Serological Relationships among some Midwestern Snakes

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Abstract

Using immunoelectrophoresis, serum samples from 24 species of midwestern snakes were reacted against antiserums raised against serums of *Elaphe obsoleta, Natrix sipedon,* and *Agkistrodon piscivorus.* On the basis of immunoelectrophoretic patterns, three clusters of species can be recognized. One consists of *Natrix* (3 sp.), *Thamnophis* (2 sp.), *Regina septemvittata, Clonophis kirtlandi, Storeria dekayi* and *Virginia valeriae.* A second consists of *Elaphe* (2 sp.), *Lampropeltis* (3 sp.) and *Pituophis melanoleucus.* The third consists of *Agkistrodon* (2 sp.), *Sistrurus catenatus,* and *Crotalus horridus.* Five species (*Coluber constrictor, Diadophis punctatus, Carphophis amoenus, Farancia abacura,* and *Heterodon platyrhinos*) do not fit well into any of the above groups nor do they appear closely related to each other. Immunoelectrophoretic patterns do not indicate a markedly closer relationship between the *Natrix* and *Elaphe* groups of nonvenomous snakes than exists between these groups and the *Agkistrodon* group of pit vipers. *Elaphe, Natrix* and *Agkistrodon* all have species in east Asia, and the American groups presumably evolved from this stock. Other relationships and their zoogeographic implications are discussed.

Introduction

About 38 species of snakes occur in Indiana and adjoining states. Traditional taxonomy divides them into two families, the venomous pit vipers (Crotalinae, now generally considered a subfamily of the Viperidae) and the "typical nonvenomous snakes" of the family Colubridae. However, work during the past decade by investigators using both morphological and nonmorphological criteria has shown the Colubridae to be a highly heterogenous group (2,6,9,12,13). This paper brings together my observations on serological relationships of midwestern snakes as based on comparison of serum immunoelectrophoretic patterns. While immunoelectrophoresis, micro complement fixation, and other serological techniques will not solve all the problems of snake systematics, they can be useful tools in estimating degrees of relationship in a group that shows much specialization and has a meagre fossil record.

Materials and Methods

Most of the snakes used were collected in Indiana and nearby states. Larger specimens were bled by heart puncture, smaller ones by decapitation. Serum was separated, divided into aliquots of 0.2 to 0.5 ml and either used immediately or stored at -20°. Samples used represented serum from one to eight individuals. Nearly all samples were used within six months of collection. Antisera were produced in rabbits against pooled serum from *Agkistrodon piscivorus, Elaphe* *obsoleta*, and *Natrix sipedon* by methods previously reported (8). Immunoelectrophoresis was carried out at room temperature on agar-coated slides in 0.025 ionic strength barbital buffer, pH 8.6. Each set of slides was run at 7.5 v/cm for 45-60 min depending on migration of a nigrosin dye marker. Development with antiserum was carried out for approximately 24 hrs at room temperature. Slides were washed, dried, and stained with amidoschwarz or Ponceau S. Many of the serum-antiserum combinations were repeated 3-10 times; some were done only once or twice because of the small volume of the serum samples.

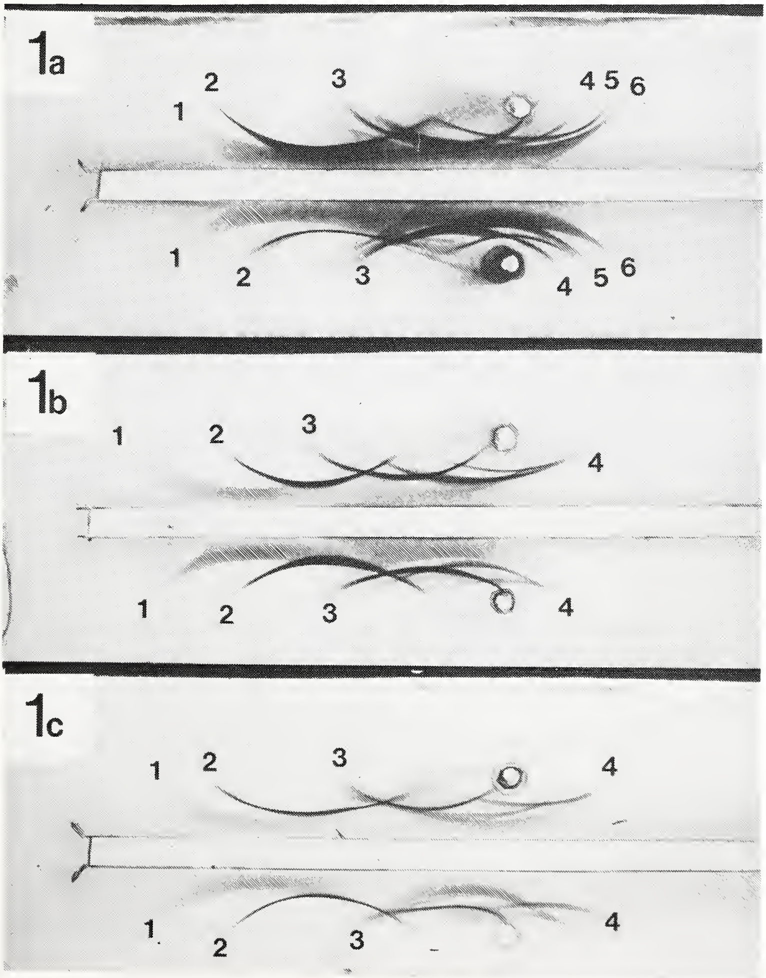

FIGURE 1. *Sera of natricine snakes developed with* ***Natrix sipedon*** *antiserum and with corresponding arcs numbered. 1A (upper)* ***Natrix sipedon,*** *(lower) N.* ***rhombifera****: 1B (upper)* ***Thamnophis radix,*** *(lower)* ***T. sirtalis****: 1C (upper)* ***Storeria dekayi,*** *(lower)* ***Virginia valeriae****. In this and other figures, the anode is to the left.*

Results

Examination of many immunoelectrophoretic patterns indicates snake serum developed with antiserum against a homologous or closely related species shows a strong pattern of 6 arcs, 3 predominantly on the anode side and 3 on the cathode side, with 2 to 4 additional minor arcs. Typical patterns are shown in Figures 1 and 2. The number of anode and cathode side arcs observed

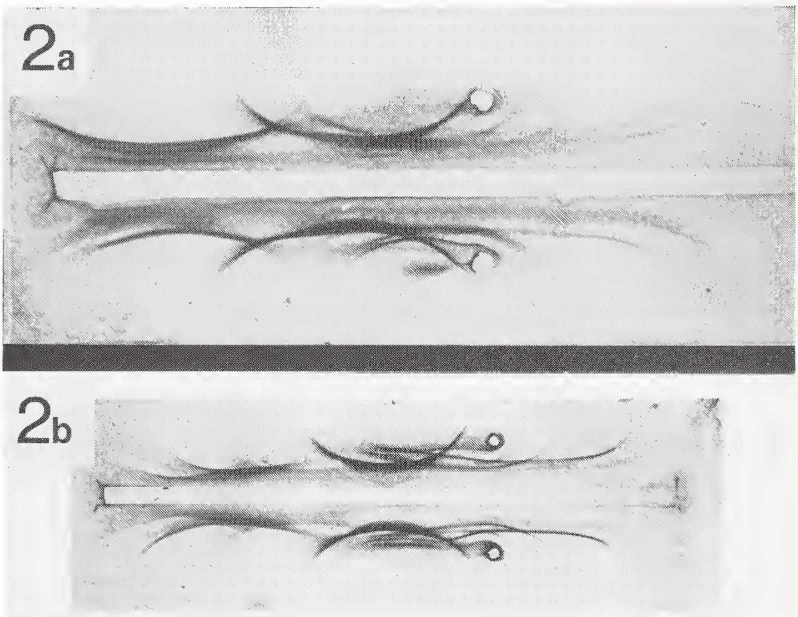

FIGURE 2. *Sera of **Lampropeltis calligaster** (upper), **L. getulus** (lower), and **Elaphe obsoleta** (both upper and lower) developed with **Elaphe obsoleta** antiserum.*

with various serum-antiserum combinations are shown in Table 1. In some cases, position of the arcs relative to the origin made their allocation arbitrary. One well defined arc, usually lying just to the anode side of the origin and often passing through it, is seen in nearly all combinations of serum and antiserum, being absent only when serums of archaic snakes such as Acrochoridids and

TABLE 1. *Numbers of anode and cathode arcs observed with combinations of snake serums with three antiserums. The figure in parentheses represent the number of times the combination was carried out.*

	Antiserum					
	Natrix sipedon		*Elaphe obsoleta*		*Agkistrodon piscivorus*	
Snake Species	Anode	Cathode	Anode	Cathode	Anode	Cathode
Natrix sipedon	4-5	4 (10)	2-3	0 (5)	2	1 (1)
Natrix erythrogaster	4	4 (3)	3	0 (2)	2	1 (1)
Natrix rhombifera	5	4 (2)	—	—	—	—
Regina septemvittata	3-4	2-3 (4)	3	0 (2)	2	1 (1)
Thamnophis sirtalis	3	3 (5)	3-4	0 (2)	—	—

Thamnophis radix	4	3 (2)	2	1 (1)	—	—
Clonophis kirtlandi	3-4	4 (4)	2-3	0 (2)	—	—
Storeria dekayi	2-3	4 (4)	2	0 (2)	2	1 (1)
Virginia valeriae	3	3 (3)	3	0 (1)	—	—
Coluber constrictor	3	2 (2)	3-4	2-3 (4)	2	1 (1)
Elaphe obsoleta	2	2 (4)	4-6	3 (8)	2	0-1 (2)
Elaphe vulpina	—	—	5	3 (3)	2	0 (1)
Lampropeltis getulus	2	2 (3)	4-5	2-3 (8)	2	0 (2)
Lampropeltis calligaster	—	—	5	3 (1)	—	—
Lampropeltis triangulum	—	—	5	0 (1)	2	0 (2)
Pituophis melanoleucus	2	3 (2)	4	2-3 (2)	—	—
Diadophis punctatus	3	2 (2)	2	1 (1)	2	1 (1)
Carphophis amoenus	2	3 (1)	2	0 (1)	2	1 (1)
Farancia abacura	2	1 (3)	2	0 (2)	—	—
Heterodon platyrhinos	2	2-3 (4)	2-3	0 (4)	3-4	1 (3)
Agkistrodon piscivorus	2	2 (2)	4	0 (4)	4-5	3-4 (5)
Agkistrodon contortrix	2	2 (2)	3	0 (1)	3-4	2-3 (5)
Sistrurus catenatus	1	2 (1)	3	1 (1)	3	2 (2)
Crotalus horridus	1-2	2 (3)	3	1 (2)	3	2 (2)

some Boids are developed with antiserums to serums of modern snakes. (Fig. 1, Arc 3) It is present in all patterns analyzed here and evidently represents a protein with similar antigenic determinants in the serum of most modern snakes. The arcs with greatest anode mobility, presumably prealbumin and albumin, appear in most of the patterns but show much variation in intensity of staining.

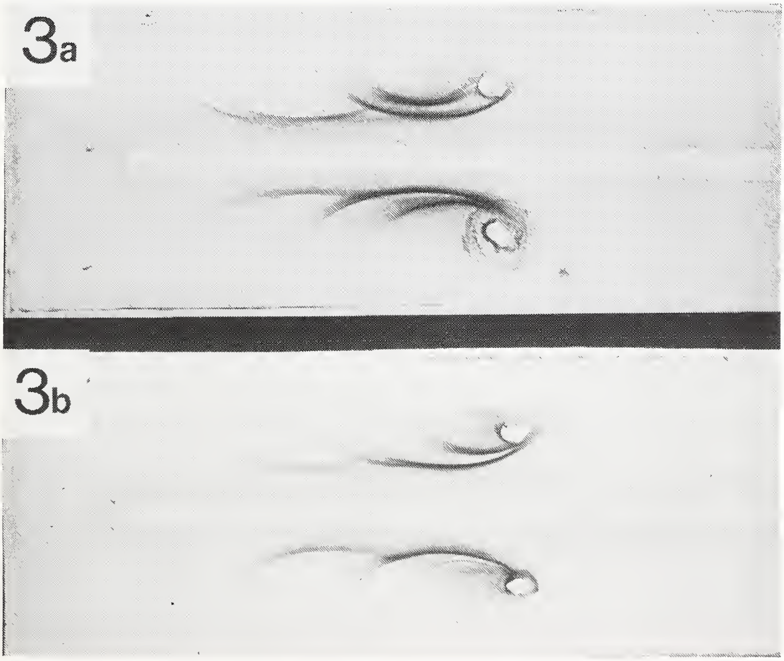

FIGURE 3. *Sera of* ***Coluber constrictor*** *and three natricine snakes developed with* ***Elaphe obsoleta*** *antiserum. 3A (upper)* ***C. constrictor****, (lower)* ***Thamnophis sirtalis****. 3B (upper)* ***Natrix erythrogaster*** *(lower)* ***Regina septemvittata****. Note virtual absence of cathode side arcs.*

Cathode side arcs, presumably globulins, show less tendency to be shared by diverse species (Fig. 3).

When the number of arcs and their intensity and distinctness all are considered, an admittedly subjective evaluation, certain clusters of patterns can be detected. One includes those midwestern snakes, *Natrix, Regina, Thamnophis, Clonophis, Storeria,* and *Virginia.* All show patterns of at least 5 arcs when developed with *Natrix sipedon* antiserum; usually no more than 3 arcs with *Elaphe* or *Agkistrodon* antiserums. The genera *Elaphe, Lampropeltis,* and *Pituophis* show 6 or more arcs when developed with *Elaphe obsoleta* antiserum; no more than 4 arcs when developed with *Natrix* or *Agkistrodon* antiserums.

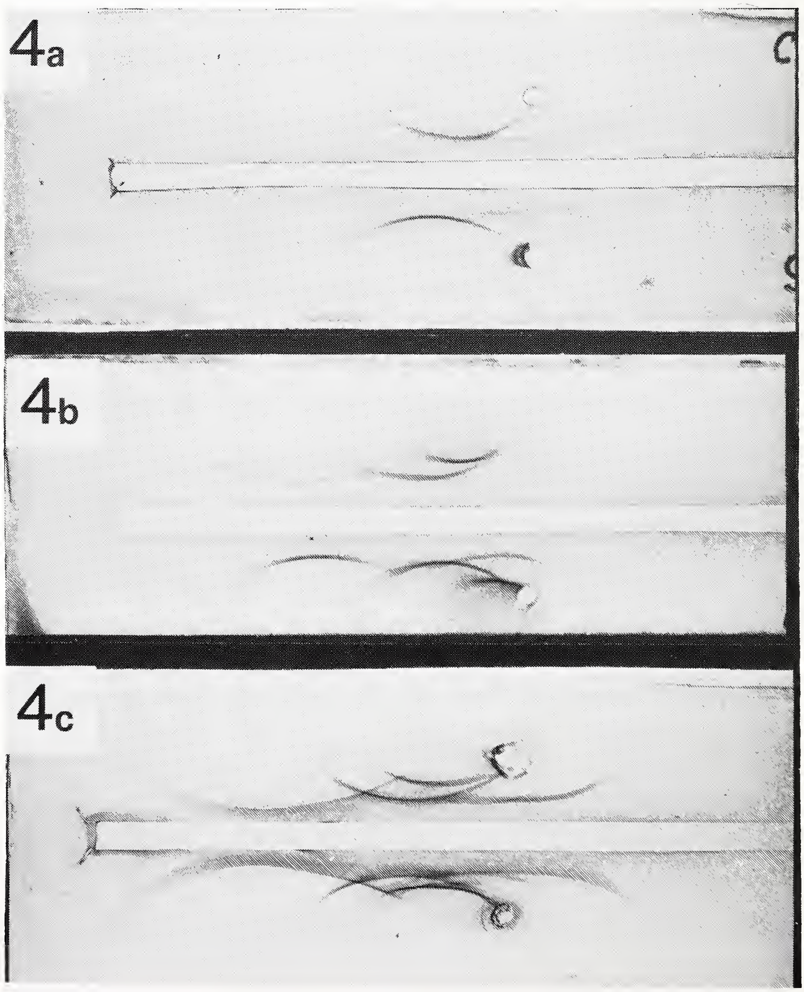

FIGURE 4. *Sera of pit vipers developed with various antisera. 4A (upper)* ***Crotalus horridus****, (lower)* ***Sistrurus catenatus*** *developed with* ***Natrix sipedon*** *antiserum. 4B (upper)* ***Sistrurus catenatus****, (lower)* ***Crotalus horridus*** *developed with* ***Elaphe obsoleta*** *antiserum. 4C (upper)* ***Sistrurus catenatus****, (lower)* ***Agkistrodon contortrix*** *developed with* ***A. piscivorus*** *antiserum.*

Serums of pit vipers, *Agkistrodon, Sistrurus* and *Crotalus*, show at least 5 arcs when developed with *Agkistrodon piscivorus* antiserum; 3 or 4 arcs when developed with *Elaphe* or *Natrix* antiserums (Fig. 4). Five genera, each represented by a single species, do not fall into any of the above groups. *Diadophis* and *Carphophis* serums show 5 relatively weak arcs with *Natrix* antiserum, 2 or 3 with *Elaphe* and *Agkistrodon*. *Farancia* serum shows no more than 3 arcs with any antiserum. *Heterodon* serum shows no more than 4 arcs with any antiserum, but its strongest reactions are with *Agkistrodon* antiserum (Fig. 5). *Coluber constrictor* serum shows 4 or 5 arcs with *Natrix* antiserum and 5 or 6 with *Elaphe*.

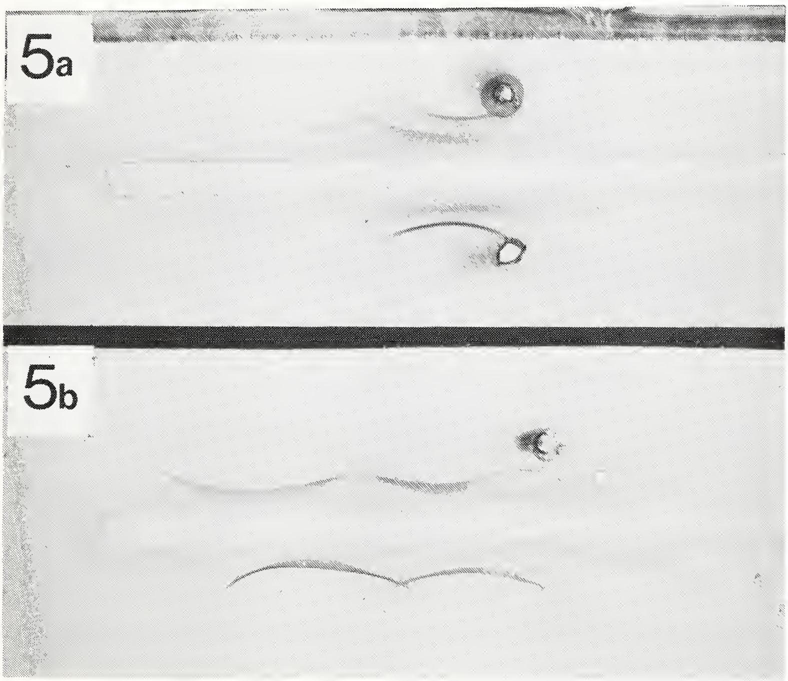

FIGURE 5A. *Serum of* ***Heterodon platyrhinos*** *(upper) and* ***Elaphe obsoleta*** *(lower) developed with* ***Agkistrodon piscivorus*** *antiserum. 5B. Serum of* ***H. platyrhinos*** *(upper) and* ***Farancia abacura*** *(lower) developed with* ***Natrix sipedon*** *antiserum.*

Discussion

The snake fauna of the Midwest, like that of most of the northern hemisphere, contains no archaic or highly aberrant genera and appears to be of comparatively recent origin. Serological data presented here show a close relationship among the 9 species and 6 genera assigned to the natricine group. The genus *Natrix* is Eurasian, and there is convincing evidence (8,11) that the Eurasian and North American species are not closely related. Rossman and Eberle (11) suggest revival of the generic name *Nerodia* for the North American species. *Thamnophis*, closely allied to North American *Natrix*, occurs from northern Canada to Costa Rica and has undergone extensive speciation. The other American natricine genera contain one to a few species and are limited in distribution. *Elaphe* is Eurasian, and limited serological evidence indicates a fairly close relationship between New and Old World species. *Lampropeltis* is American with a range from Canada to Ecuador. *Pituophis* is also American and is found from Canada to southern Mexico. Serologically both these genera are closely allied to American *Elaphe*. The pit vipers are well represented in both Asia and America with one genus, *Agkistrodon*, common to the two regions. Serum immunoelectrophoretic patterns indicate a relatively remote relationship between two Asian *Agkistrodon* examined and the three American species. The same criterion indicates relationship between American *Agkistrodon* and the rattlesnakes (*Sistrurus* and *Crotalus*) is more remote than that between *Thamnophis* and American *Natrix* and between *Lampropeltis* and American *Elaphe*. For each of these three groups, the morphological, serological, and zoogeographic evidence indicates an Asian origin with migration to North America and extensive adaptive radiation with evolution of new genera. In the case of the pit vipers there has been an extensive adaptive radiation with evolution of new genera. In the case of the pit vipers there has been an extensive South American radiation as well.

Serologically *Coluber*, as represented by its sole New World species, *C. constrictor*, is intermediate between the *Natrix-Thamnophis* and *Elaphe-Lampropeltis* groups although somewhat closer to the latter. On the basis of serum immunoelectrophoretic pattern, I cannot distinguish *C. constrictor* from two species of *Masticophis* that have been examined, and there are few morphological differences between the genera. *Coluber* contains numerous Eurasian species, but their relationships with one another and with their New World allies are obscure.

Morphologically *Heterodon* appears closely related to *Xenodon* and *Lystrophis* of tropical America. The relatively strong immunoelectrophoretic pattern of *Heterodon* serum developed with *Agkistrodon* antiserum and also with *Crotalus* antiserum (9) suggests that *Heterodon* may belong to a stock from which the viperid snakes evolved. The data presented here tell little of the relationships of the North American genera *Carphophis, Diadophis,* and *Farancia.* The former two show a tenuous affinity with the natricine group, but this may be fortuitous.

Of the midwestern snake genera not included in this study, there is serological data indicating *Tropidoclonion* is a typical natricine (7). *Opheodrys* is reported to be serologically related to *Coluber* and *Cemophora* to *Lampropeltis* (10). I have not examined serum from either midwestern species of *Tantilla,* but serum of the western *T. nigriceps* shows only weak reactions with *Natrix* and *Elaphe* antiserums.

In summary, 19 of the 24 species studied can be allocated on the basis of serum immunoelectrophoretic patterns to one of three groups. All the groups have Eurasian affinities, but the species themselves represent a distinctively American evolutionary radiation. Fossil evidence indicates *Elaphe, Lampropeltis, Natrix,* and unidentified pit vipers were present in the Middle and Upper Miocene of central North America and were replacing an older boid fauna. A species ancestral to *Heterodon* was also present (3,4,5). Most of the genera and many of the species have been identified in Pliocene material (1). If a migration of Asian species to North America took place, it must have begun early in the Miocene some 25 million years ago and continued into the Pliocene. Serological differences between congeneric New and Old World species are compatible with a separation in time of this magnitude (8).

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