lead to changes in morphological complexity. We suggest the following evolutionary scenario for secondary simplification of the caecilian brain. We start with the simplest form of developmental delay—all processes are affected equally. Animals with large cells and a presumed low rate of cellular proliferation would require a longer time to reach a certain level of morphological complexity than animals with smaller cells and a higher rate of cellular proliferation. If the total time of development is the same in both groups, those animals possessing small cells would be morphologically more complex than would animals with large cells. However, if development stops early in animals with small cells, they would have the same degree of morphological complexity as an animal with large cells and a longer developmental time. If development stops very early in species possessing small cells, the degree of complexity would be even less than in animals with large cells. However, if development stops early in animals with small cells, they would have the same degree of morphological complexity as a simple animal with large cells and a longer developmental time. If development stops very early in species possessing small cells, the degree of complexity would be even less than in animals with large cells. This may be the case in I. russelli, a miniaturized progenetic (see Wake, '86) caecilian species that possesses very small cells. Since cell size is correlated with the rate of cell proliferation (Sessions, '84), the occurrence of small cells in miniaturized species may be a compensatory effect for an early cessation of development. Developmental trajectories that affect different processes in different ways complicate this simple model. If some processes are retarded while, simultaneously, others are accelerated, a mosaic of simplified (remaining from early ontogenetic stages) and advanced (representing later ontogenetic stages) characters, an ontogenetic re patterning such as that described for direct-developing salamanders (Wake and Roth, '89), occurs. While changes in genome size lead to a general slow-down of development that affects all structures in the same way, patterns of development that affect different structures in different ways require additional factors. Studies on the salamander P. waltl (Schmidt and Böger, '93) suggest that ontogenetic repatterning and miniaturization are under hormonal control. An increase in thyroxine during the aquatic larval stage in that species leads to miniaturization and the development of a mosaic of characters that resembles that of bolitoglossine salamanders.

Even though there is no strict correlation between developmental strategy and brain morphology, our studies on cell migration and lamination in the tectum mesencephali and telencephalon show that direct development, together with miniaturization, may lead to the greatest simplification of the brain (compare together data in Tables 1 and 2). I. diocrinum russelli possesses a tectum that is rather simple, and it has the lowest degree of cellular migration in the tectum. This is in concordance with data obtained in salamanders (Roth et al., '90a, '94) showing that the simplest brain morphology (in terms of lamination and degree of cellular migration) occurs in direct-developing miniaturized species. Similarly, most species of caecilians that are characterized by a high degree of morphological complexity are species that have an aquatic phase of development with free-living larvae.

The most striking feature in viviparous caecilians is the degree of cellular migration within the telencephalon. All species that are characterized by extensive cellular migration within the telencephalon (D. mexicanus, G. multiplicata, G. seraphini, and T. natans) are viviparous (Tables 1, 2). These are species that have a poor or moderate degree of tectal morphological complexity. In contrast, the least cellular migration within the telencephalon occurs in E. bicolor and E. petersi, those species that have the greatest degree of lamination within the tectum and are oviparous with free-living larvae. It remains an open question whether intrinsic constraints in the development of the brain exist, in the sense that the reduction of the morphological complexity in one brain region is correlated with an increase in complexity in another brain region, or whether
the simplification of the telencephalon in E. bicolor and E. petersi is due to a retardation of the development of the telencephalon particular to these species, probably due to paedomorphosis, or whether the simplification of the telencephalon represents a plesiomorphic condition in caecilians.

Visual system reduction and tectum morphology

Gymnophione amphibians are characterized by a reduction of the visual system, ranging from loss of the components of accommodation and covering the eye with skin to the eye being represented only by an amorphous retina in an orbit deeply embedded in musculature (Wake, '85). Stimulation by afferents has been reported to be a major factor to trigger the morphogenesis of brain regions (Finlay et al., '87). An hypothesis that the reduction of the visual system is correlated with concomitant reduction or simplification of tectal morphology is testable with our data. We recognize three groups of species, based on components of reduction of the visual system (Table 2). Group 1 has the most reduced eyes (extrinsic musculature absent, except for the derived retractor tentaculi; lens absent, rudimentary, or amorphous; optic nerve attenuated or highly attenuated; retina reduced, net-like or amorphous). Taxa included in group 1 are S. uluguruensis, G. multiplicata, B. taitana, and B. boulengeri. These taxa are also the species that have the least tectal laminataion. However, they have moderate to extensive cell migration in the tectum. Group 2 includes forms with moderately reduced eyes (two to four extrinsic muscles present in addition to the retractor tentaculi; lens diffuse to cellular; optic nerve intact; retinal layers well developed but with reduced numbers of cells); C. occidentalis, S. kirkii, and C. indistinctum. These taxa vary in morphological complexity in the tectum, S. kirkii having very little, C. indistinctum having moderate, and C. occidentalis having extensive morphological complexity. The two latter taxa have considerable numbers of migrated tectal cells. S. kirkii possesses a moderate number of migrated cells. Forms with well-developed eyes (all extrinsic muscles present; lens well developed; optic nerve intact; retina well developed) constitute group 3 and include E. bicolor, E. petersi, I. kohtaoensis, U. narayani, D. mexicanus, H. rostratus, G. seraphini, S. grandisonae, I. russelli, and T. natans. The tecta of these taxa all have moderate to great morphological complexity, except I. russelli, which has minimal lamination. Most of these taxa also have rather extensive (E. bicolor, E. petersi, U. narayani, G. seraphini, H. rostratus, D. mexicanus, T. natans) or moderate (I. kohtaoensis and S. grandisonae) tectal cell migration. The specimens of S. grandisonae examined for our neuroanatomical work were larvae, which appear to have better developed visual systems than do adults (Wake, '85). Idiocranium russelli again may constitute a special case, it has a well-developed visual system but has minimal laminataion and the least number of migrated tectal cells. Extreme paedomorphosis (for caecilians) may be implicated in having a well-developed eye but a very simplified tectum. taxa with well-developed visual systems, therefore, have all degrees of morphological complexity and of cell migration in the tectum; species with reduced visual systems all have limited to no laminataion and moderate to extensive tectal cell migration. Several aspects of the biology of the animals may influence these generalizations. For example, the closely related S. uluguruensis and S. kirkii of the Family Scolecomorphidae have rather different visual system morphologies, but their tectal structure is remarkably similar. It is possible that there is a developmental effect, in that the brain may be more conservative than the visual system in its modification. A similar situation may obtain with the two members of the Family Typhlonectidae examined, T. natans and C. indistinctum, which have somewhat different visual systems but very similar tectal morphologies. It is not surprising that the two species examined of Epicrionops, which have very similar eye development, also have virtually the same tectal morphologies. Based on this small taxonomic and morphological sample (but inclusive of representatives of three of the five families of caecilians), we see no strict correlation of visual system reduction with tectal morphologies. This may be the case because visual afferents are not the only afferents reaching the tectum. Afferents belonging to other systems also may regulate tectal development. In this context, the correlation between the relative amount of tectal neuropil and the degree of cellular migration is important. In those species in which the percentage of white matter is relatively high, cell migration is more extensive than
in species that have a lower percentage of white matter. An exception is B. boulengeri, which has a moderate amount of white matter but no more migrated cells than a species with relatively little white matter (i.e., S. uluguruensis). This suggests that a certain size of the tectal neuropil is a necessary but not sufficient precondition for cellular migration.

In summary, our data suggest that changes in brain morphology are consequences of a mosaic of different influences. Whereas changes in the peripheral sensory system do not necessarily lead to different brain morphologies, phylogenetic characteristics and life history strategies that are related to changes in patterns of development may influence the modification of brain morphology.

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LITERATURE CITED


