

TESTING MODULARITY AND DISSOCIATION: THE EVOLUTION OF REGIONAL PROPORTIONS IN SNAKES

P. David Polly

Molecular and Cellular Biology Section, Biomedical Science Division, Queen Mary & Westfield College, London, United Kingdom

Department of Palaeontology, The Natural History Museum

Jason J. Head

Department of Geological Sciences, Southern Methodist University, Dallas, Texas

Martin J. Cohn

Division of Zoology, School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading, United Kingdom

INTRODUCTION

In this chapter, we test a hypothesis about dissociation in the evolution of body and tail proportions in snakes and examine whether that dissociation represents heterochronic change. Heterochrony can be defined as a dissociation in timing between two developmental modules. This definition – subtly different from the usual “evolutionary change in developmental timing” – was used by Raff

(1996) after he explored the concepts of dissociation and modularity in relation to the evolution of development. Raff defined *modularity* as the division of an organism into units, each with its own genetic specification, hierarchical organization, and interactions with other similar units. Through the homeostatic influences of functional and developmental integration, modules are usually linked. Raff defined *dissociation* as the process of unlinking. A heterochronic change thus requires dissociation between two dissociable modules. Distinguishing heterochronic change from its alternatives therefore requires analysis of at least two key elements: that evolutionary change can be attributed to shifts in the timing between modules and that those modules are dissociable. Attention is now being paid to the former (the papers in this volume are a notable example) but less so the latter. In this paper we test two possible developmental modules – those controlling the number of vertebrae in the body region and in the tail region of snakes – for dissociation.

Despite the simplicity of the concepts, the empirical recognition of modularity and dissociation can be difficult. One reason is that developmental modules often do not correspond with obvious morphological boundaries. Lovejoy *et al.* (2000) recently identified an unexpected module in the mammalian forelimb. By analyzing growth patterns in the forearm of several primates, they identified a surprising suite of skeletal elements that scale together during growth. The bones affected by the scalar are the distal radius and the digits II-V, which have a linear relationship and behave as a developmental module, but not the proximal radius or digit I. Interestingly, the *Hoxd11* gene is expressed in precisely in this region (Lovejoy, et al., 2000), and is known to play a role in growth of long bones. (Goff and Tabin, 1997). A second complication is that developmental processes may have overlapping physical domains. For example, several topographically disjunct skeletal elements – notably membranous bones – are linked through the common expression of *CBFA1*, a transcription factor that plays multiple roles in bone formation (Mundlos, 1999). Humans with mutations in *CBFA1* may have open skull sutures, supernumerary teeth, absent or reduced clavicles, and unfused pubic symphyses – symptoms clinically diagnosed as “cleidocranial dysplasia”. The combined targets of *CBFA1* mutational effects do not function as a module (they do not form a hierarchical unit), but the pattern of *CBFA1* expression *links* modules making complete dissociation between them difficult. Dissociation may thus be more a matter of degree than an absolute phenomenon.

Furthermore, dissociation is an *evolutionary* phenomenon. Unlike modularity, which is a property of the individual organism created by genetic and developmental correlation, dissociation is a process of unlinking over evolutionary time. Modules become dissociated along branches of a phylogenetic tree. Because modules may be linked by many independent interactions – some direct (like induction), others less so (like the influence of *CBFA1*) – dissociation may be subtle enough to require a statistically significant sample of comparisons. The scalar identified by Lovejoy, et al. (2000) was apparent only after several species were compared. A comparison of distal limb elements in only two species reveals simply that each species has distal limb elements that differ

in size and shape from those in the other species. Only when a large enough sample of species is studied does it become apparent that the distal radius and digits II-V are correlated with one another relative to the proximal radius and digit I. It is also possible that links between modules may not be linear, but the scalar may cause them to change allometrically. A single between-species comparison might reveal that the relationship between two modules has shifted, but many such comparisons may reveal that those shifts are correlated. The identification of modules and dissociation is thus a type of morphological integration study (Olson & Miller, 1959; Cheverud, 1982; Atchley, 1991; Zelditch, 1995; Leamy, et al., 1999).

Because of the variation in vertebral segment number, snakes are ideal for studying the evolutionary interactions between developmental processes determining segment number and those determining regional segment identity. Unlike in limbed vertebrates, snake locomotory and ecological diversity has been attained exclusively through specialization of the vertebral column and axial muscular system. The range of snake ecomorphology has been realized through modification of vertebral proportions (Gasc, 1976), vertebral number (Lindell, 1996), body length (Lindell, 1996), body mass (Shine, 1986), and muscular arrangements (Jayne, 1982, 1988). The extremes of snake ecomorphology – arboreal versus terrestrial versus fossorial versus aquatic forms – can often be recognized simply by the proportion of body to tail length. Many arboreal snakes, for example, have long bodies with proportionally long tails, terrestrial taxa have long bodies with shorter tails, and fossorial snakes are short with extremely short tails (Fig. 9.1). These proportional differences are found both in the linear lengths of these regions and in the number of vertebral segments in each. Specifically we test the hypothesis that control of the number of body segments is “modularized” and can be dissociated from the tail. As reviewed below, patterns of development in the two regions suggest that they are modularized; however, several developmental processes are common to both and may prevent them from being dissociated. Our hypothesis is thus based on known developmental mechanisms; we test that hypothesis against adult comparative data in a phylogenetic context. Our test thus goes further than looking at simple correlations between the number of body and tail segments in adult snakes, we examine their correlation as evolutionary *transformations*.

MORPHOLOGY AND DEVELOPMENT OF THE SNAKE VERTEBRAL COLUMN

Vertebral morphology in snakes is highly derived, and the primary regions of the vertebral column can be recognized based on discrete morphological features. All snakes possess zygosphene-zygantral articular processes along the dorsolateral margins of the neural canal throughout the column, as well as synapophyses (paired dia- and parapophyseal articulations for the ribs), corresponding to the absence of vertebral transverse processes. The primary division in the snake vertebral column is the recognition of pre- and post-cloacal (that is,

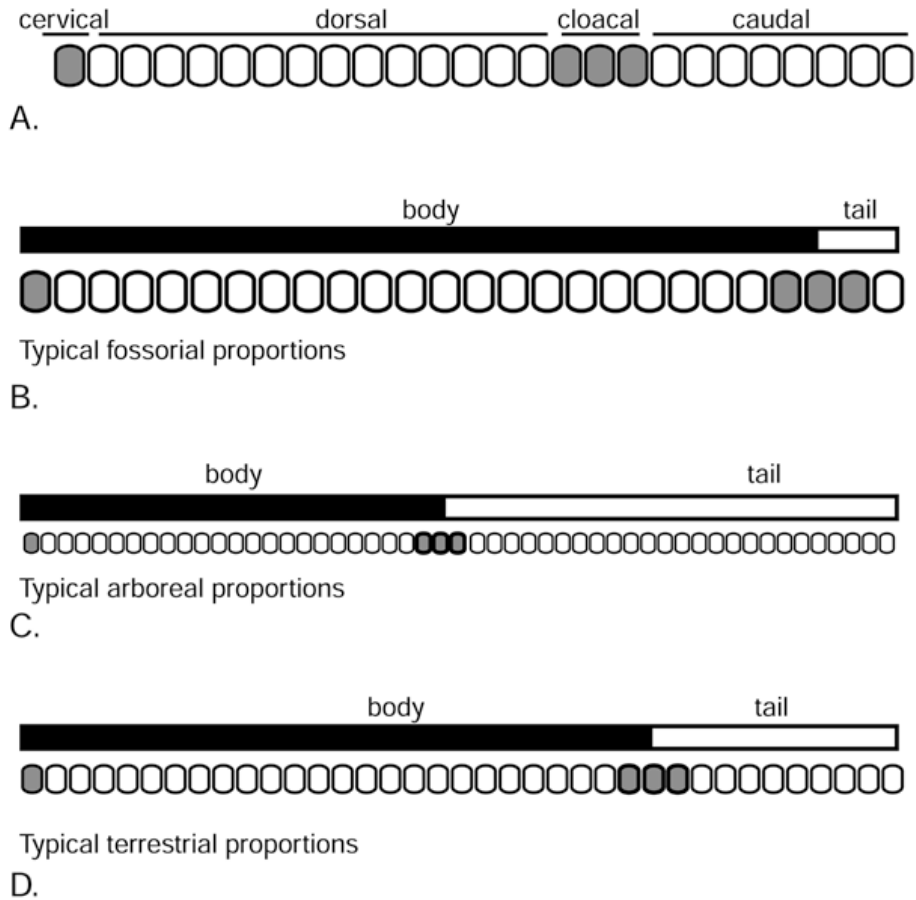


Figure 9.1. Differences in regional proportions in terrestrial, arboreal, and fossorial snakes. **A.** Morphologically defined axial regions within snakes. Snakes have a cervical vertebra, which articulates with the back of the skull, a large number of rib-bearing dorsal vertebrae (homologous with thoracic and lumbar vertebrae in mammals and birds), a small number of cloacal vertebrae (homologous with sacral vertebrae in other tetrapods), and a series of caudal vertebrae. **B.** Typical proportion between body and tail (pre- and post-cloacal) segment numbers in a typical fossorial snake (e.g., *Typhlops*). There are few vertebrae overall and the proportional number in the tail is small. **C.** Proportion of body and tail in a typical arboreal snake (e.g., *Ahaetulla*). There are many vertebrae, almost half of which are found in the tail. **D.** Proportion of body and tail in a typical terrestrial snake (e.g., *Python*). There are a large number of vertebrae, but the proportion found in the tail is considerably less than in arboreal snakes.

caudal) regions that are separated by a series of three to five cloacal vertebrae. These regions are differentiated morphologically by the transition from pre-cloacal synapophyses to forked cloacal lymphapophyses to caudal pleurapophyses (sensu Hoffstetter and Gasc, 1969), the transition from a single ventral hypapophysis to paired hemapophyses at the cloaca, and an abrupt short-

ening of the centrum at the cloaca (Thireau, 1967). Within the pre-cloacal column, regions have been recognized for some taxa, including “cervical” or anterior trunk, mid-trunk, and posterior trunk regions (e.g., LaDuke, 1991a). Prominent hypapophyses are restricted to the “cervical” region, and subcentral paramedian lymphatic fossae to the “posterior trunk” region. However, these characteristics are restricted to inclusive clades of snakes, and are not present in the majority of taxa. Among all snakes, the division of the precaudal vertebral column into discrete regions can only be achieved by recognition of relative placement. The only universally recognizable regions are pre- and post cloacal (Hoffstetter and Gasc, 1969).

The development of the snake axial skeleton can be thought of as three conceptually separate processes: segmentation, regionalization, and skeletogenesis. During early development the embryo’s paraxial mesoderm is partitioned into segments, the somites, which then differentiate into axial bones, muscles and dermis. As development progresses, the structures within each segment continue to grow, accentuating regional differences along the axis. The degree to which these three processes are integrated is an open question and at least two of them, segmentation and skeletogenesis, are common to both body and tail. In this paper we ask whether the number segments—and therefore the number of vertebrae—in the body and tail regions can be dissociated evolutionarily.

Most of the work on somitogenesis has been carried out using the chick embryo, but similar studies of other vertebrate embryos suggest that mechanisms are generally conserved. Cells that emerge from the primitive streak and Hensen’s node during gastrulation form the embryonic mesodermal layer. The streak deepens into a *primitive groove* with a thickening known as Hensen’s node at the anterior end (Fig. 9.2, A and B). Pre-mesodermal epiblast cells move toward the midline of the embryo and ingress through the streak. After ingress, cells move laterally and anteriorly, giving rise to the mesodermal layer of the embryo. Fate mapping of the streak and node has identified regional sub-populations of cells that act as a source of somitic mesoderm (Tam and Tan, 1992). After the anterior (pre-lumbar) part of the body is laid down (LeDouarin, et al., 1996), the primitive streak and node regress posteriorly, leaving the notochord in their wake, and are replaced by a bulb of mesenchymal cells in the tail bud (Fig 9.2C).

In the tail-bud, cells continue to ingress from the surface, and many genes associated with gastrulation continue to be expressed (Knezevic, et al., 1998). The tail bud continues to produce somites that are added onto the posterior end of the segmental plate (Fig. 9.2D), beginning with the first lumbar vertebra (LeDouarin, et al., 1996). The embryonic distinction between Hensen’s node and the primitive groove on the one hand and the tail-bud on the other thus presumably corresponds with the two primary regions into which adult snake axial morphology is divided –pre-cloacal and post-cloacal, or body and tail. Experimental manipulations of embryos provide evidence for the idea that the tail bud retains competence to generate somites beyond the stage at which the

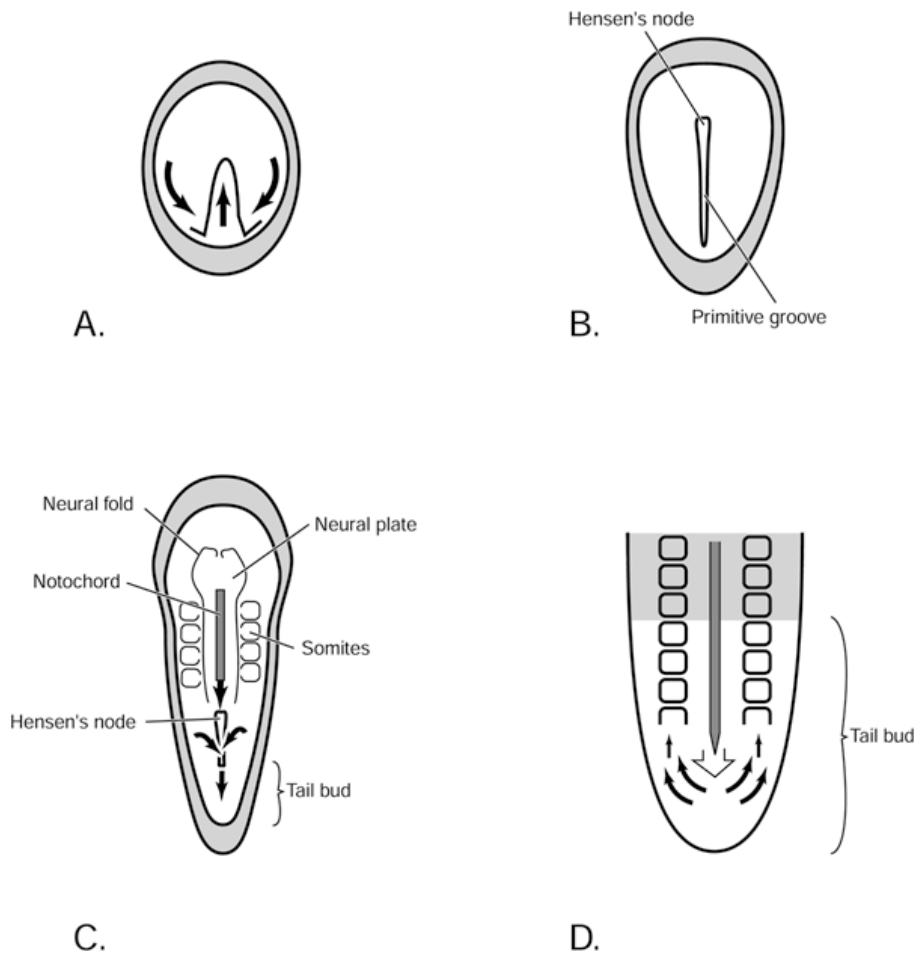


Figure 9.2. Early development and segmentation. **A:** early gastrulation. Primitive streak forms and extends anteriorly as cells migrate through it to form mesodermal tissue. **B:** Streak extends and deepens to become the primitive groove, with Hensen's node at the anterior end. **C:** further differentiation takes place as Hensen's node and the primitive groove regress posteriorly. Pre-cloacal body segments are formed in the paraxial mesoderm lateral to the full length of the groove. **D:** gastrulation-like processes continue in the tail-bud behind the posterior end of the primitive groove. Cells are added to the end of the growing bud, allowing the segmentation process to continue posteriorly. See text for details.

expected number of somites are laid-down. For example, transplanting the tail bud of a 13.5-day mouse embryo into an 8.5-day embryo shows that tail bud cells can "continue to participate in somitogenesis well beyond their expected developmental life span", although somitogenesis ceases when the appropriate number of host somites have formed (Tam and Tan, 1992, p 714). These results suggest that posterior segment number is not solely determined by

the competence of tail bud cells to form mesoderm, but rather by extrinsic cues acting on those cells.

The process of neurulation begins on the ectodermal surface as gastrulation and segmentation proceed posteriorly. Above the notochord and anterior to the regressing Hensen's node two lateral folds develop around the open neural plate (Fig. 9.2C). These arch medially over the plate to form an enclosed neural tube, which sinks into the embryo over the notochord. The mesodermal layer is now divided by the tube and notochord into two lateral halves, known as paraxial mesoderm. Following neurulation, the paraxial mesoderm is segmented beginning at the anterior end, as somites condense within the segmental plate. The timing of individual somitogenesis is foreshadowed by the *hairy1* and *lunatic fringe* genes, which are expressed in a wave-like pattern spreading anteriorly from the caudal end of the presegmental plate (Palmeirim, et al., 1998). As the wave reaches its anteriormost point, an expression pathway involving the *Delta-1* and *Notch-1* genes polarizes a package of mesoderm one segment in length into rostral and caudal halves. A new somite is thus added to the posterior end of the growing somitic chain (Palmeirim, et al., 1997). The process continues down the axis and into the tail-bud following the addition of new mesoderm and somites at the caudal end (LeDouarin, et al., 1996). Once formed, the somites differentiate into dermatome, which becomes the connective tissues of the skin, sclerotome, which condenses around the notochord and neural tube as vertebral elements and ribs, and myotome, which migrates ventrolaterally to become the axial and appendicular muscles.

The number of vertebrae does not change after early development, and at hatching pre-cloacal, cloacal, and post-cloacal vertebrae are clearly distinguishable. Subsequent differentiation within these regions can be quite complex (LaDuke, 1991b), but does not seem to be mediated by sharp Hox boundaries. This may not be surprising given the complexity and number of gene expression pathways contributing to vertebral development (Monsoro-Burq, et al., 1996). Unlike vertebrates with forelimbs, Hox gene expression is not regionalized in the pre-cloacal segments (Cohn and Tickle, 1999). In the chicken, for example, the expression boundaries of *Hoxb5*, *Hoxc8*, and *Hoxc6* correspond to the boundaries of the adult cervical, thoracic, and lumbar regions, while in snakes the expression of these genes is coextensive along the entire pre-cloacal region. The vertebrae are at first also uniform in size. In *Python molurus* at ten days post-hatching, the first vertebra is the largest in both length and width. Posteriorly all segments are approximately the same length, but their width tapers very gradually along the body, dropping sharply about halfway down the tail. Snakes grow not by adding additional segments, but by growth in segments. In amniotes, the number of vertebrae in an individual is constant once established during early embryogenesis, except in species capable of regenerating their tails. At hatching the vertebrae of snakes (and other limbless vertebrates) are subequal in size (see Wake, 1980 for data on caecilian axial skeleton growth). As the snake grows to maturity each of the segments increases in both length and width, although not all segments increase at the

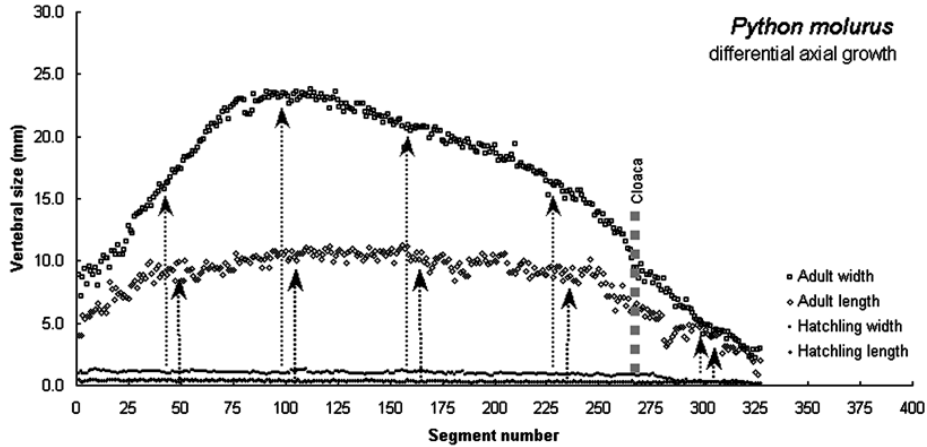


Figure 9.3. Differential growth in length and width of vertebrae in *Python molurus*. Horizontal axis shows vertebral position from head to tail; vertical axis shows linear dimension (length or width) in millimeters. Two individuals are shown, a ten day old hatchling (shown as the parallel lines made of solid shapes along the bottom of the graph) and an adult (shown as the two curved lines made up of open shapes). The length (diamonds) and width (squares) of each vertebrae of each snake are shown. The position of the cloacal vertebrae is shown by the broken gray line. The amount of post-hatching growth in each segment is indicated by the difference in adult and hatching sizes (indicated by the broken vertical arrows). Growth in tail vertebrae is less than in body vertebrae, and growth in width is greater than growth in length. The region of maximum growth is in the body, about 1/3 way back from the head. The hatchling snake had more vertebrae (349) than the adult (327) so some were removed to make the two data series equal length for the purposes of this figure.

same rate or to the same final size (Fig. 9.3). Both rate of growth of an individual snake and its maximum adult size are correlated with the total number of vertebrae (Lindell, 1996).

Because of the variation in both segment number and in regional proportions, snakes are ideal for studying the evolutionary interactions between developmental processes determining segment number and those determining regional segment identity. Snakes have greater than average among-species variation in vertebral number, including the proportion found in the pre- and post-cloacal regions (Lindell, 1994). They also have unusually high within-species variation, a phenomenon that is common in elongate vertebrates (Jockusch, 1997; Lindell, 1996). *Vipera berus*, for example, has vertebral counts ranging from 139 to 157 – about plus or minus about 6% of the total number – but extreme variation is apparently not common since the standard deviation is only about 3.0 (Lindell, 1996). Thus, among species variation in vertebral counts is much greater than within-species. This is an important point for our analysis since it implies that we are actually measuring evolutionary changes in vertebral number rather than sampling an artifact of within-species variation.

Related to this is the question of intraspecific variation in body and tail proportions, including sexual dimorphism. For example, male snakes are generally smaller than females, and often have longer tails (Shine, 1993; Lindell, 1996). The latter is possibly due to the fact that males store their hemipenes in the proximal part of the tail. Snake dimorphism may be more due to variation in growth rather than variation in vertebral number. Lindell (1996) found small but statistically significant differences in the vertebral counts of *Vipera berus*, the European Adder. On average, males had 144.9 vertebrae (SD : 2.21) and females 148.8 (SD : 3.14). Within- and between-sex variance in snout-vent length, however, was much greater, with males being on average 500.9 mm (SD : 75.5) and females 523.2 mm (SD : 105.7). Like axial regionalization, dimorphism seems to develop during maturation since it is rarely apparent at hatching (Shine, 1993). The time to maturation is usually longer in females, leading to an overall greater size. Since growth of mid-body vertebrae is greater than tail vertebrae, a longer period of growth should result in a proportionally shorter tail (Fig. 9.3). The snout-vent length of females should, therefore, increase more than it does in males during maturational growth. Thus dimorphism in growth pattern and overall size may also be factors explaining the between-sex differences in regional proportion (King, et al., 1999).

TESTING FOR DISSOCIATION

We hypothesize that – as regards number of vertebrae – the body and tail of snake are modules that can be dissociated during evolution (Fig. 9.4). The modularity of the two regions is suggested by the differences in the formation of their mesoderm and by the ecomorphological differences among snake species show in Figure 9.1. However, modularity does not necessarily imply dis-sociability. Because segmentation and somitogenesis are continuous across the body/tail bud boundary, differences in regional proportion may be an allo-

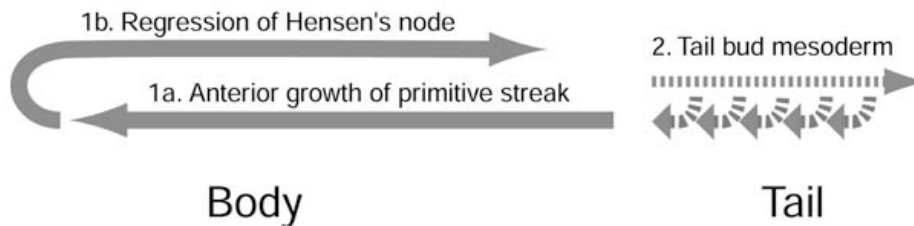


Figure 9.4. Two modules in snake axial development. The pre-cloacal vertebrae are derived from somites that formed in paraxial mesoderm along the margins of the primitive streak. Post-cloacal vertebrae are derived from somites laid down in segmented mesoderm that is added to the end of a growing tail-bud. The adult cloacal region is thus located at the embryonic junction between tail-bud and posterior-most streak. See text for details.

metric function of the total number of segments. In other words, when the total number of segments is changed, vertebrae may be added or removed from the two regions in some constant proportion. Such a situation could explain the difference between the fossorial and arboreal snakes in Figure 9.1 without invoking dissociation. The fossorial snake has few total segments and a very short tail. If evolutionary changes in the two regions are correlated and changes in the tail are proportionally greater than in the body, then one would expect a snake with lots of vertebrae to have a proportionally longer tail. Thus, inability to dissociate the two regions might imply that the long *tail* of arboreal snakes is not related to its habitat so much as a long body is; however, dissociability would allow body and tail to evolve independently, permitting a wider variety of ecomorphologies. The fact that some taxa (like the one in Fig. 9.1B) have lots of vertebrae but relatively few in the tail hints at such a possibility.

We test this hypothesis by looking at evolutionary changes in the number of body vertebrae relative to the number in the tail. We ask (A) whether simple change in the number of segments is responsible for the differences among snakes both in segment number and relative length of body and tail regions or (B) whether there is evidence for independence in the number of segments and the relative proportion of body and tail. We first investigate the relationship between number of body vertebrae, the number of tail vertebrae, and the total number of vertebrae to determine the extent to which they are correlated. This is used to determine whether simple allometry explains the diversity of body-to-tail proportion among snakes. We also look at changes in vertebral number along the branches of snake phylogeny. In particular, we look for correlation between change in the number of body vertebrae and change in the number of tail vertebrae. The relationship should be constant if there is no dissociation. We identify exceptions as cases of dissociation.

In this study dissociation is therefore a departure from the usual relationship between change in the number of body vertebrae and in the number of tail vertebrae. Dissociation could manifest itself in three ways: (1) segments are added or removed to the tail without affecting the number in the body region, (2) segments are added or removed in the body region without affecting the tail, or (3) segments are added or removed from one region and the opposite done in the other. We do not invoke dissociation when segments are added or deleted from both regions, even if in different proportions. After identifying dissociations, we return to the question of whether they represent heterochronic change in **CONCLUSIONS**.

MATERIALS AND METHODS

We examined the skeletons of 32 species of snakes, recording the number of pre-cloacal (body) and post-cloacal (tail) vertebrae in each (Table 9.1). Data were collected from articulated skeletons. Not all specimens were fully mature

individuals, but all specimens were at least 28 % of reported adult body lengths. Post-hatchling vertebral counts reflect the number of segments produced during early embryonic development. Neither the number of vertebrae nor their regional identities change after being established in the embryo. We also examined embryos of *Python molurus* and *Thamnophis ordinoides*. The species considered in this study represent all of the higher orders of living snakes, as well as the range of their ecological diversity.

The ratio of tail-to-body vertebrae (TtB) and the percentage of body vertebrae relative to the total were calculated from the raw data. These are reported as “odds ratios” of the number of tail to body vertebrae and as pie graphs showing the percentage of body (white circles) and tail (black wedges). Odds ratios are useful for clearly representing differences between the number of segments in the body and tail among snakes. A ratio of 0.5:1 indicates both that there is one half tail vertebra per body vertebra and that the tail is half as many segments long as is the body. The ratios provide a more intuitive summary of differences in the number of vertebrae in each region than do equivalent percentages of the total number of segments (Sokal and Rohlf, 1995).

The phylogeny we employ in this paper is derived from analyses by Ashe and Marx (1987), Cadle (1988), Cundall, et al. (1993), Dowling, et al. (1993), Keogh (1998), and Kluge (1991, 1993). Morphological and molecular data are generally congruent in their expression of snake relationships (Kluge, 1989; Cundall, et al., 1993; Heise, et al., 1995): Serpentes consists of a sister-taxon relationship between Scolecophida (blind snakes) and Alethinophidia (all other snakes), with Alethinophidia composed of a gradation of aniliid (pipe snakes), xenopeltid (sunbeam snakes), “henophidian” (boas, pythons), and acrochordid (file snakes) taxa, culminating in Colubroidea, which is composed of Viperidae (vipers, rattlesnakes), Colubridae (racers, grass snakes), Elapidae (cobras, kraits, sea snakes), and Actraspidae (stiletto snakes). Differences in phylogenetic hypotheses include monophyly versus paraphyly of Anilioidea and Boidae (Cundall, et al., 1993; Rieppel, 1988), as well as the relative relationships of various “henophidian” taxa with respect to Colubroidea (Cundall, et al., 1993; Heise, et al., 1995). Additionally, interrelationships of many colubrid taxa are poorly known.

Because of uncertainty about certain aspects of snake phylogeny, we considered two alternate trees (Fig. 9.5). The first makes a minimum of assumptions about those colubrines whose relationships have not been studied or are controversial and it supports paraphyly of *Cylindrophis* and *Anilius* (Cundall, et al., 1993). The second tree assumes both relationships among Colubrinae based on continental-scale geographic provenance and the monophyly of Anilioidea (*Cylindrophis*+*Anilius*, Rieppel, 1988; Kluge, 1991). Divergence times for key clades were determined using oldest known occurrences (*e.g.*, Rage, 1984; Rage & Richter, 1994; Gardner & Cifelli, 1998). Dates for other clades were extrapolated linearly from those. For example, if the parent clade originated

Table 9.1: Pre- and Post-cloacal proportions in selected snake species

Species	Pre-cloacal	Post-cloacal	Total	Tail: Body and Percent Tail	Estimated SVL _c , cm	Typical Adult SVL _c , cm
<i>Acrochordus javanicus</i>	204	67	271	0.3:1 f	98	130
<i>Agkistrodon piscivorus</i>	138	41	179	0.3:1 f	78	120
<i>Ahaetulla nasuta</i>	181	134	315	0.7:1 j	50	160
<i>Anilius scytale</i>	217	22	239	0.1:1 c	70	70
<i>Arizona elegans</i>	225	57	282	0.3:1 e	99	90
<i>Bitis arietans</i>	143	27	170	0.2:1 d	98	150
<i>Crotalus molossus</i>	177	28	205	0.2:1 d	99	105
<i>Cylindrophis rufus</i>	203	20	223	0.1:1 c	44	
<i>Dendroaspis viridis</i>	226	122	348	0.5:1 h	181	220
<i>Epicrates chenchria</i>	265	64	329	0.2:1 e	163	200
<i>Erythrolamprus aesculapii</i>	198	46	244	0.2:1 e	98	100
<i>Eunectes murinus</i>	252	59	311	0.2:1 e	199	900
<i>Hydrophis fasciatus</i>	228	27	255	0.1:1 c	92	90
<i>Lachesis muta</i>	232	45	277	0.2:1 d	273	225
<i>Laticaudata laticaudata</i>	245	37	282	0.2:1 d	89	110
<i>Masticophis flagellum</i>	237	53	290	0.2:1 e	62	225
<i>Micrurus fulvius</i>	213	49	262	0.2:1 e	78	75
<i>Morelia spilota</i>	286	75	361	0.3:1 e	186	245
<i>Naja nigricollis</i>	203	63	266	0.3:1 f	144	220
<i>Oxybelis fulgidus</i>	206	142	348	0.7:1 i	140	130
<i>Pareas carinatus</i>	173	64	237	0.4:1 f	48	90
<i>Python molurus</i>	259	68	327	0.3:1 e	281	400
<i>Python regius</i>	215	33	248	0.2:1 d	116	155
<i>Storeria dekayi</i>	114	35	149	0.3:1 f	28	35
<i>Thamnophis ordinoides</i>	142	60	202	0.4:1 g	43	95
<i>Typhlops jamanicus</i>	200	15	215	0.1:1 c	22	25

<i>Daboia russelli</i>	175	48	223	0.3:1 e	91	135
<i>Xenodon severus</i>	143	36	179	0.3:1 e	155	130
<i>Xenopeltis unicolor</i>	188	30	218	0.2:1 d	64	90

The number of pre-cloacal vertebrae, post-cloacal vertebrae, and the total number of vertebrae in the axial skeleton are reported for each species considered in this study. The proportion of post-cloacal to pre-cloacal vertebrae is reported both as an odds ratio and as a pie graph showing the percentage of post-cloacal vertebrae relative to the total. An estimated snout-vent length for the specimen used to represent each species is reported with an adult average for that species.

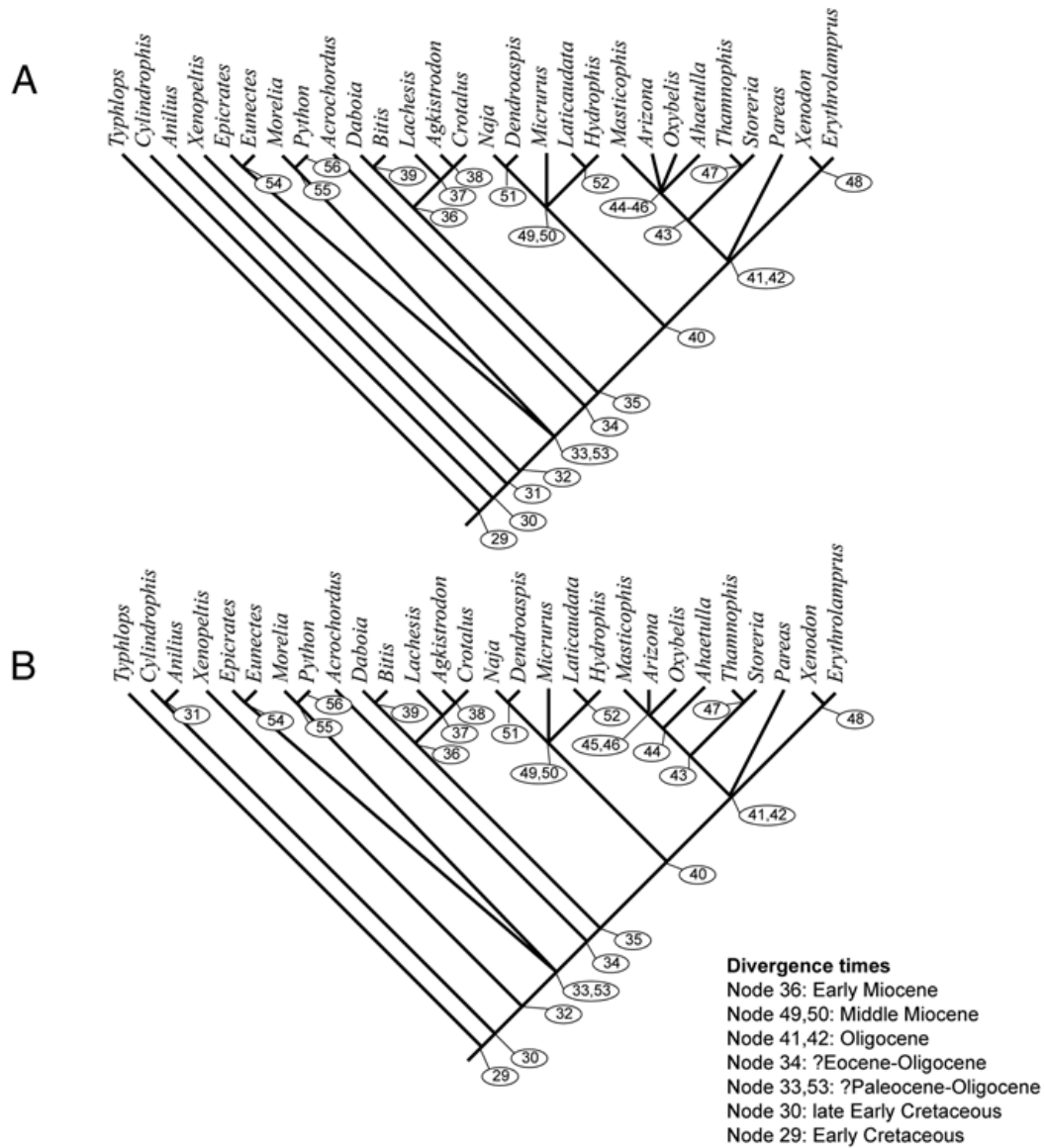


Figure 9.5. Cladograms of the snakes considered in this study. Because of uncertainty about the relationship of some of the snakes considered in this study, we considered two trees (A and B). The nodes are numbered as discussed in the text. Divergence times for seven nodes were estimated from paleontologic data. See text for details.

15 million years ago and has two nested sub-clades the latter were estimated to have originated 10 and 5 million years ago respectively. These dates were used to estimate branch lengths for the tree in millions of years (Table III). The phylogeny-based analyses below require fully dichotomous trees; polytomies were accommodated by assigning very small branch lengths (0.0001 in this case) to branches connecting nodes forming the polytomy as suggested by Martins (1995). For repeatability, the two trees are presented here with branch lengths in New Hampshire format:

Tree A

(Typhlops:140.0,((Anilius:80.0,(Xenopeltis:65.0,((Acrochordus:34.0,(((Lachesis:12.0,(Agkistrodon:7.0,Crotalus:7.0):5.0):8.0,(Bitis:10.0,Daboia:10.0):10.0):13.0,((Pareas:30.0,(((Oxybelis:5.0,(Ahaetulla:5.0,(Arizona:5.0,Masticophis:5.0):0.001):0.001):15.0,(Storeria:10.0,Thamnophis:10.0):10.0):10.0,(Erythrolamprus:15.0,Xenodon:15.0):15.0):0.001):1.0,(Micrurus_:15.0,((Dendroaspis:7.0,Naja:7.0):7.99,(Hydrophis:7.0,Laticaudata:7.0):7.99):0.01):16.0):2.0):1.0):6.0,((Epicrates_:15.0,Eunectes:15.0):25.0,(Morelia:15.0,(Python_m:10.0,Python_r:10.0):5.0):25.0):0.01):25.0):15.0):20.0,Cylindrophis:100.0):40.0);

Tree B

(Typhlops:140.0,((Anilius:60.0,Cylindrophis:60.0):40.0,(Xenopeltis:65.0,(Acrochordus:34.0,(((Lachesis:10.0,(Agkistrodon:5.0,Crotalus:5.0):5.0):10.0,(Bitis:5.0,Daboia:5.0):15.0):13.0,((Pareas:30.0,(((Oxybelis:5.0,(Ahaetulla:5.0,(Arizona:5.0,Masticophis:5.0):0.001):0.001):15.0,(Storeria:10.0,Thamnophis:10.0):10.0):10.0,(Erythrolamprus:15.0,Xenodon:15.0):15.0):0.0010):2.0,(Micrurus_:15.0,((Dendroaspis:7.0,Naja:7.0):7.99,(Hydrophis:7.0,Laticaudata:7.0):7.99):0.01):17.0):1.0):1.0):6.0,((Epicrates_:15.0,Eunectes:15.0):25.0,(Morelia:15.0,(Python_m:5.0,Python_r:5.0):10.0):25.0):0.001):25.0):35.0):40.0);

Correlations among the number of body vertebrae, the number of tail vertebrae, and the total number of vertebrae were assessed using model I regression. This is the appropriate regression because we wished to assess the degree to which each y variable (either pre- or postcloacal number) is predictable given a particular value of the x (either total number or precloacal number). Because our purpose was to determine whether evolutionary change in the number of axial segments results in change exclusively in the tail, either in the body or tail, or in both body and tail, we also looked at the correlation of changes in these traits along branches of the tree. To do this we optimized the three traits—the number of body, tail, and total vertebrae—on both trees using Martins and Hansen's (1997) generalized linear model. Node values and their standard errors (or variances) are estimated by the method and are reported in Table 9.2. The node values are the most probable given the topology of the tree, the data at the tips, and certain assumptions about evolutionary change in the traits. Those assumptions are that gain and loss of segments are equally probable and that the direction of change at one point in time is dependent neither on preceding nor subsequent changes (*i.e.*, a Brownian motion model of evolution). As performed here, this optimization is the same as a squared-change parsimony optimization (Maddison, 1991).

Changes along each branch were calculated from the optimized node values by subtracting the end value from the beginning value. These were standardized by dividing through by the length of each branch in millions of years, resulting in an estimate of change in each trait per million years per branch. Both standardized changes and branch lengths are reported in Table 9.3. Two sorts

Table 9.2. Tree node reconstructions and their standard errors.

	Total Vertebrae				Preloacal Vertebrae				Postloacal Vertebrae			
	Tree A	Tree B	Mean:	Var	Tree A	Tree B	Mean :	Var :	TreeA	Tree B	Mean :	Var
	22.1	21.8			22.7	22.6			26.9	27.1		
RSS	191.4	189.8			117.9	121.2			41.8	46.1		
sigma	250	254	252	9	187	191	189	7	63	64	63	0
Node 29	95	94	95	0	75	76	75	1	44	47	45	3
SE	244	249	247	16	182	187	184	12	62	63	62	0
Node 30	58	58	58	0	45	46	46	0	27	28	28	1
SE	254	248	251	16	188	171	180	146	65	77	71	65
Node 31	56	62	59	17	44	50	47	16	26	31	28	10
SE	250	246	248	6	194	197	196	4	55	49	52	18
Node 32	53	55	54	2	41	44	43	3	25	27	26	3
SE	247	247	247	0	196	197	196	0	51	50	51	0
Node 33	34	34	34	0	26	27	27	0	16	17	16	0
SE	261	261	261	0	206	207	206	0	55	54	55	0
Node 34	29	28	28	0	22	23	23	0	13	14	14	0
SE	263	263	263	0	208	208	208	0	56	55	55	0
Node 35	27	27	27	0	22	22	22	0	13	13	13	0
SE	262	261	262	1	206	205	206	0	56	55	56	0
Node 36	31	32	32	1	24	26	25	1	14	16	15	1
SE	221	215	218	21	184	180	182	7	37	34	36	4
Node 37	27	26	26	1	21	21	21	0	13	13	13	0
SE	217	214	216	4	188	187	187	0	29	27	28	2
Node 38	23	20	21	5	18	16	17	2	11	10	10	0
SE	313	327	320	102	232	240	236	27	81	88	84	24
Node 39	27	21	24	22	21	17	19	12	13	10	11	3
SE	267	265	266	2	210	209	210	1	57	56	56	1
Node 40	27	27	27	0	21	21	21	0	12	13	13	0
SE	269	268	268	0	212	213	213	0	57	55	56	1
Node 41	27	27	27	0	21	22	21	0	13	13	13	0
SE												

Node 42	269	268	268	0	212	213	213	0	57	55	56	1
SE	27	27	27	0	21	22	21	0	13	13	13	0
Node 43	274	273	273	1	223	224	223	0	51	49	50	2
SE	31	30	31	0	25	24	24	0	15	15	15	0
Node 44	281	275	278	16	238	233	236	12	43	42	42	0
SE	15	25	20	48	12	20	16	32	7	12	10	14
Node 45	281	286	283	15	238	241	239	3	43	46	44	5
SE	15	17	16	1	12	13	13	1	7	8	8	1
Node 46	281	286	283	15	238	241	239	3	43	46	44	5
SE	15	17	16	1	12	13	13	1	7	8	8	1
Node 47	275	275	275	0	224	225	224	0	51	50	51	0
SE	27	27	27	0	21	22	22	0	13	13	13	0
Node 48	299	298	298	0	234	234	234	0	65	64	65	0
SE	32	32	32	0	25	26	25	0	15	16	15	0
Node 49	269	269	269	0	198	197	198	0	72	72	72	0
SE	26	26	26	0	20	21	20	0	12	13	12	0
Node 50	269	269	269	0	198	197	198	0	72	72	72	0
SE	26	26	26	0	20	21	20	0	12	13	12	0
Node 51	278	278	278	0	210	210	210	0	68	68	68	0
SE	23	23	23	0	18	18	18	0	11	11	11	0
Node 52	220	220	220	0	175	175	175	0	45	45	45	0
SE	23	23	23	0	18	18	18	0	11	11	11	0
Node 53	247	247	247	0	196	197	196	0	51	50	51	0
SE	34	34	34	0	26	27	27	0	16	17	16	0
Node 54	217	217	217	0	177	177	177	0	41	40	41	0
SE	34	34	34	0	27	27	27	0	16	17	16	0
Node 55	213	218	215	9	173	176	174	3	40	42	41	2
SE	29	33	31	7	23	26	24	6	13	16	15	4
Node 56	203	202	203	1	168	168	168	0	35	35	35	0
SE	20	21	20	0	16	16	16	0	9	10	10	0

The estimated total number of vertebrae, pre-cloacal vertebrae, and post-cloacal vertebrae are reported for each node of the phylogenetic trees in Figure 9.5. Estimates were made using the topologies of both Tree A and Tree B. Martins and Hansen's (1997) Generalized Linear Model was used to estimate the values from the species data in Table I and the phylogenetic branching pattern. Standard errors (SE) for each estimate are also reported. The mean and variance of the Tree A and Tree B node estimates is reported as an index of discrepancy between the two optimizations.

Table 9.3 Evolution of vertebral numbers.

	<u>Branch Length, MY</u>		<u>Δ Total Vertebrae</u>		<u>Δ Preloacal</u>		<u>Δ Postloacal</u>	
	TreeA	TreeB	TreeA	TreeB	TreeA	TreeB	TreeA	TreeB
Node29-->Node30	40	40	-0.15	-0.13	-0.12	-0.1	-0.03	-0.02
Node29-->Typhlops	140	140	-0.25	-0.28	0.09	0.07	-0.34	-0.35
Node30-->Cylindrophis	100	N/A	-0.21	N/A	-0.41	N/A	-0.42	N/A
Node30-->Node31	20	100	0.5	-0.01	0.5	-0.01	0.18	0.14
Node30-->Node32	N/A	10	N/A	-0.3	N/A	-0.31	N/A	-1.34
Node31-->Anilius	80	40	-0.19	-0.23	0.36	1.14	-0.54	-1.37
Node31-->Cylindrophis	N/A	40	N/A	-0.6	N/A	-1.12	N/A	-1.42
Node31-->Node32	15	N/A	-0.27	N/A	0.41	N/A	-0.68	N/A
Node32-->Node33,53	25	25	-0.12	0.04	0.06	-0.01	-0.17	0.04
Node32-->Xenopeltis	65	65	-0.49	-0.48	-0.1	-0.14	-0.39	-0.29
Node33,53-->Node34	6	6	2.33	2.33	1.74	1.63	0.68	0.74
Node33,53-->Node54	25	25	-1.2	-1.2	-0.77	-0.8	-0.77	-0.8
Node33,53-->Node55	25	25	-1.36	-1.16	-0.91	-0.85	-0.43	-0.32
Node34-->Acrochordus	33	33	0.3	0.3	-0.07	-0.08	0.36	0.38
Node34-->Node35	1	1	2	2	1.19	1	0.62	0.67
Node35-->Node36	13	13	-0.08	-0.15	-0.15	-0.23	0.05	0.03
Node35-->Node40	1	1	4	2	2.6	1.28	1.15	0.64
Node36-->Node37	8	8	-5.14	-5.75	-2.74	-3.1	-2.4	-2.64
Node36-->Node39	10	10	5.07	6.66	2.63	3.44	2.44	3.21
Node37-->Lachesis	12	12	4.64	5.18	3.99	4.29	0.65	0.89
Node37-->Node38	5	5	-0.86	-0.11	0.69	1.27	-1.55	-1.38
Node38-->Agkistrodon	7	7	-5.43	-5.04	-7.08	-6.98	1.65	1.94
Node38-->Croatalus	7	7	-1.72	-1.33	-1.51	-1.4	-0.2	0.08
Node39-->Bitis	10	10	-14.31	-15.74	-8.94	-9.68	-5.38	-6.06
Node39-->Daboia	10	10	-9.01	-10.44	-5.74	-6.48	-3.28	-3.96
Node40-->Node41,42	2	2	1	1.5	1.03	1.96	-0.17	-0.29
Node40-->Node49,50	16	16	0.13	0.25	-0.77	-0.72	0.92	0.99
Node41,42-->Node43	10	10	0.5	0.5	1.1	1.06	-0.55	-0.6
Node41,42-->Node48	15	15	1.99	2.02	1.44	1.4	0.55	0.61

Node41,42-->Pareas	30	-1.06	-1.04	-1.31	-1.33	0.25	0.29
Node43-->Node44	N/A	N/A	0.2	N/A	0.96	N/A	-0.73
Node43-->Node44-46	15	0.47	N/A	0.99	N/A	-0.55	N/A
Node43-->Node47	10	0.1	0.2	0.12	0.1	0	0.12
Node44-->Ahaetulla	N/A	N/A	3.99	N/A	-5.22	N/A	9.13
Node44-->Node45,46	N/A	N/A	2.24	N/A	1.48	N/A	0.76
Node44-46-->Ahaetulla	5	6.86	N/A	-11.4	N/A	18.26	N/A
Node44-46-->Arizona	5	0.26	N/A	-2.6	N/A	2.86	N/A
Node44-46-->Masticophis	5	1.86	N/A	-0.2	N/A	2.06	N/A
Node44-46-->Oxybelis	5	13.46	N/A	-6.4	N/A	19.86	N/A
Node45,46-->Arizona	N/A	N/A	-0.85	N/A	-3.11	N/A	2.25
Node45,46-->Masticophis	5	N/A	0.75	N/A	-0.71	N/A	1.45
Node45,46-->Oxybelis	N/A	N/A	12.35	N/A	-6.91	N/A	19.26
Node47-->Storeria	10	-12.64	-12.59	-16.14	-11.05	-1.6	-1.54
Node47-->Thamnophis	10	-7.34	-7.29	-8.24	-8.25	0.9	0.96
Node48-->Erythrolampris	15	-3.64	-3.63	-2.38	-2.4	-1.26	-1.23
Node48-->Xenodon	15	-7.97	-7.96	-6.05	-6.07	-1.92	-1.89
Node49,50-->Micrurus	15	-0.5	-0.47	1.01	1.04	-1.51	-1.51
Node49,50-->Node51	7	1.29	1.29	1.81	1.84	-0.56	-0.56
Node49,50-->Node52	7	-7.05	-7	-3.31	-3.27	-3.74	-3.73
Node51-->Dendroaspis	7	9.98	10	2.22	2.24	7.76	7.76
Node51-->Naja	7	-1.74	-1.71	-1.07	-1	-0.67	-0.71
Node52-->Hydrophis	7	4.98	5	7.62	7.57	-2.64	-2.64
Node52-->Laticaudata	7	8.86	8.86	10.05	10	-1.21	-1.14
Node54-->Epicrates	15	7.44	7.44	5.88	5.87	1.56	1.57
Node54-->Eunectes	15	6.24	6.27	5.02	5	1.23	1.24
Node55-->Morelia	15	9.84	9.56	7.52	7.36	2.32	2.2
Node55-->Node56	5	-2	-3.2	-1.03	-1.62	-5.34	-1.44
Node56-->Pythonmolurus	10	12.35	12.47	9.09	9.15	3.26	3.32
Node56-->Pythonregius	10	4.45	4.57	4.69	4.7	-0.24	-0.2

The changes in pre-cloacal, post-cloacal, and total vertebral numbers are reported as a rate. The evolutionary change in vertebral number was inferred for each branch using the species data in Table 9.1 and the node estimates in Table 9.2. Because branch-length varies considerable across each tree, the change along each branch was standardized by dividing it by its branch length to yield an index of change in number per million

of “odds ratios” summarizing change in regional proportions were also calculated, the difference in body-to-tail at the end of each branch compared to the same ratio at the beginning (ΔTtB) and the ratio of change-in-body to change-in-tail ($\Delta\text{T}:\Delta\text{B}$). The first of these, ΔTtB , was calculated for each branch by first calculating the TtB odds ratio for the node at the beginning and end of each branch as described above. The end value was then subtracted from the beginning and divided through by the length of the branch in millions of years. The ΔTtB ratio thus represents change in the number of tail vertebrae per body vertebra per million years per branch. The second ratio, $\Delta\text{T}:\Delta\text{B}$, is the ratio of change in the number of tail vertebrae per million years per branch to each body vertebra per million years per branch. This was calculated by dividing the change in tail vertebrae per million years per branch (Table III) into the change in body vertebrae per million years per branch. Because the two ratios are easily derived from the data in Tables 2 and 3, they number are not separately reported. The two ratios were compared to changes in total number of vertebrae to assess whether the addition and deletion of axial segments is normally associated with change in the body region, in the tail region, or is equally distributed between the regions.

RESULTS AND DISCUSSION

The number of vertebrae in snakes was variable among species, both in total and region by region (Table I). The total number ranged from 149 in *Storeria* to 361 in *Morelia*, a twofold difference. The mean was 257, the standard deviation (*SD*) was 56.8, and the coefficient of variation (*CV*) was 22.1. Among-species variation in the tail was found to be greater than in the body. The number of body vertebrae ranged from 114 in *Storeria* to 286 in *Morelia*. The mean was 203, *SD* was 41.5 and *CV* was 20.4. The number in the tail ranged from 15 in *Typhlops* to 142 in *Oxybelis* with a mean of 54, *SD* of 31.6, and *CV* of 58.6. The large coefficient of variation in the number of tail vertebrae indicates that most changes in total vertebral number are probably concentrated in that region. Also, the fact that the species with the longest and shortest tails are not the species with the longest and shortest bodies indicates that body and tail change independently. If the two were universally correlated then the same species would be at the extreme ends of the ranges of both regions. The variation in regional numbers confirms that the number of segments can be modified, both in individual regions and *in toto*. While this suggests that dissociation between body and tail may occur, it does not rule out an allometric relationship between evolutionary changes in the two regions.

There is considerable variation in the ratio of tail to body (TtB) among snakes (Table 9.1). The smallest TtB ratio is 0.08:1 in fossorial *Typhlops*. In other words, *Typhlops* has the smallest number of tail vertebrae in proportion to its body of any of the snakes we examined. The largest TtB is 0.74:1 in the arboreal snake *Ahaetulla*, giving it the largest number of tail vertebrae in pro-

portion to its body of any snake examined. *Ahaetulla* thus has more than nine times as many tail vertebrae per body segment than does *Typhlops*. The mean TtB was 0.27:1, its SD was 0.16:1, and its CV was 58.3. This is again suggestive of dissociation, but does not rule out the possibility that the proportion of tail to body is allometric.

Both the numbers of body and tail vertebrae are positively correlated with the total number in the skeleton (Fig. 9.6 A and B). This is expected because the total is, by definition, the sum of body and tail. As one or both of the components increases, so does the total. This autocorrelation means that the slopes of the two regressions in Fig. 9.6 A and B sum to 1.0 because, together, the two regions account for the total number of vertebrae. This means that the slopes do not tell us anything of interest about the evolution of regional proportions. The R^2 values do, however. The total number of vertebrae better predicts the number of body vertebrae than it does the number in the tail. Total number explains 70% of the variance in the body, but only 49% of variance in the tail. This suggests that, regardless of how many segments long a snake is, the number of vertebrae in the tail is more variable than the number in the body. There is not a constant relationship between total number of vertebrae and the proportion of body to tail, meaning that the proportion between regions can be changed without changing the total number of vertebrae. This could not be accomplished without some dissociation. The lack of correlation between the numbers of body and tail vertebrae substantiates this (Fig. 9.6C).

The relationship between proportion of body relative to tail is easier to see in a regression of the tail-to-body odds ratio (TtB) on the total number of vertebrae (Fig. 9.7). There is a positive and significant ($P=0.036$) correlation between the ratio of tail to body segments and the total number of vertebrae, but the latter only explains 15% of the variance of the former. The positive correlation between TtB and total vertebrae number suggests that on average body and tail evolution are not dissociated; as segment number increases so does the number of tail vertebrae relative to the number in the body. But the low predictive value of the regression means that quite a lot of change in regional proportions is independent of changes in number of axial segments.

Evolution in the number of tail vertebrae relative to both body vertebrae and the total number of segments can be more fruitfully examined more by looking directly at phylogenetic changes in these three variables (Table 9.3). Looking at the branch-by-branch correlation between change in the total number of vertebrae per million years (ΔTot) and change in body number per million years (ΔB) we find a very tight linear relationship in which ΔTot explains 46% of the variance in ΔB as optimized on Tree A and 59% when optimized on Tree B (Fig. 9.8, A and B). Conversely, ΔTot is positively correlated with change in tail number (ΔT) but only accounts for 36% of the variance on Tree A and 39% on Tree B (Fig. 9.8, C and D). This means that adding vertebrae to the body almost always increases the total number of vertebrae, but adding them to the tail may either increase the total or happen while the total decreases (*i.e.*, vertebrae are deleted from the body and added to the tail). This implies that long-

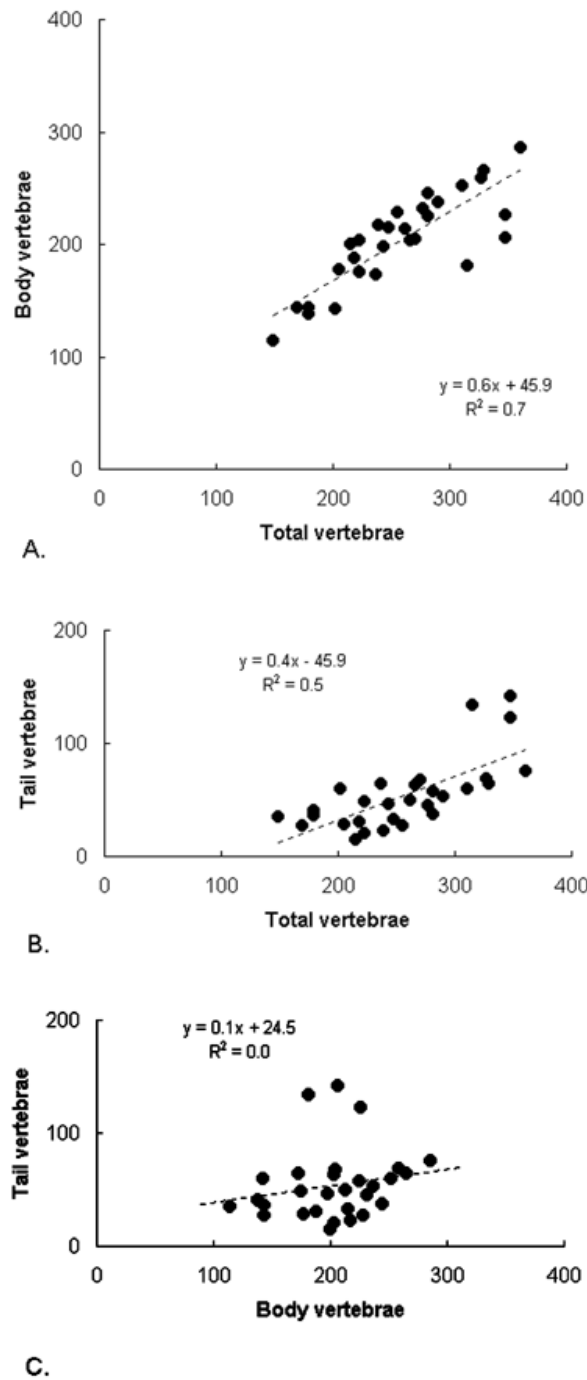


Figure 9.6. Correlation between (A) the total number of vertebrae in the axial skeleton and the number of pre-cloacal (body) vertebrae; (B) the total number and the number of post-cloacal (tail) vertebrae; and (C) the number of pre- and post-cloacal vertebrae in twenty-nine snake species. A positive relationship between the total number of vertebrae and both pre-cloacal and post-cloacal number is always expected because of autocorrelation. The wide spread of outliers along the y-axis in C indicates that some snake species have substantially different regional proportions than others given the same total number of vertebrae.

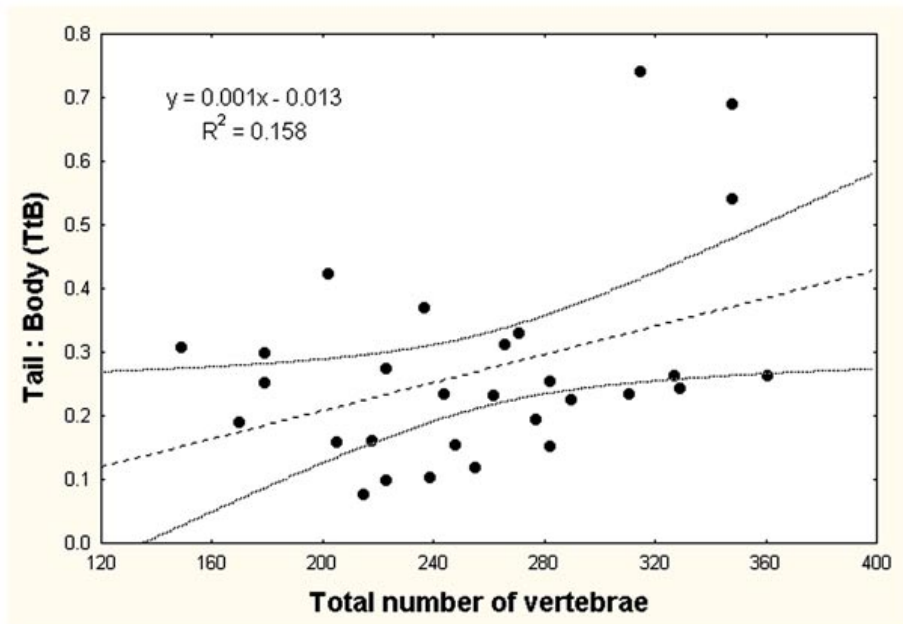
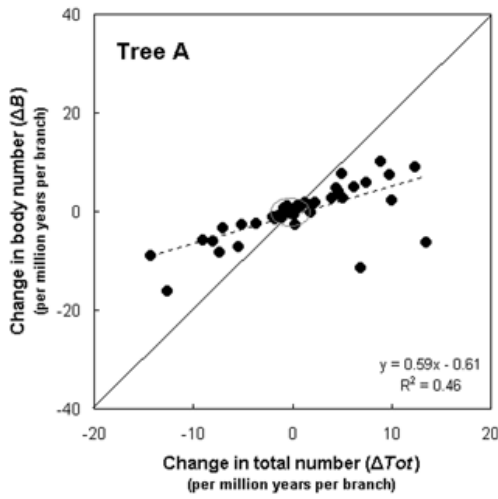


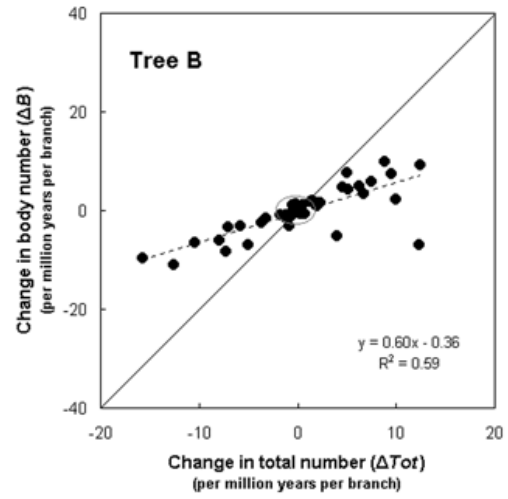
Figure 9.7. Correlation between tail-to-body ratio and total number of vertebrae. The y-axis is the “tail” part of the ratio as the “body” part always equals one (*i.e.*, Y:1). Linear regression line and 95% prediction intervals for the mean and individuals are indicated. There is a positive correlation between the ratio of tail-to-body and the total number of vertebrae, $R^2=0.15$, $P=0.036$.

bodied snakes evolve by increasing the total number of vertebrae, but that long-tailed snakes have evolved both by adding to the total number of vertebrae or adding to the tail at the expense of the body. A comparison of the change in tail number (ΔT) relative to body number (ΔB) makes this clearer (Fig. 9.8, E and F). The scatter of points above the regression line and to the left of the line of isometry indicates that when large numbers of tail vertebrae are added, the number of body vertebrae often simultaneously decreases. But when body vertebrae are added (points to the right of the line of isometry) the number of tail vertebrae does not usually change. These data strongly suggest that dissociation between body and tail is possible. Note, however, that it is quite common for their to be no change in either the number of tail or the number of body vertebrae as indicated by the large number of data points clustered at 0,0 (Fig. 9.8, A-F).

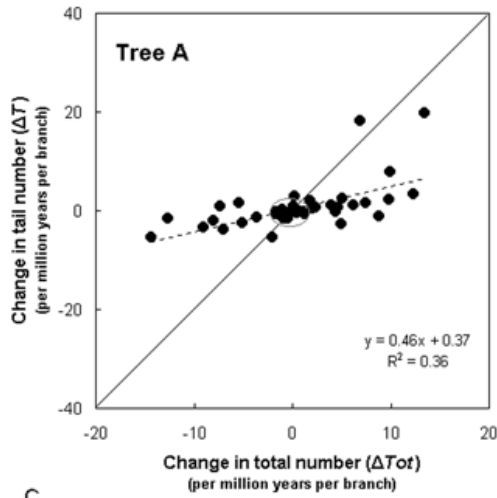
The strongest evidence for occasional dissociation between body and tail in the evolution of snakes is shown in Figure 9.9, which shows the relationship between ΔTtB and ΔT . The points in the upper left and lower right quadrant represent branches on which the total number of vertebrae has either increased or decreased, but the tail has done the opposite. When the odds ratio on the vertical axis is positive, the number of tail relative to the number of body vertebrae has increased. The most common mode of change – indicated



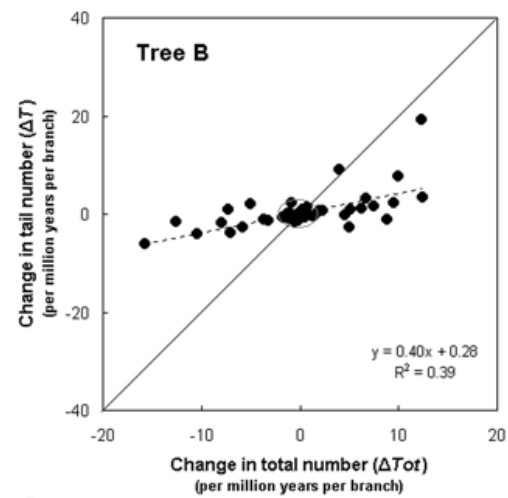
A.



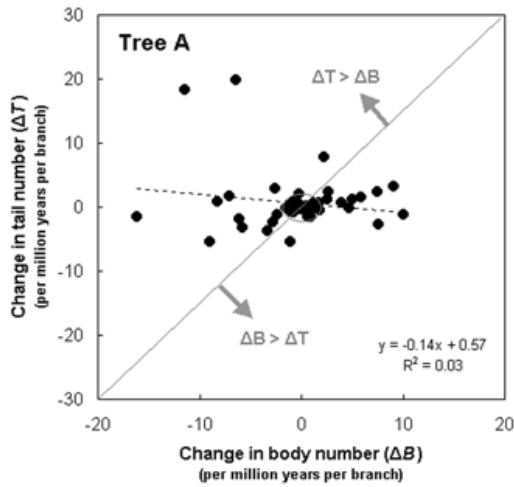
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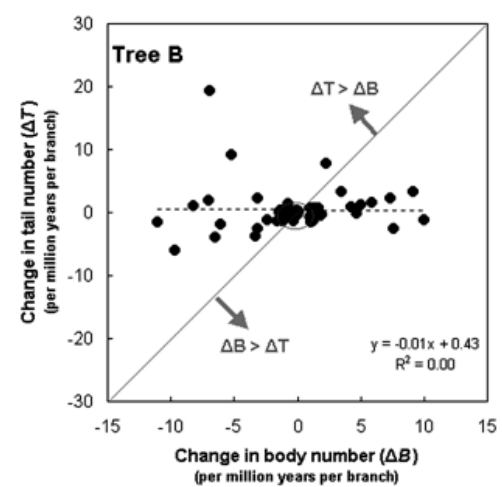
C.



D.



E.



F.

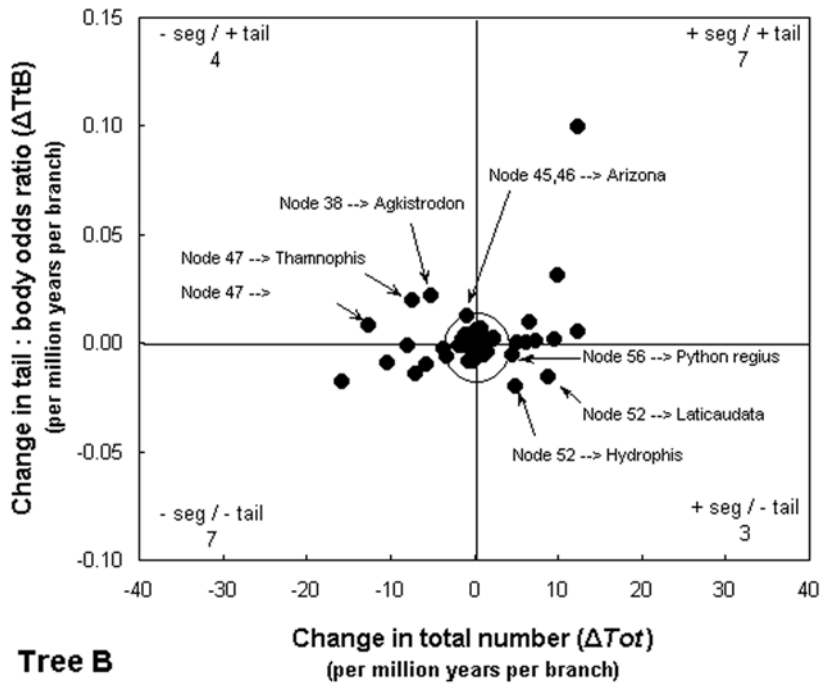
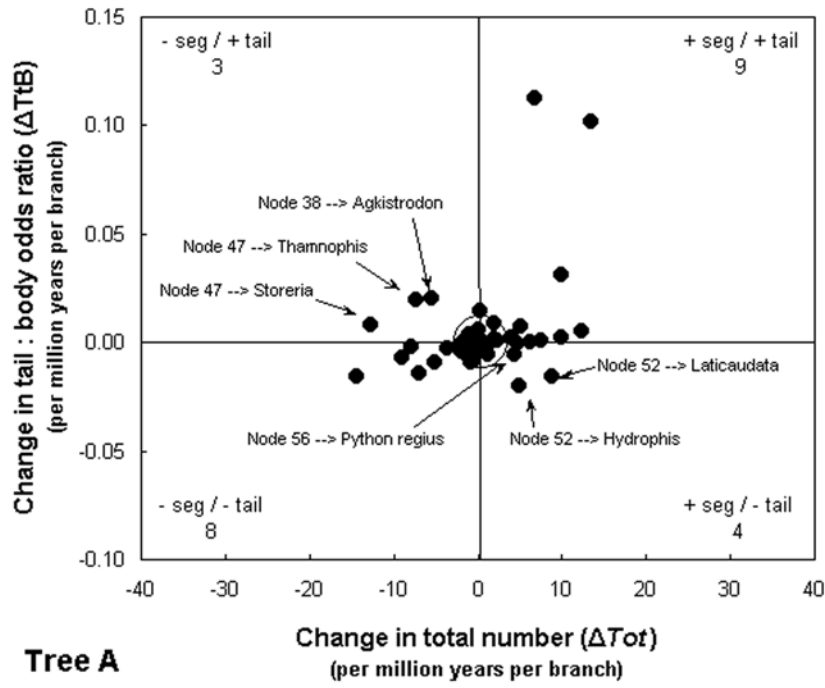
by the circled points at 0,0 – is for neither the total number nor the ratio to change. Counting the number of non-zero data points in the various quadrants allows us to estimate the probability of various modes of change. The most likely explanation is that the addition of tail vertebrae corresponds to addition of total body segments or, conversely, deletion of tail vertebrae to deletion of total segments (17 points fall into this category on Tree A and 18 on Tree B). In this mode, the proportion of body-to-tail is changed as a result of change in the overall number of tail vertebrae rather than by shifting the border between regional boundaries. It is much less likely that the ratio of tail to body segments increases at the same time as the total number of segments decreases (3 points fall into this category). In this mode, the border between the boundaries is shifted anteriorly (increasing the ratio of tail to body vertebrae) while at the same time the number of segments in the body is decreased. For this to happen vertebrae must be lost from the body and added to the tail, indicating a dissociation. This mode of change is localized in two clades *Thamnophis+Storeria* and in *Agkistrodon*. In Tree B, *Arizona* creeps into this category also. The inverse mode of change, addition of total vertebrae but reduction of the number in the tail, lies in the bottom right quadrant. Three data points fall into this category. This mode occurred within the clade containing *Hydrophis* and *Laticaudata* (perhaps associated with aquatic specialization) and independently in *Python regius*, a long-bodied snake with a relatively short tail. Thus only six out of 59 branches show evidence for dissociation between body and tail, indicating that it is possible but rare.

CONCLUSIONS

In this chapter, we tested evolutionary transitions in the number of body and tail vertebrae in snakes for patterns of modularity and dissociation. Based on experimental embryology of other amniotes, we hypothesize that, in snakes, these two regions are modules (sensu Raff, 1996). The adult body vertebrae (pre-cloacal vertebrae in snakes) forms from the segmented mesoderm that originates from the primitive streak and node, while the post-cloacal (tail) skeleton is formed from mesoderm that originates from the tail bud. The physical point of contact between these two processes is at what becomes the cloacal region in the adult (in most other vertebrates it is more easily recognized as the sacral region). The total number of pre-cloacal segments may then be controlled by anterior growth of the primitive streak and groove and the



Figure 9.8. Changes in segment number along branches per million years. Each data point represents changes along one of the branches in Tree A and Tree B (Fig. 9.5). **A, B)** the relationship between change in total number of vertebrae and the number of body segments. **C, D)** the relationship between change in total number of vertebrae and number of tail segments. **E, F)** the relationship between change in body vertebrae and tail vertebrae. Solid diagonal lines represent isometric change.



number of tail segments may be controlled by the amount of mesoderm that is generated by the tail bud. There is evidence for links between the two modules, particularly the processes of segmentation and somitogenesis, which traverse both the body and tail regions. Our data indicate that evolutionary changes in the number of body and tail vertebrae is usually coordinated, with segments being either gained or lost simultaneously in both regions. The tail region, however, is more evolutionarily labile than the body because changes in the number of vertebrae happens with greater frequency and magnitude there. The number of vertebrae in the body does also change, however, but more rarely and less radically than in the tail. Our data also indicate that the two regions can be dissociated so that vertebrae are gained in one and lost in the other. Such dissociation is rare, however. Of the 59 branches of the phylogenetic trees we considered, dissociation only happened for 6 of them. As far as we can localize these, they occur in a clade of terrestrial North American colubrids (*Thamnophis* plus *Storeria*), in a clade of marine elapids (*Laticaudata*+*Hydrophis*), in the branch leading to *Python regius* (the ball python), and in the branch leading to *Agkistrodon* (the cottonmouth). These four dissociations are the only candidates for heterochronic change among the data and taxa we considered.

But as Raff (1996) pointed out, heterochrony requires dissociation, but not all dissociations are heterochronic. Does heterochrony play a role in the evolution of body and tail proportions in snakes? Our data are suggestive, but, we argue, it is difficult to test for heterochrony without knowing the exact developmental mechanism responsible for an observed morphological transition. Given what is known about vertebrate development, some aspects of body-tail evolution may be heterochronic while others are not. The maximum growth of the tail might be a function of the total volume of mesoderm produced by the posterior streak and tail bud. If very little is produced, then the tail might be small or absent. One gene involved in axial elongation is the *T* or *Brachyury* gene (Herrmann, 1991). BRACHYURY is required for movement of cells through the streak, and in its absence, posterior elongation of the axis is prevented due to lack of mesodermal differentiation (Wilson & Beddington, 1997). Although a role for BRACHYURY in evolutionary changes to the vertebrate body axis has not been established, it is helpful to think of axial elongation in this developmental mechanistic sense. One way of generating more



Figure 9.9. Relationship between changes in tail-to-body ratio and change in total number of vertebrae. Each data point represents changes along one of the branches in Tree A or Tree B (Fig. 9.5). The lower left and upper right quadrants contain points that are not dissociations. In the lower left, both the total number of vertebrae and the number in the tail decrease; in the upper right the two both increase. The upper left and lower right contain dissociations. In the upper left, the number of vertebrae in the tail increases while the total number decreases. In the lower right, the number in the tail decreases while the total number increases. In both cases, body and tail must be dissociated for the changes to occur. The data points in the circle are branches on which there was virtually no change in either the total number of vertebrae or the ratio of tail-to-body.

posterior vertebrae would be to increase the number of cells passing through the posterior streak/tail bud. This process would not be heterochronic; it would be a change in volume rather than a change in timing. But of course the volume of mesoderm may be a function of the rate and duration of cell production and migration. For example, sustained outgrowth of the tail bud beyond the point at which it normally ceases to produce mesoderm would cause the posterior end of the body axis to grow longer, resulting in a greater number of caudal vertebrae. If so, the non-heterochronic process becomes heterochrony again. Furthermore, what was first imagined as a two variable process—growth of the primitive streak and the tail bud explain both the total number of vertebrae in a snake plus its regional proportions—has now become a multivariate process in which the rate of mesoderm production during gastrulation, the duration of gastrulation, the rate of anterior growth of the primitive groove, the onset of neurulation, and the rate of *hairyl* cycling all contribute to adult snake vertebral number, but identity is determined by superimposition of differential Hox gene expression on these segments. The nature of the relationship between mechanisms that generate of somitic mesoderm and those that determine its Hox code has not yet been resolved.

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