

RATTLE LOSS IN PYGMY RATTLESNAKES (*SISTRURUS MILIARIUS*): CAUSES, CONSEQUENCES, AND IMPLICATIONS FOR RATTLE FUNCTION AND EVOLUTION

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ABSTRACT: The unusually small rattles of the Pygmy Rattlesnake (*Sistrurus miliarius*) produce barely audible rattling sounds, suggestive of the earliest stages of rattle evolution. We combine a comparative analysis of rattle morphology of 20 rattlesnake species, obtained from museum specimens, with an ecological field study of *S. miliarius*. We use these data to investigate hypotheses concerning the current function(s) and evolutionary origin of the rattle. First, it was necessary to counter Klauber's (1940, 1972) caveat that preserved rattlesnakes be avoided in studies of rattle chain lengths, as museum specimens might exhibit either unusually short (from breakage due to handling) or unnaturally long (collector bias) rattle chains. We found no evidence to support this warning. Our museum and field data indicate that *S. miliarius* is unique among mainland species in that its rattle chains are very short, often possessing no free rattle segments (and are thus effectively mute). Both of these traits, short rattle chains and high incidence of being rattleless, apparently result from frequent rattle segment loss during ecdysis. The high rate of segment loss is due to rattle morphology (poorly developed longitudinal grooves between the first and second lobes of each segment) rather than small rattle size. The extremely high rate of rattle loss we report for *S. miliarius* (38.9% overall; 52.3% for *S. m. miliarius*) argues that this species be added to the list of so-called rattleless rattlesnakes, which include *Crotalus catalinensis* and *C. ruber lorenzoensis*.

Although the diminutive and easily shed rattle of *S. miliarius* may be ancestral, several lines of evidence suggest that it is derived. Rattle chain length currently does not appear to be influenced by natural selection in *S. miliarius*, as this trait is not heritable, is highly variable, and has no impact on fitness. Accordingly, reduction in rattle chain length and high rates of rattle loss in *S. miliarius* appear to be the products of relaxed selection, but relaxed selection from what? *Sistrurus miliarius* is hesitant to rattle when threatened by potential enemies, but use their tails for luring ectothermic prey. Thus, rattles appear unimportant for attracting frogs and lizards, a finding at odds with the hypothesis that rattles enhance caudal luring. We argue instead that the tiny, barely audible, and frequently lost rattle of *S. miliarius* has been emancipated from selection related to either warning away or deflecting the bites of their enemies.

INTRODUCTION

Arguably, no single morphological structure in snakes has been the object of more interest and speculation than the rattle of rattlesnakes. Of the approximately 2,700 species of snakes, only members of the genera *Crotalus* and *Sistrurus* (ca. 29 species; Murphy et al., this volume) possess this remarkable appendage. Indeed, the rattle was almost certainly a key evolutionary innovation that promoted their radiation (Greene, 1988). Although some authors purport that the rattle evolved independently in each genus (e.g., Garman, 1888), the complexity of this structure and its similarity across all rattlesnake species indicate that it arose only once in a shared common ancestor of *Crotalus* and *Sistrurus* (Greene, 1988).

What was the adaptive significance of the rattle in proto-rattlesnakes? Hypotheses concerning the origin of the rattle range from the superficially silly (e.g., rattling charms and immobilizes the snake's prey) to the sensible (e.g., rattling is aposematic), and were reviewed by Klauber (1940, 1972). Three hypotheses are currently in vogue; the most popular is that the

rattle evolved as an aposematic warning of the snake's venomous nature (Klauber, 1940, 1972; Greene, 1988, 1992, 1997; Sisk and Jackson, 1997; Tiebout, 1997). Historically, the targets of this warning were thought to be large ungulates, such as the American Bison (*Bison bison*), that might trample the snake (Hay, 1887; Garman, 1889; Barbour, 1922). More recently, however, Greene (1997) argued that original targets of the proto-rattle's warning might have been small carnivores such as the White-nosed Coati (*Nasua narica*) and Ringtail (*Bassariscus astutus*), enemies that are more abundant than Bison in the montane regions of Mexico where rattlesnakes are thought to have evolved (Klauber, 1972; Greene, 1997).

The strongest evidence in support of the warning hypothesis is that all extant species of rattlesnakes are thought to use rattling only in defensive contexts (Greene, 1988, 1992, 1997). The current utility of a trait, however, may not always reveal its original function (Tinbergen, 1963; Gould and Vrba, 1982). Defensive tail vibration is common in snakes, including many non-venomous species. One presumed function of defensive tail vibration, in taxa other than noxious species such as rattlesnakes, is to deflect attention of a predator away from the snake's more vulnerable head (Greene, 1973, 1979, 1988). Several authors have sug-

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gested that rattles originated because they enhanced this distraction for rattlesnakes as well (Garman, 1889; Williams, 1966).

A third hypothesis is that rattles evolved neither to warn away nor divert attack from enemies, but instead to enhance feeding (Schuett et al., 1984). Many species of snakes use caudal luring to attract prey, including several species of rattlesnakes (Neill, 1960; Jackson and Martin, 1980; Schuett et al., 1984; Reiserer, this volume). According to Schuett et al. (1984), a proto-rattle might have either enhanced the attractiveness of the lure and/or prevented damage to the snake's tail when bitten by the attracted prey. That tail injuries can be fatal, especially for small snakes, has been demonstrated in three species of *Thamnophis* (Willis et al., 1982). The protection that proto-rattles might provide to vulnerable tails could also apply to the distraction hypothesis outlined above, as a keratinous tail tip could blunt the attacks of predators as well as prey.

There has been only a single attempt to systematically test these hypotheses. Sisk and Jackson (1997) argued that the first step in the origin of modern rattles was the appearance of a bilobed terminal end-scale. Without such bilobation, the end-scale would have nothing to "snag" during ecdysis, and there would be no retention of the loose, noise-making segments, since rattlesnakes that possess only a basal or attached segment are mute. Sisk and Jackson (1997) showed that epoxy models of a bilobed rattle were no more effective in luring prey than were models of a conical, unlobed rattle, failing to support the hypothesis of Schuett et al. (1984) that proto-rattles might enhance caudal luring. Unfortunately, the bilobed models were actually quieter than the conical models when scraped across a paper and wood substrate, a finding that also fails to support the aposematism hypothesis. The idea that rattling might better deflect the attention of a predator was not tested, nor was the hypothesis that proto-rattles might originally have protected a snake's tail from bites by prey or by enemies.

Although the gross morphology of rattles is similar in all rattlesnakes, differences between species occur with respect to relative size and dimensions of rattle segments (Klauber, 1940, 1972). This variability may reflect different selection pressures across species, and might therefore illuminate the current function or the historical genesis of the rattle. Cook et al. (1994), for example, showed that Pygmy Rattlesnakes (*Sistrurus miliarius*) possess unusually small rattles, even when body size was adjusted. The rattling sounds produced

by these tiny rattles are exceptionally quiet and high-pitched, and seem unlikely to be effective in warning away enemies. Cook et al. (1994) suggested that the diminutive rattle of *S. miliarius* might be an apomorphic by-product of selection that favors thin tails for caudal luring.

Differences in rattle size are not the only interspecific comparisons suggesting that selection pressures on rattles vary across species. There are at least two insular populations of so-called rattleless rattlesnakes in the Gulf of California: the Santa Catalina Island Rattlesnake (*Crotalus catalinensis*) and the San Lorenzo Island Rattlesnake (*C. ruber lorenzoensis*). Both populations are characterized by a high frequency of individuals possessing no loose rattle segments, ranging from ca. 55% in *C. r. lorenzoensis* (Radcliffe and Maslin, 1975), to 85–100% in *C. catalinensis* (Shaw, 1964; Klauber, 1972). A proximate explanation for the high rate of rattle loss in both populations focuses on poor development of the lobes and grooves that "lock" an older segment onto a younger one during ecdysis (Klauber, 1972; Radcliffe and Maslin, 1975). Ultimate explanations for the poor segment retention in both populations include: (1) founder effect, with the original colonists possessing abnormal rattle morphology (Klauber, 1972); (2) relaxed selection from enemies, as both islands may be depauperate in rattlesnake predators (Shaw, 1964; Johnson, 1972; Radcliffe and Maslin, 1975); and (3) direct selection on snakes that retained loose segments as these individuals might inadvertently reveal themselves while climbing to hunt birds, a suggestion consistent with the unusual, arboreal habits of both populations (Hollingsworth and Mellink, 1996; Greene, 1997). All of these citations suggest that the condition of being rattleless is derived, and several mention that snakes from both populations still rattle, albeit silently, when disturbed by humans (Shaw, 1964; Johnson, 1972; Radcliffe and Maslin, 1975). Detailed investigations of the loss of previously functional traits can help identify the costs and benefits of the original trait and its vestigial manifestation (Fong et al., 1995). A thorough account of the causes and consequences of rattle loss in *C. catalinensis*, *C. r. lorenzoensis*, or other species of rattlesnakes might, therefore, elucidate the function of rattles and rattling.

Sistrurus miliarius is, for several related reasons, an excellent model for examining questions regarding both the origin and current utility of the rattles. First, the genus *Sistrurus* is considered by most rattlesnake systematists to be basal to *Crotalus* (e.g., Knight et al.,

1993; Murphy et al., this volume). Thus, rattle structure and function in *Sistrurus* may be plesiomorphic. Second, it is generally assumed that proto-rattles were small (e.g., Schuett et al., 1984). Because *S. miliarius* has tiny rattles, both in terms of their absolute and relative size (Cook et al., 1994), the manner in which it currently employs its rattles may thus reflect the original (ancestral) uses of this structure. Third, *S. miliarius* has rates of rattle loss that approach those of *C. catalinensis* and *C. r. lorenzoensis* (Cook, 1992). An analysis of the proximate and ultimate causes of rattle loss in *S. miliarius* could elucidate the costs and benefits of rattles. Last, the natural history of *S. miliarius* is well known, including information on feeding and defensive biology (Farrell et al., 1995; Bishop et al., 1996; May et al., 1996; Rabatsky and Farrell, 1996; Roth et al., 1999; Rabatsky, 2002; Greene et al., this volume).

MATERIALS AND METHODS

Sources of Data

We used two sources of data to test our hypotheses regarding rattle loss in *S. miliarius*. The first involved morphometric analyses of preserved specimens from seven institutions. Museum collections have been successfully used to assess the functions of defensive tail displays in snakes (e.g., Greene, 1973), suggesting that such collections might also be useful for exploring rattle evolution. Our second data set comes from an intensive, long-term study of individually marked animals in the field.

Museum data.—Morphometric data were obtained from ca. 1,700 specimens from the following institutions: the Department of Biology, University of Texas at Arlington; the North Carolina Museum of Natural Sciences; the Field Museum of Natural History in Chicago; the Florida Museum of Natural History; the University of Kansas Museum of Natural History; the U. S. Museum of Natural History; and the Department of Ecology and Evolutionary Biology, University of Arizona. We measured snout-vent length (SVL), tail length (TL), number of rattle segments (hereafter called rattle chain length), and the depth of the exposed lobe of the basal (i.e., attached) rattle segment (see Klauber, 1972; Cook et al., 1994; Rowe and Owings, 1996). We did not make detailed measurements of more distal rattle segments, as previous research demonstrates that spectral characteristics of a snake's rattling sounds are determined primarily by the size of the snake's most proximal rattle segments, and primarily by the depth of the exposed lobe of its

basal segment (Young and Brown, 1993; Cook et al., 1994; Rowe and Owings, 1996). Body measurements were made to the nearest millimeter and rattle measurements to the nearest 0.01 mm (using Mitutoyo calipers model 505-647-50). We made a special effort to obtain montane rattlesnakes from Mexico (e.g., *C. lannomi*, *C. pusillus*, and *C. stejnegeri*), as the ecology of these small-rattled species may best represent the conditions in which rattles first evolved (Klauber, 1972; Greene, 1997). We excluded specimens possessing only their natal button from our analyses, as these individuals had no opportunity to "lose" a segment (during ecdysis) prior to their collection. This criterion reduced the total sample size to 1,488 individuals representing 20 species of rattlesnakes. Sample sizes for museum specimens of each species are shown in Table 1.

We scored rattles for each specimen as either "complete" (i.e., possessing their natal button; Fig. 1a) or "incomplete" (i.e., having lost one or more of their rattle segments, including the button; Fig. 1b). A rattle might break, and thus be incomplete, in one of two ways; a full segment (and thus all distal segments) can slip off the lobes of its proximal segment, or a rattle segment can weaken and tear. In the latter case, the first lobe of the broken segment typically remains as a "ring" in between the first and second lobes of the more proximal segment (Fig. 1b). These partial segments, which we scored as "fragments," nicely illustrate the mechanism that locks an older more distal segment to its younger, proximal neighbor (i.e., the lip or opening of the older segment sits within the longitudinal groove between the first and second lobes of the newer segment). We sought to quantify the robustness of this locking mechanism by examining specimens with incomplete strings and without a fragment, as these individuals have terminal segments with fully visible grooves between the first and second lobes. We examined the groove from the side (which allows an assessment of the dorsal and ventral dimensions of the groove; Fig. 1c) and above (providing an assessment of the lateral dimensions of the groove; Fig. 1d). The groove was ranked from each perspective on a seven-point ordinal scale, ranging from a maximum of 4 (for a deep groove generating a good grip between that segment and the next older segment) to a minimum of -2 (for a tapered second lobe providing no groove and thus no grip at all for retaining the older segment).

Field data.—Our field data on *S. miliarius* were collected over an eight year period (1992–2000). The

Table 1. Rattle characteristics of 20 rattlesnake species (*Crotalus* and *Sistrurus*) represented by museum data collected in this study.

Species	N ¹	% snakes rattleless	Mean chain length ²	CV of chain length ³	Rattle size ⁴ (mm)	% rattles broken at terminus ⁵
<i>C. aquilus</i>	30	0.0	7.30	0.40	6.0	13.8
<i>C. atrox</i>	70	1.4	5.54	0.50	13.0	51.7
<i>C. durissus</i>	52	11.5	5.02	0.69	10.4	22.2
<i>C. horridus</i>	76	6.6	5.80	0.64	13.4	10.9
<i>C. intermedius</i>	34	8.8	6.56	0.59	5.1	11.1
<i>C. lepidus</i>	115	6.9	6.31	0.54	7.3	20.0
<i>C. mitchellii</i>	17	11.8	4.53	0.67	11.3	0.0
<i>C. polystictus</i>	44	9.1	4.75	0.63	6.7	20.5
<i>C. pricei</i>	35	22.9	5.29	0.65	4.7	3.5
<i>C. pusillus</i>	28	7.1	4.21	0.49	3.2	11.5
<i>C. ruber</i>	56	3.6	6.59	0.38	13.5	48.7
<i>C. stejnegeri</i>	4	0.0	3.50	0.37	3.0	33.3
<i>C. tigris</i>	16	0.0	8.13	0.25	12.2	57.1
<i>C. transversus</i>	3	0.0	4.00	0.43	4.3	33.3
<i>C. triseriatus</i>	22	13.6	3.86	0.56	3.8	15.0
<i>C. viridis</i>	255	4.7	5.64	0.44	11.4	46.0
<i>C. willardi</i>	55	10.9	4.29	0.59	5.8	9.1
<i>S. catenatus</i>	124	9.7	3.47	0.54	6.0	11.9
<i>S. miliarius</i>	378	38.9	2.72	0.72	2.7	2.7
<i>S. ravus</i>	74	4.1	5.51	0.50	5.0	5.2

¹Sample size. ²Average number of rattle segments, including the attached (basal) segment. ³Coefficient of variation in rattle chain length (standard deviation/mean). ⁴Mean depth of the exposed lobe of the basal segment. ⁵Percentage of incomplete rattle chains in which the terminal segment(s) of the chain had “broken” rather than “slipped” (as determined by the presence of a fragment).

study sites were four areas of mesic forest on the floodplain of the St. Johns River in Volusia County, Florida. Each study site was adjacent to freshwater marsh and less than 4 km from any of the other three study sites. We focused our research effort most intensely in a single, 10 ha patch of forest (site HI). Site HI was visited an average of three times a week during the entire study period, with almost daily visits during late July and August. The three other areas were visited less frequently. We used mark-recapture techniques to study the snakes. We marked *S. miliarius* with PIT-tags, a technique that does not affect the growth, movement, or recapture probability of these snakes (Jemison et al., 1995), and found them by visually searching the study sites. We measured the mass of captured snakes with a spring-loaded scale (Pesola, Switzerland) and measured SVL by using the squeeze-box technique (Quinn and Jones, 1974); we

determined sex by probing. We also counted the number of segments on a snake’s rattle chain and noted if the rattle chain was complete or incomplete. Individuals were typically released at their capture site within 10 minutes of capture.

We kept track of the age of most individuals. At the start of the study (early 1992), the population consisted of two distinct size classes: the young-of-the-year snakes (1991 cohort) and a group of larger snakes that were born in 1990 or earlier (the 1990 “cohort”). Most of the snakes born in 1991–2000 were PIT-tagged in their first 18 months of life, when their size and rattle characteristics allowed us to accurately determine their age. In addition to these “known-age” snakes, there were also a few individuals that were too old when first captured to accurately determine their age. These individuals were PIT-tagged and their age was classified as unknown.

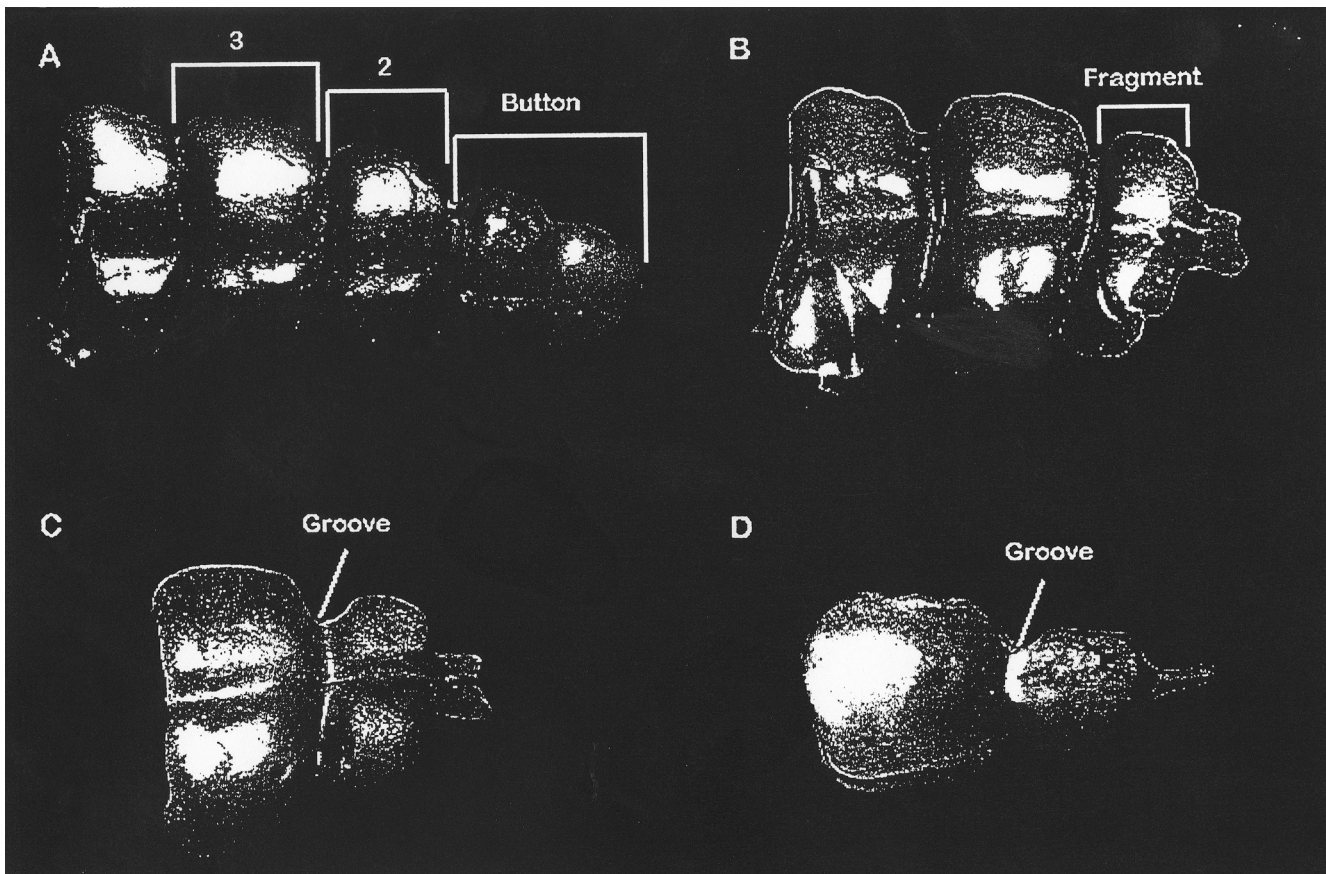


Fig. 1. Morphology of the rattlesnake rattle (from *Crotalus adamanteus*). (A) Complete rattle chain with button; exposed lobes of 2nd and 3rd segments are indicated. (B) Incomplete rattle chain resulting from “breakage” of a segment; bracket indicates attached portion (fragment) of broken segment. (C) Lateral view of isolated rattle segment. Longitudinal groove between first and second lobes is marked. (D) Dorsal view of isolated segment with longitudinal groove marked.

Natural History of Tail Use by *Sistrurus miliarius*

To determine whether *S. miliarius* uses its tail in defensive situations, we observed the response of 1,350 individuals to an aggressive encounter. Wearing a heavy glove, we approached each snake head-on immediately after it was observed in the field, and tapped it on the head with our gloved finger. We recorded the snakes' response to this provocation, noting the number of snakes that rattled (defined as rapidly vibrating their tail). Only 3.0% of the snakes (41 of 1,350 individuals) rattled in response to provocation. The length of the rattle chain did not influence the proportion of snakes that rattled. While very few mute snakes (those with only a basal segment) vibrated their tail (2.7%, 13 of 490 snakes), neither did many individuals possessing two or more segments (3.2%, 28 of 856 snakes). A 2 x 2 contingency table analysis indicated there was no significant association between rattle chain length and the number of individuals that rattled ($\chi^2 = 0.187$; $df = 1$; $P = 0.66$). Rabatsky (2002) has recently reported similar results from another population of *S. miliarius*.

Although *S. miliarius* does not appear to use its rattles to warn away enemies, they frequently use their tail to attract prey. Caudal luring has been reported in numerous snakes (e.g., Neill, 1960; Heatwole and Davison, 1976; Pough, 1988; Strimple, 1992), including *S. miliarius* (Jackson and Martin, 1980; Rabatsky and Farrell, 1996; Rabatsky, 2002). In most species the young show this behavior almost exclusively (Greene, 1992), although adult *S. miliarius* have also been reported to use their tails in attracting prey (Jackson and Martin, 1980). In our field study (Rabatsky and Farrell, 1996), however, we have never observed caudal luring in adults. Indeed, older snakes lose the brightly colored tail of juveniles, and they are less frequently found in foraging postures with visibly exposed tails (Rabatsky and Farrell, 1996). These observations suggest that caudal luring is not as commonly employed by adult *S. miliarius* as by younger conspecifics.

It appears that *S. miliarius* possesses traits that several authors suggest may have characterized proto-rattlesnakes. The rattle of *S. miliarius* is tiny

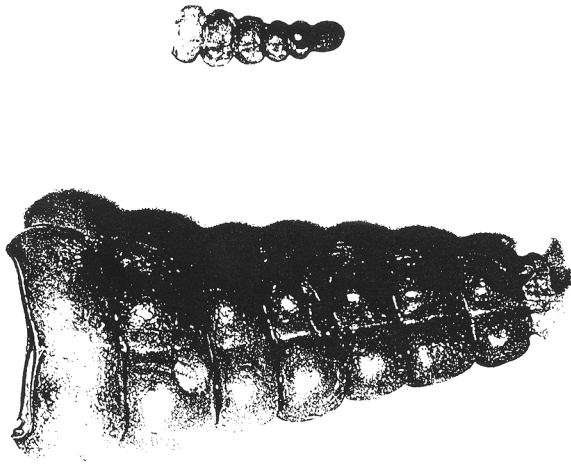


Fig. 2. Typical rattle chains from an adult (33.6 cm SVL) *Sistrurus miliarius* (above) and an adult (69.8 cm SVL) *Crotalus viridis*.

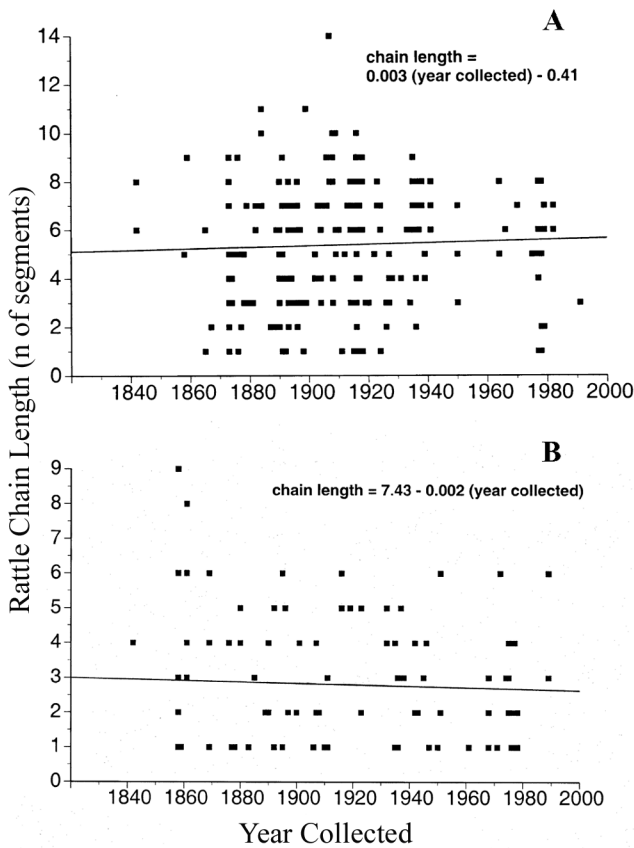


Fig. 3. (A) Rattle chain length in *Crotalus viridis* as a function of the year collected. (B) Rattle chain length in *Sistrurus miliarius* as a function of the year collected. Both samples from the United States National Museum.

and when shaken produces a barely audible, unusually high-pitched sound (Cook et al., 1994). Klauber (1972:266 and references cited therein) suggests that such tiny rattles would have “little value as warning devices.” Schuett et al. (1984) argued that incipient rattles of proto-rattlesnakes were likely to be small and more important in luring prey than in warning away predators. Our field data show that *S. miliarius* uses its tail primarily for caudal luring (although solely by juveniles), but only infrequently shakes its tail in warning displays. Moreover, many *S. miliarius* retain only the basal segment of their rattle, rendering any such warning mute (Cook, 1992). A detailed examination of the causes and consequences of rattle loss in *S. miliarius* might, therefore, tell us something about rattle evolution.

Can Museum Specimens be used to Document Rattle Characteristics?

Before we offer explanations why *S. miliarius* are so often rattleless, it is important that we address an explicit caveat against using museum specimens for making inferences about rattle chain lengths in wild populations. Klauber (1940, 1972) argued strongly that wear and tear on museum specimens coupled with the softening effect of liquid preservatives on rattles could lead to significant rattle loss after specimens were collected. Thus, museum samples might generate both low average chain lengths and high rates of rattlelessness, compared to those that occur naturally. Alternatively, Klauber cautions that collectors may have decided to collect only those specimens with long chains, thereby leading to overestimation of mean chain lengths and underestimates of the frequency of rattleless individuals.

If the “wear and tear” argument has merit, we might expect an inverse relationship between the age of a specimen (i.e., the date the specimen was added to a collection) and both chain length and the percent of the sample that has no loose segments. We looked for these relationships in two species for which we had large samples spanning a broad range of collection dates. The two species, *S. miliarius* and *C. viridis*, were also selected because they are dramatically different in body size and rattle size (Fig. 2). To minimize the possibility that specimens at different museums might suffer inconsistent rates of post-preservation segment loss (e.g., due to differences in specimen use and curatorial techniques at different institutions), we restricted our analyses to the collections of *S. miliarius* and *C. viridis* housed in the U.S.

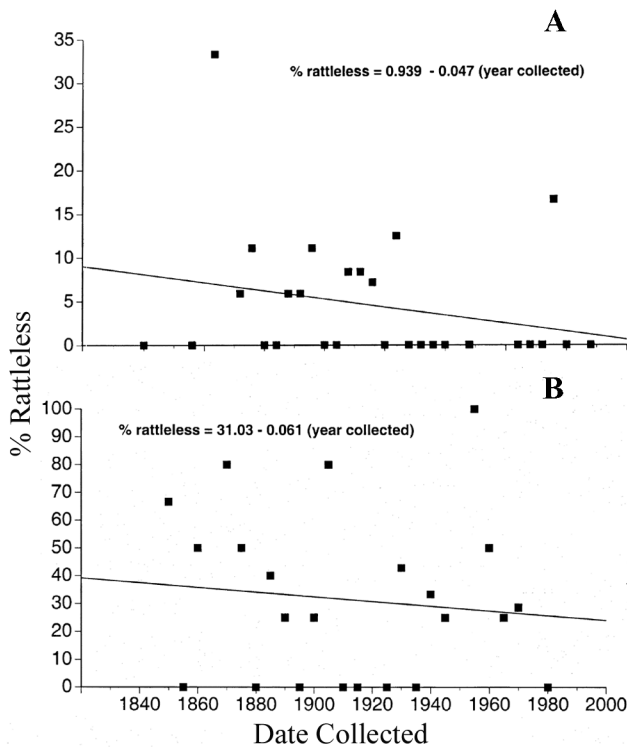


Fig. 4. (A) Percent rattlesless *Crotalus viridis* by date collected (snakes divided into groups; each group includes individuals collected during a 4-year span). With the outlier from 1862 to 1866 excluded from this analysis, correlation between the two variables drops from 0.25 to 0.09, while the level of significance increases from 0.20 to 0.66. (B) Percent rattlesless *Sistrurus miliarius* by date collected (snakes divided into groups; each group includes individuals collected during a five-year span). Samples the same as in Figure 3.

National Museum (USNM). The rattlesnake collections at the USNM are arguably one of the oldest and most intensively studied in the world; if frequent handling of pickled rattlesnakes damages their rattle strings, collections at the USNM should provide such evidence.

Our results fail to support the first half of Klauber’s caveat. There is no relationship between the date a specimen was added to the USNM collection and the number of rattle segments that specimen still retains, either for *C. viridis* ($r = 0.038$; $F_{1,207} = 0.30$; $P = 0.59$; Fig. 3a) or for *S. miliarius* ($r = 0.057$; $F_{1,101} = 0.32$; $P = 0.57$; Fig. 3b). Nor is there a greater fraction of rattlesless individuals among older specimens, either for *C. viridis* ($r = 0.250$; $F_{1,26} = 1.74$; $P = 0.20$; Fig 4a) or for *S. miliarius* ($r = 0.017$; $F_{1,22} = 0.006$; $P = 0.94$; Fig. 4b). For these samples, there appears to be little evidence of rattle breakage due to preservation and handling.

The flip side to Klauber’s caveat, that collectors may prefer collecting only those rattlesnakes with

longer strings, cannot be tested in the same manner. Instead, we can compare the average chain lengths for different species for which there exist both field and museum data. We have comparative data for eight such species (Table 2). If museum collections are biased towards specimens with long rattles, average chain lengths should be higher in collections than from field populations. Because the field data represent chain lengths recorded only from mature individuals, we delimited the museum data to those individuals larger than the minimum SVL of sexually reproductive females (values were obtained from Klauber, 1972; Ernst, 1992). A paired *t*-test shows no significant difference, for these eight species, between the average chain lengths of free-ranging vs preserved snakes (5.87 segments vs. 5.60 segments, respectively; $t = 0.711$; $df = 7$; $P = 0.50$). Indeed, there appears to be no consistent pattern across categories; five species show greater chain lengths in museum samples, while three show greater chain lengths in field samples (Table 2). These results fail to support Klauber’s “biased collector” warning.

These comparisons are coarse, as we know that geographically distinct populations of the same species of rattlesnake have different rattle chain characteristics (e.g., Table 3). A finer-grained analysis, then, would be to compare the rattle chains of free-living rattlesnakes with museum collections obtained from the same general locations. We have these kinds of data for *S. miliarius* from four populations in central Florida, and have compared their rattle-chain characteristics with a museum collection obtained from this same region (Fig. 5). This figure illustrates two points. First, individuals from the preserved collection have shorter rattle chains than free-living individuals (e.g., approximately 50% of the museum specimens have three or fewer segments, while the same proportion of wild individuals have four or fewer). This suggests, as Klauber cautioned, that there may be some segment loss in preserved rattlesnakes. Nonetheless, the percent of rattlesless snakes in the museum collection falls within the range we found for the four field populations. If preservation and handling lead to rattle loss, these data indicate that such loss may be confined to the more distal segments.

Collectively, our results highlight the utility of museum collections in studies of rattle chains. Although Klauber’s caveat should not be ignored, comparative investigations of rattle morphology can ill afford to not use the specimens in these valuable collections. Many rattlesnake species are rare and

Table 2. A comparison of mean rattle chain lengths in museum specimens to individuals from field studies for *Crotalus* and *Sistrurus*. Mean rattle chain length refers to the number of rattle segments.

Species	Mean rattle chain length		Source of field data
	Museum specimens	Field studies	
<i>C. atrox</i>	7.32	6.74	A. Holycross, pers. comm.
<i>C. horridus</i>	5.80	4.14	Keenlyne, 1978
<i>C. lepidus</i>	6.89	6.79	A. Holycross, pers. comm.
<i>C. ruber</i>	7.21	7.57	Klauber, 1972
<i>C. viridis</i>	5.64	5.09	Klauber, 1972
<i>C. willardi</i>	4.63	4.29	A. Holycross, pers. comm.
<i>S. catenatus</i>	4.15	5.97	A. Holycross, pers. comm.
<i>S. miliarius</i>	3.00	3.86	This study

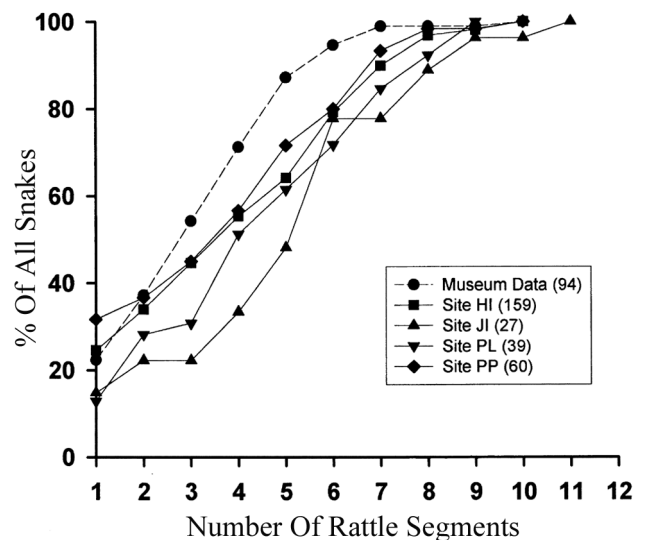
Table 3. Rattle characteristics for the three subspecies of *Sistrurus miliarius* derived from museum specimens. Table columns as in Table 1. Total sample size here (N = 355) is smaller than for *S. miliarius* in Table 1 (N = 378) as 23 individuals could not be positively assigned to subspecies. CV = coefficient of variation.

Subspecies	N	% snakes rattleless	Mean chain length	CV of chain length	Rattle size (mm)	% rattles broken at terminus
<i>S. m. barbouri</i>	78	25.6	3.22	0.61	2.81	4.2
<i>S. m. miliarius</i>	197	52.3	2.15	0.76	2.76	2.0
<i>S. m. streckeri</i>	80	21.3	3.54	0.60	2.47	14.0

inhabit regions where access is difficult. This is especially true for some Mexican species that may have the most to tell us about rattle evolution (e.g., *C. lannomi*, *C. pusillus*, and *C. stejnegeri*).

Intraspecific Comparisons of Rattle Chain Length

We collected data on the chain lengths of adult specimens for 20 species of rattlesnakes from museum collections (Table 1). For 16 of them, we had large enough sample sizes ($N \geq 20$) to plot cumulative frequency distributions (Fig. 6a–c). These data indicate that individuals of most species are rarely found with only a single segment. In contrast, *S. miliarius* (Fig. 6c), and to a lesser degree *C. pricei* (Fig. 6b), are unusual in that many individuals were “rattleless” (i.e., their rattle chain consisted of only the basal segment). Although the high rate of rattle loss in *C. pricei* deserves further investigation, there is no question that *S. miliarius* is an outlier with regard to segment retention. Although *C. pricei* is frequently rattleless, the average chain length for this species is almost twice as long as that for *S. miliarius* (5.29 vs 2.72 segments, respectively; Table 1). Moreover, approximately one-half of the *C. pricei* individuals possess chains of five or greater segments (Fig. 6b), whereas

**Fig. 5.** Comparison of cumulative frequency distributions of *Sistrurus miliarius* rattle chain lengths from museum specimens collected in central Florida, and data from field studies at four central Florida study sites. Sample sizes for each cumulative frequency distribution in parentheses.

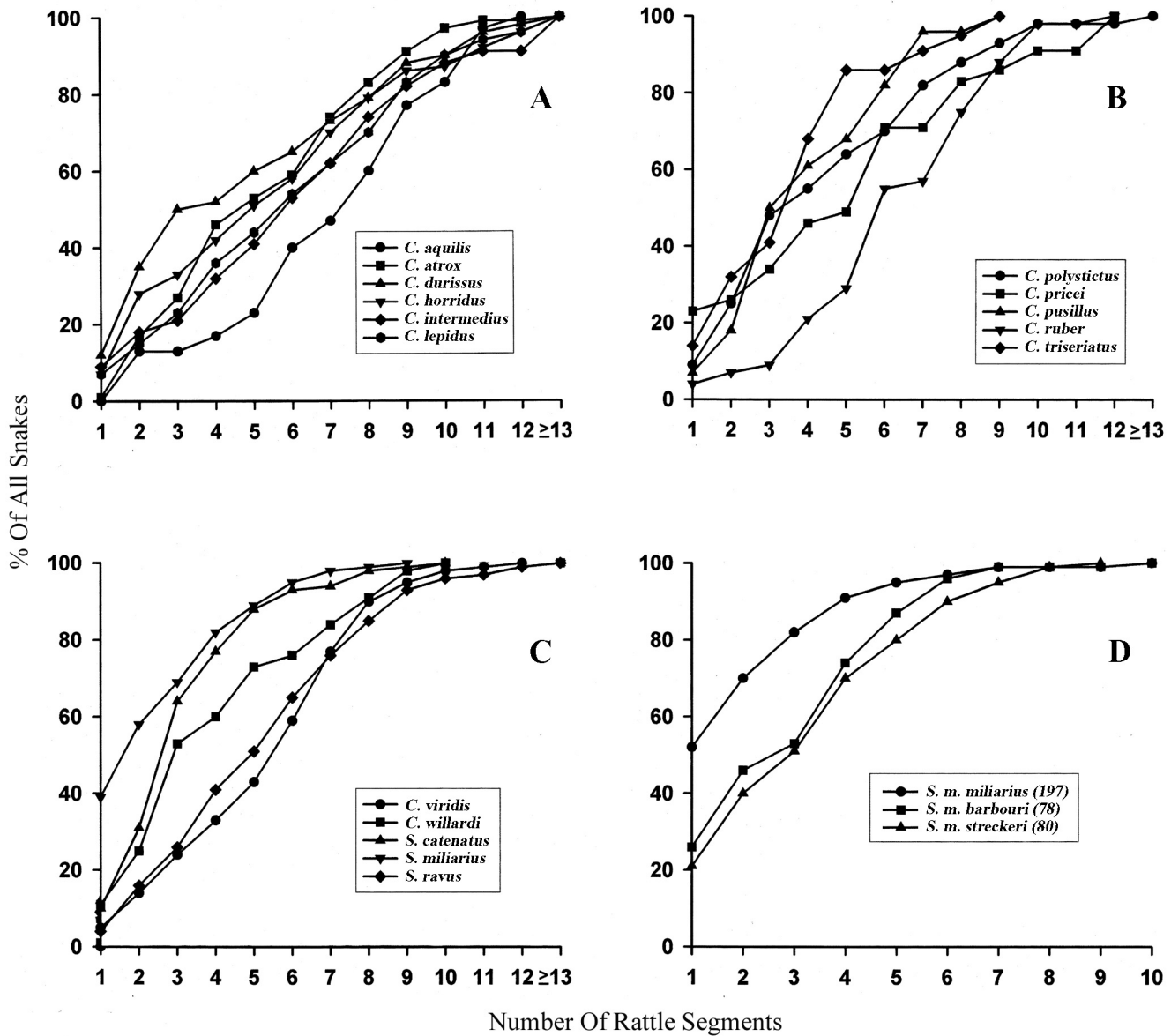


Fig. 6. (A–C) Frequency distributions of rattle chain lengths for 16 species of rattlesnakes. (D) Cumulative frequency distribution of rattle chain lengths for the three subspecies of *Sistrurus miliarius*. Samples represent museum specimens (Table 1).

one-half of the *S. miliarius* individuals have fewer than two (Fig. 6c).

It is worth noting that the high proportion of rattleless individuals in *S. miliarius* is not a geographically localized phenomenon; rattle loss appears to be common in individuals from all areas of the species' geographic range (Fig. 6d). However, individuals from the northeastern corner of this species' distribution (*S. m. miliarius*) have a higher probability of being rattleless than conspecifics from the southern (*S. m. barbouri*) and western (*S. m. streckeri*) parts of their range.

Why does *Sistrurus miliarius* have Short Rattle Chains?

There are several potential proximate causes for short rattle chain length in *S. miliarius*, including: (1) low rates of shedding in *S. miliarius* compared to other species of rattlesnake (a new segment is created with each shed), (2) populations of *S. miliarius* composed of very young snakes compared to other species of rattlesnakes, giving them less time to accumulate rattle segments, and (3) high rates of rattle segment loss in *S. miliarius* relative to other species of rattlesnakes.

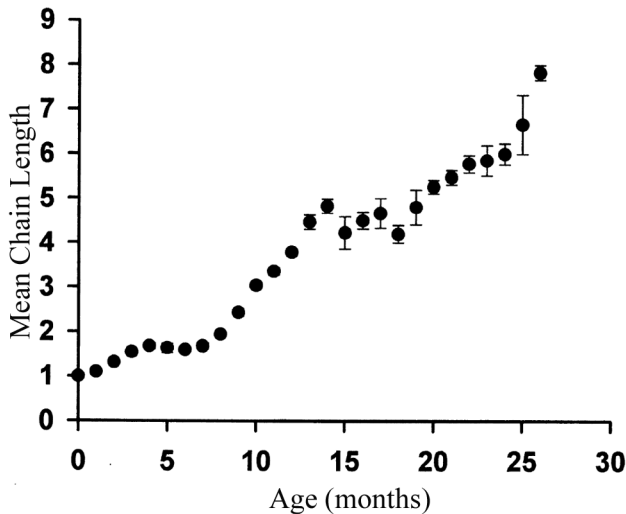


Fig. 7. Relationship between age and mean number of sheds (mean rattle chain length in snakes with complete chains) for a field population of *Sistrurus miliarius*. Error bars indicate \pm one standard error (in some cases bars are obscured by the symbol).

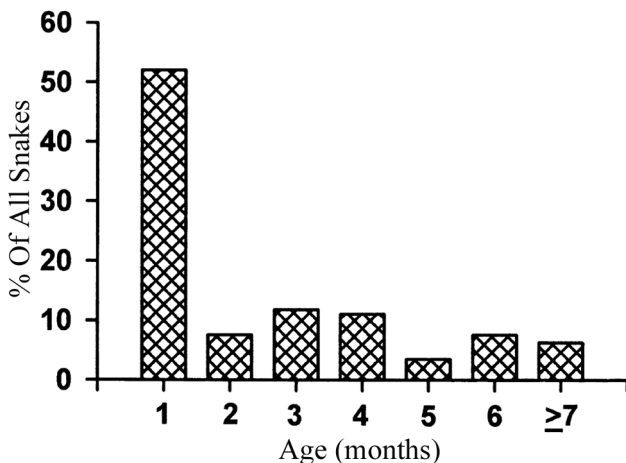


Fig. 8. Age structure of a field population of *Sistrurus miliarius* in 1994.

To determine the shedding rates of *S. miliarius*, we looked at the length of rattle chains in our field populations among known-aged snakes with complete, unbroken rattles (i.e., including the button; Fig. 7). A regression between snake age and chain length indicates that there is a strong linear relationship between these variables (chain length = $0.218 \times$ age in months + 0.884 , $r = 0.91$; $F_{1, 2377} = 11325$; $P \ll 0.001$). The slope of this regression indicates that *S. miliarius* typically completes ca. 2.6 sheds/year. This level of shedding frequency is higher than many other species of rattlesnakes. For example, Brown (1991) estimates that *C. horridus* typically completes between 1.26 to 1.58 sheds/year, depending on a snake's age. Using

data from captive individuals representing ten species, Klauber (1972) found that rattlesnakes averaged 2.3 sheds/year. Clearly, low rattle chain lengths in *S. miliarius* are not due to a low frequency of shedding.

We estimated the age structure of our field population of *S. miliarius* for the summer of 1994 (before the arrival of neonates). At that time there was a large cohort of 1-year-old snakes. *Sistrurus miliarius* exhibits unstable age structures due to annual variation in reproductive output by adult females (1993 was a year with high reproduction). This cohort constituted slightly more than 50% of the population (Fig. 8). There were, nonetheless, many snakes (43.9% of the population) that were at least 3 years old; Pygmy Rattlesnakes are not as long-lived as larger species of rattlesnakes (e.g., Brown, 1991; Fitch, 1985). However, given the relationship between age and number of sheds in *S. miliarius*, a large fraction of our study population (individuals three or more years of age) would have typically shed at least eight times and, therefore, had the potential to accumulate long rattle chains. Low rattle chain lengths in *S. miliarius* are not due to the age structure of the population.

Instead, rattle segment loss is the cause for low rattle chain lengths in *S. miliarius*. The relationship between age and mean rattle chain length in our field population (Fig. 9) shows that by the third year *S. miliarius* typically loses segments as rapidly as they are added by shedding. By the fourth year the rate of rattle segment loss typically exceeds the rate of segment addition by shedding, so that mean chain lengths decline as the snake ages.

Why Does *Sistrurus miliarius* Have High Rates of Segment Loss?

Segment loss: slippage vs breakage.—How might rattle segment loss occur? A rattle chain can break in one of two ways, which we will hereafter call “slippage” and “breakage.” In slippage, a full segment (and thus all more distal segments) slides off the lobes of its proximal segment, whereas in breakage, a rattle segment weakens and tears. In the latter case, the first lobe of the broken segment normally remains as a fragment or “ring” in between the first and second lobes of the more proximal segment (Fig. 1b). Both slippage and breakage result in incomplete rattle chains (chains lacking the button). We examined the terminal segments of incomplete rattle chains of snakes to determine if segment loss was due to breakage (in which case a segment fragment was attached to the terminal segment) or slippage (no

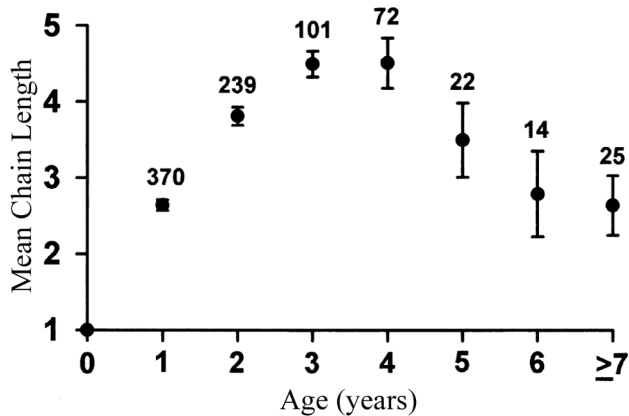


Fig. 9. Relationship between *Sistrurus miliarius* age and mean rattle chain length (number of rattle segments) for our field population. Sample sizes shown above each mean.

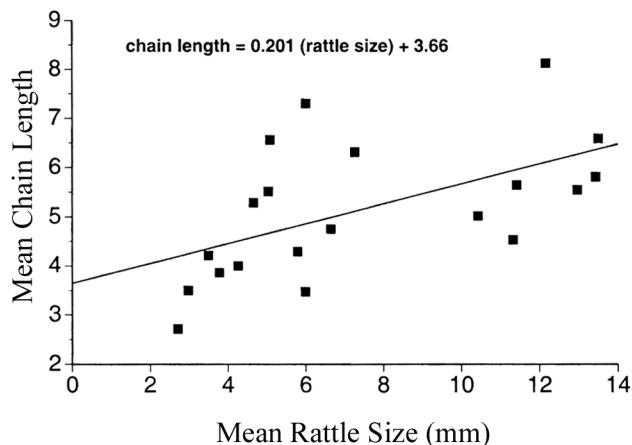


Fig. 10. Mean rattle chain length (number of rattle segments) as a function of average rattle size in each species (as measured by depth of exposed lobe of basal segment). Data from our museum samples (Table 1).

segment fragment present). Based on our museum data, *S. miliarius* had the second lowest breakage frequency of any species (2.7% of specimens with incomplete rattle chains experienced breakage, while 97.3% experienced slippage; Table 1). Only *C. mitchellii* had a lower frequency of breakage, a result that may be the product of a small sample size ($N = 17$).

Moreover, the low rates of breakage for *S. miliarius* are not an isolated geographical event, as all three subspecies had low breakage values (Table 3). In short, the high rates of rattle segment loss in *S. miliarius* result from segment slippage, and not segment breakage.

Allometric artifact.—The short rattle strings, high rates of rattlessness, and high rates of slippage in *S. miliarius* may simply reflect the fragility of this species' tiny rattle. Klauber (1940, 1972) has shown,

in *C. atrox*, that the thickness of the wall of a rattle segment increases with the size of the segment. Moreover, Klauber implies that interspecific differences in average chain lengths may result from this allometry (i.e., small species of rattlesnakes will have small, thin-walled rattles that are easily broken while larger species, with thicker-walled and more robust rattles, should possess longer rattle chains). Small species of rattlesnakes, therefore, might more frequently be rattless. We tested the "allometric artifact" hypothesis by regressing average chain lengths and percent of rattless individuals on average basal segment depth for each species. There is a significant relationship between a species' average chain length and its average rattle size, as measured by the depth of the exposed lobe of basal segments ($r = 0.554$; $F_{1,18} = 7.95$; $P = 0.011$; Fig. 10); this finding supports the allometric artifact hypothesis. However, there is only a weak, nonsignificant trend for small-rattled species to also have higher rates of being rattless ($r = 0.347$; $F_{1,18} = 2.46$; $P = 0.135$; Fig. 11). This nonsignificant trend is driven solely by the high rate of being rattless in *S. miliarius*. When *S. miliarius* is removed from the analysis, any hint of a relationship between the size of a species' rattle and its frequency of complete rattle loss disappears ($r = 0.199$; $F_{1,17} = 0.70$; $P = 0.414$). Thus, species with diminutive rattles may possess short chains because distal segments are fragile and easily lost. The fragility of tiny segments, however, does not typically lead to high rates of rattlessness. For example, neither *C. pusillus* nor *C. stejnegeri* have high rates of being rattless (*C. lannomi* is known from a single specimen), although both have small rattles and low average chain lengths (Table 1). *Sistrurus miliarius*, in other words, is frequently more rattless than we would expect, given its diminutive rattle morphology.

What proximate mechanism in *S. miliarius* causes the higher frequency of being rattless than in other small-rattled species? The answer appears related to differences among these species in the depth and robustness of the longitudinal groove separating the first and second lobes of each segment (i.e., the groove that locks older on to younger segments; Fig. 1c–d). There is an allometric trend in the depth of this groove. Species with small rattles tend to have significantly less well-developed grooves than species with large rattles; this trend is apparent when the rattles are viewed either from the side ($r = 0.776$; $F_{1,18} = 62.21$; $P < 0.0001$; Fig. 1c) or from above ($r = 0.779$; $F_{1,18} = 63.45$; $P < 0.0001$; Fig. 1d). Such allometry probably

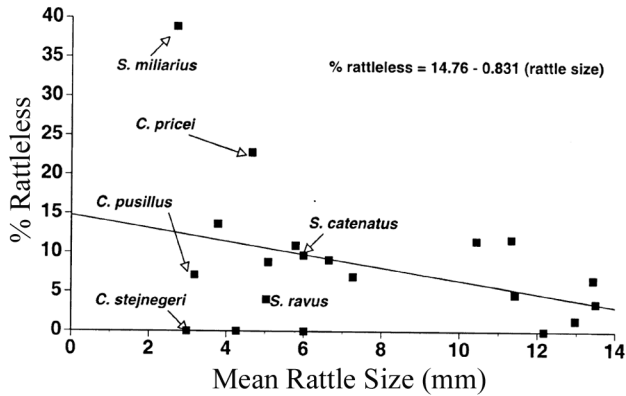


Fig. 11. Percent of individuals that possess no loose rattle segments as a function of that species' average rattle size (measured by depth of exposed lobe of basal segment). When *Sistrurus miliarius* is removed from the analysis, the already weak relationship ($r = 0.347$; $P = 0.135$) completely disappears ($r = 0.199$; $P = 0.414$). Data from our museum samples (Table 1).

explains why large-rattled species usually lose rattle segments by breakage, while small-rattled species lose them via slippage. The relative depth of this groove, however, might also explain the differences between *S. miliarius*, *C. pusillus*, and *C. stejnegeri*. The most dramatic disparity in groove morphology among these three small-rattled species is revealed from a dorsal view of the rattle (Fig. 1d). Both *C. pusillus* and *C. stejnegeri* have a moderate groove separating the first from second lobes of a rattle segment (mean scores of 1.500 and 1.348, respectively), while *S. miliarius* has extremely tapered second lobes with no groove (mean score of -0.356). Results from a one-way ANOVA show highly significant differences in groove morphology among these three species ($F_{2, 320} = 52.26$; $P < 0.0001$); *S. miliarius* has significantly shallower grooves than either *C. pusillus* or *C. stejnegeri*, which do not differ from each other (post hoc comparisons using a Tukey-Kramer HSD).

Is Rattle Chain Length Currently Subject to Natural Selection in *Sistrurus miliarius*?

Endler (1986) identified three necessary and sufficient conditions for natural selection. There must be: (1) variation in the trait of interest, (2) fitness differences resulting from this variation, and (3) heritability of the trait. We collected data on *S. miliarius* to determine if these conditions were met. There is great variation in chain length in *S. miliarius*. Indeed, the coefficient of variation for *S. miliarius* rattle chain length is higher than the other 19 species of rattlesnakes we measured (Table 1), with high levels of variation characterizing each of the three subspecies (Table 3).

There is clearly sufficient variation in chain length in *S. miliarius* for natural selection to occur.

Measuring fitness (lifetime reproductive success) is particularly difficult for long-lived species such as rattlesnakes. We investigated several fitness components (traits that often strongly influence fitness) to determine if there appeared to be any impact of rattle chain length on each fitness measure. These fitness components included measures of growth rate, survivorship, male reproductive success, and female reproductive success.

Impact of chain length on juvenile growth.—If the presence of a well-formed rattle enhances foraging success by increasing the effectiveness of caudal luring, we would expect young snakes with several segments to feed more frequently and, therefore, grow faster than conspecifics with only a single rattle segment. We investigated first-year growth by collecting SVL data on yearling *S. miliarius* (first year snakes captured between 1 June and 31 July, when they are approximately 10–12 months old). The mean SVL of yearling *S. miliarius* with a single segment was 26.5 cm (SE = 0.49; N = 62). The mean SVL of yearling snakes with two or more segments was 27.3 cm (SE = 0.23; N = 153). It appears that rattle chain length has little or no impact on juvenile growth rate since there was no statistically significant difference between these two means ($t = -1.75$; $df = 213$; $P = 0.0816$).

Impact of chain length on survival.—Chain length might influence survival since only individuals with more than one segment can make rattling sounds. To test this hypothesis we compared the survival rates of *S. miliarius* with only one segment with the survival rate of other members of the study population that had two or more segments. If a functional rattle is needed to warn predators, we would predict that mute snakes (those with only a single segment) would suffer greater mortality than snakes that can make rattling sounds (those with two or more segments). We used data on adult snakes in our study population that were observed in 1994 and determined if they were found again more than one year later. The percentage of snakes found one or more years later gives a conservative estimate of the percentage of adult snakes that survived for that period. Fifty percent (30 of 60 individuals) of snakes with a single segment in 1994 are known to have survived more than one year. Similarly, 51.5% (101 of 106 individuals) of the snakes with two or more segments in 1994 are known to have survived more than one year. There was no

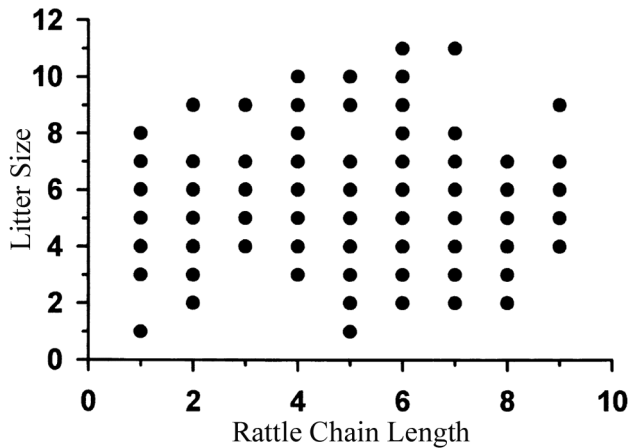


Fig. 12. Relationship between female rattle chain length and litter size in a free-living population of *Sistrurus miliarius*.

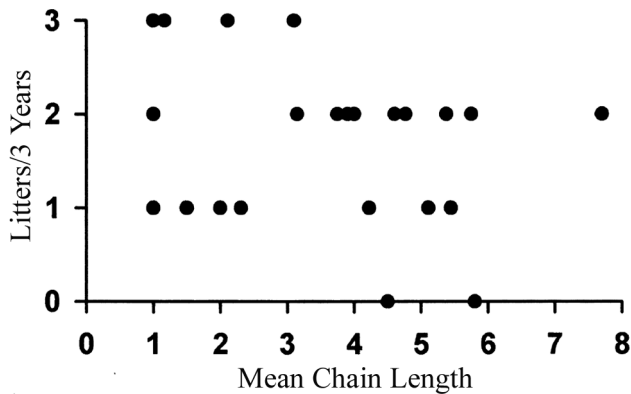


Fig. 13. Relationship between a female's rattle chain length (over a three-year period) and frequency of reproduction (number of litters produced during the three-year period) in a free-living population of *Sistrurus miliarius*.

significant difference among these groups ($\chi^2 = 0.0036$; $df = 1$; $P = 0.95$), indicating that rattle chain length does not appear to influence survival.

Impact of chain length on male reproductive success.—The mating season for *S. miliarius* extends from late August to January (May et al., 1996). During this period we often found adult males associated with (and often coiled on) adult females that were about to shed. Research on *C. viridis* indicates that accompaniment (i.e., a male trailing a female) always precedes copulation, and that accompaniment can be used as an index of male reproductive success (Duvall et al., 1992). To determine if associated male *S. miliarius* differed in rattle chain length from single males, we compared each associated male with two other males. These males were single and of the same age as the associated male captured closest in time, both before and after the capture of an associated male (sample

size of single males, therefore, was twice that of associated males). Mean rattle chain length for the associated males was 4.58 segments ($N = 33$) while the mean chain length for the single males was 4.02 segments ($N = 66$). There is no significant difference between these two means ($t = -0.997$; $df = 97$; $P = 0.32$) indicating that rattle chain length does not appear to influence male reproductive success.

Impact of chain length on female reproductive success.—We investigated three traits (the number of offspring per litter, hereafter referred to as litter size; the age at first reproduction; and the frequency of reproduction) that contribute to female reproductive success. We collected pregnant females several weeks before parturition and held them in field enclosures until they gave birth. Immediately following parturition, and after data were collected, the female and her young were returned to their site of capture (see Farrell et al., 1995). Each female was used only once in this analysis; for females with data from several litters, we randomly selected one litter for inclusion in the test. Correlation between the rattle chain length of the adult female and litter size was not statistically significant ($r = 0.03$; $F_{1, 135} = 3.94$; $P = 0.72$; Fig. 12).

The age of first reproduction often has a strong influence on fitness. Individuals that start reproducing earlier having higher lifetime reproductive success than those that begin reproducing at a later age (Stearns, 1992). There is significant variation in the age of first reproduction for female *S. miliarius*. In a small sample of known-age snakes, we have sufficient recaptures to determine the age of first reproduction (the age when they produced their first litter). Age of first reproduction ranged from 2 to 5 years (Table 4). There was no significant relationship between mean chain length for a female and her age of first reproduction ($r = 0.10$; $F_{1, 16} = 0.16$; $P = 0.69$). We should caution, however, that this analysis has little power given our small sample size.

There is also significant variation in the frequency of reproduction in female *S. miliarius*. Adult females often skip one or more years between parturition events (Farrell et al., 1995). To determine if rattle chain length influences the frequency of reproduction, we analyzed data only from females with high recapture rates. For 24 females we had enough recaptures to determine if that female reproduced in each of three consecutive years. Of the 24 females, two never reproduced, seven produced a single litter, 11 produced two litters, and four produced three litters. There was no significant association between the

Table 4. The relationship between age of first reproduction and mean rattle chain length in a free-living population of *Sistrurus miliarius*.

	Age at first reproduction (years)			
	2	3	4	5
Number of females	1	13	3	1
Rattle chain length	6.0	3.5	6.0	3.0

mean chain length for a female over the three year period and the number of litters she produced ($r = 0.26$; $F_{1,22} = 1.60$; $P = 0.22$; Fig. 13).

Is chain length heritable?—The repeatability of a trait (the degree to which that trait maintains a stable expression in an individual) sets an upper limit on heritability (Falconer, 1989). To determine the level of repeatability in rattle chain length we compared adult snakes from our field populations that had two captures separated by more than two years. The correlation between the initial rattle chain length for an individual and its later rattle chain length was low ($r = 0.248$), indicating that the heritability of rattle chain length was less than 0.25. To estimate heritability more directly, we compared rattle chain length in mothers with rattle chain length in their offspring, after the offspring had survived for more than one year in the field (and had a chance to add segments to their rattle chains). For 38 mother-offspring pairs for which we had data, there was no statistically significant correlation between maternal rattle chain length and offspring rattle chain length (offspring chain length = $0.0945 \times$ maternal chain length + 3.09; $r = 0.16$; $F_{1,36} = 0.94$; $P = 0.34$). The heritability of a trait is twice the slope obtained when using a mother-offspring regression (Falconer, 1989). In this case our estimate of the heritability of chain length (0.189 with a standard error of 0.195) is not significantly greater than zero, suggesting little or no heritability for this trait.

In the field population of *S. miliarius* that we studied, there does not appear to be current selection on rattle chain length. While there is great within-population variation in this trait, the variability does not appear to influence fitness and does not appear to be heritable.

GENERAL DISCUSSION

Our combined results suggest that *S. miliarius* should be added to the list of so-called rattleless rattlesnakes, a label previously applied only to *C. catalinensis* and *C. r. lorenzoensis* from the Gulf of

California. While *S. miliarius* is not as frequently mute as *C. catalinensis*, the high rate of rattle loss in this species (38.9% overall, Table 1; 52.3% for *S. m. miliarius*, Table 3) is comparable to that reported for *C. r. lorenzoensis* (54.0%; Radcliffe and Maslin, 1975).

The mechanistic explanation for such high rates of rattle loss in *S. miliarius* appears to be the poorly developed longitudinal groove separating the first and second lobes of the rattle, especially the lateral sections of this groove as viewed from above (Fig. 1d). Indeed, *S. miliarius* has little or no constriction in this plane. With nothing to “snag” the older segments during ecdysis, the entire rattle chain simply slips off the basal segment, rendering the snake mute. High rates of rattle loss in *S. miliarius* are not an allometric by-product, as two other species possessing diminutive rattles (*C. pusillus* and *C. stejnegeri*) have rattle loss rates (7.1 % and 0.0 %, respectively) typical of other rattlesnakes (Table 1). Not surprisingly, the rattles of both of these species have grooves that, in their lateral dimensions, are significantly deeper than the grooves in *S. miliarius*.

Evolutionary implications.—It is important to explore the question whether the condition of being rattleless in *S. miliarius* is ancestral or derived. Several phylogenies place the genus *Sistrurus* as basal to all rattlesnakes (Knight et al., 1993; Murphy et al., this volume). The tiny, easily shed rattles of *S. miliarius* might thus be considered plesiomorphic. What are the implications for understanding rattle evolution if *S. miliarius* represents the ancestral condition? Such a scenario would present problems for the warning hypothesis. Our field data suggest that *S. miliarius* is reluctant to use its rattle to frighten enemies. Moreover, our museum results show that they are often mute (38.9% overall; 52.3% for *S. m. miliarius*), having lost all free segments of their rattles. Last, mute individuals survive as well as those that can “sound off” when they rattle (50.0 vs 51.5% yearly survival, respectively). Thus, predation is no more intense on *S. miliarius* that are incapable (or much less capable) of generating aposematic sounds than on those who can. The rattles of this species thus appear unimportant as warning devices.

If the rattles of *S. miliarius* are plesiomorphic, our results may support the hypothesis that rattles evolved to enhance caudal luring or to protect vulnerable tails during luring. The rattle origin scenario of Schuett et al. (1984) suggests that the segmented, keratinous tail tip of a proto-rattlesnake might effectively mimic the insect prey of frogs and lizards. Randall Reiserer

(pers. comm.) suggested that a simple, bulbous end scale (e.g., a rattlesnake's button) would itself resemble an arthropod head; bilobation of the button might further enhance the appearance of segmentation. Sisk and Jackson (1997) make a similar but less explicit argument. In other words, the "caudal luring hypothesis" for rattle evolution may not require that rattlesnakes retain multiple segments of their rattle; longer chains may add to the optical attractiveness of the lure, but this is not required. In this scenario, the fitness of a proto-rattlesnake would not necessarily be enhanced by the retention of older rattle segments. This hypothesis, of course, begs the question of what selection pressures eventually favored the retention of such segments, thereby generating rattle chains. Nonetheless, Reiserer's idea deserves more attention, and our findings do not contradict his suggestions. For example, results from our field studies show no significant relationships in *S. miliarius* between several measures of fitness and rattle chain length or the presence of at least one loose segment. Perhaps all that is important for *S. miliarius* now, and for proto-rattlesnakes in the Miocene (Holman, 1979), is the presence of an enlarged terminal scale. Similar arguments might apply to the hypothesis that rattles evolved to prevent injuries to tender tail tips; a bulbous end scale may be all that was necessary to minimize vertebral damage delivered by bites from either duped prey or distracted predators. Regardless, if the rattles of *S. miliarius* are primitive, both their current and historical utility appears to have had little to do with sounding a warning.

Several arguments, however, suggest that rattle loss in this species is derived. First, the phylogenies that place *Sistrurus* as basal to *Crotalus* (Knight et al., 1993; Murphy et al., this volume) show *S. catenatus* as a sister species to *S. miliarius* (with *S. ravus* as a basal member of *Crotalus*). Yet, *S. catenatus* possesses neither tiny rattles (Cook et al., 1994) nor high rates of rattle loss (9.7%, Table 1). The second argument relates to the proposal that rattlesnakes evolved in the highlands of Mexico (Klauber, 1972; Greene, 1997). Given this perspective, the current geographical distribution and habitat preferences of *S. miliarius* (e.g., various riparian habitats in the southeastern United States; Ernst, 1992) are recently evolved in this clade. Third, the evidence that we have presented, both here and earlier (Cook et al., 1994), shows that the rattle of *S. miliarius* is nearly useless as a warning device. Nonetheless, the tail muscles of *S. miliarius* appear specialized for the fast-twitch vibrations of rattling

(Moon, 2001). The most parsimonious explanation of this paradox is that the tiny, barely audible, and frequently lost rattle segments of *S. miliarius* is an apomorphic condition, perhaps the result of relaxed selection from predation (see below). Not only can relaxed selection lead to the disintegration of complex adaptations, but also the rate of decay may differ dramatically among different components of the trait (e.g., Coss, 1999). In short, *S. miliarius* may still possess the physiology for rattling, even though the effectiveness of this behavior is precluded by the degenerative morphology of their rattle. Last, our previously mentioned results showing no significant fitness differences between *S. miliarius* that can and those that cannot rattle. The lack of fitness differences between mute and audible individuals is consistent with the idea that the rattle of *S. miliarius* is derived, disintegrating, and nonfunctional. The implications of this scenario for understanding the original function of rattles, that the rattle of *S. miliarius* is apomorphic, are problematic but provocative. For example, we would need to know whether the shrunken lobes and shallow constrictions of pygmy rattles represent atavisms or evolutionary novelties (e.g., McCune, 1990), a difficult question given the poor fossil record for rattlesnakes (Holman, 1979).

Vestigialization.—What processes can lead to the loss of a previously functional trait? Fong et al. (1995) provide an excellent review and offer three scenarios that might produce vestigialization. First, the trait may be under relaxed selection. Mutations affecting the trait are no longer subject to natural selection, accumulate through genetic drift, and ultimately lead to the eventual loss of the structure. Reduction in the eyes of cave-dwelling organisms may be such an example (Culver, 1982). Second, the trait is actively selected against. Diving birds, for example, often have small wings and reduced pulmonary air sacs. These are apparently adaptations that reduce buoyancy and, thereby, enhance the ability of these birds to swim underwater (Gill, 1995). Third, the trait may not itself be maladaptive, but instead is indirectly selected against because, if nothing else, the trait costs energy to develop and maintain. In secondarily flightless insects, for example, females are more often wingless than males, possibly because not producing wings increases the energy available for egg production (Roff, 1990). The three processes are not mutually exclusive and can be difficult to separate, as demonstrated by the loss of venom resistance in California Ground Squirrels (*Spermophilus beecheyi*) no longer

subject to predation from rattlesnakes (Poran et al., 1987; Biardi et al., 2000). This loss of resistance may simply be due to relaxed selection and subsequent genetic drift, or to indirect selection because of metabolic or immunological costs associated with producing serum proteins providing unneeded resistance to rattlesnake venom.

Which of the three scenarios best accounts for rattle loss in *S. miliarius*? Is selection, for instance, favoring mute individuals? Small *C. v. oregonus* are at a disadvantage when confronting sophisticated predators that use rattling to assess the body size and body temperature of the snake (Rowe and Owings, 1978, 1990, 1996; Swaisgood et al., 1999). Pygmy Rattlesnakes are not only small, but they also have abnormally small rattles (Cook et al., 1994). The rattling sounds they produce could invite injury or death by indicating their small size to a Gray Fox (*Urocyon cinereoargenteus*) or Bobcat (*Felis rufus*). Perhaps this is why *S. miliarius* in this study rarely rattled when aggressively provoked by humans. We caution, however, that the defensive behavior of rattlesnakes appears to be risk-sensitive (e.g., Duvall et al., 1985; Rowe and Owings, 1990, 1996; Clarke et al., 1996; May et al., 1996; Kissner et al., 1997). Humans may represent a threat so formidable that the best tactic for *S. miliarius* is crypsis, retreat, or head-hiding. The “selection against rattles and rattling” hypothesis would be more strongly supported if *S. miliarius* rarely rattled when confronted by important predators in their evolutionary history.

In contrast, two of our results strongly contradict predictions made by the “direct selection” and “indirect selection” hypotheses, but are consistent with the idea that rattle loss in *S. miliarius* is a product of relaxed selection. As Endler (1986) noted, a trait undergoing selection must be heritable and variable, and the variability must have fitness consequences. Highly selected traits typically exhibit low variability and low heritability, as the additive genetic variance will have been weeded out by selection (Mousseau and Roff, 1987). Thus, a trait experiencing relaxed selection should be highly variable, often exhibit high heritability, and the variability should have little if any effect on individual fitness. Our results show there are no significant differences between the fitness of *S. miliarius* that can or cannot produce rattling sounds. Mute individuals grew as quickly and survived as well as individuals that could rattle. There were no differences in average chain lengths between males that accompanied females and those that did not. None of the measures

of female fitness that we recorded were correlated with rattle chain length. Furthermore, the variability in rattle chain length is higher in *S. miliarius* than in any other species we measured (Table 1). The lack of fitness consequences and high phenotypic variability in chain length are exactly what we would predict if *S. miliarius* was no longer undergoing selection for functional rattles. The fact that chain length in *S. miliarius* shows low heritability is a bit perplexing but may not contradict our argument. We suspect, even with the poor lobe and groove morphology evident in rattles of *S. miliarius*, that there is a large stochastic component to rattle loss in this species. In contrast to middle-aged snakes, young snakes have short chains because they require time to accumulate many segments (Fig. 9). Older snakes have short chains not only because they have had more opportunity to lose their rattle during shedding, but also because longer chains may slip off more easily when being shed. These non-genetic chance influences on chain length could generate the low heritability and repeatability estimates we have observed in *S. miliarius*.

We propose that *S. miliarius* is losing its rattles due to relaxed selection—but relaxed selection from what? The answer to this question might provide insight into the current function, and possibly, the historical genesis of the rattle. Three currently popular hypotheses are that the rattle evolved to enhance caudal luring, to better warn away enemies, or to distract attention to the snake’s more expendable tail and away from the head. Our results contribute, we believe, to a growing body of evidence that casts doubt on the first of these hypotheses. Moon (2001) argued that the slow, wavy motions associated with caudal luring do not require the specialized muscle physiology found in rattlesnake tails and, therefore, that defensive tail vibration must be the precursor to rattling. Sisk and Jackson (1997) have shown that an artificial and mechanically waved bilobed tail tip, a structure they consider a necessary prerequisite to the evolution of a rattle chain, does not appear to enhance caudal luring. Here, we demonstrate that the presence or absence of a rattle chain contributes nothing to the fitness of *S. miliarius*, even though this species is known to caudal lure (Jackson and Martin, 1980; Rabatsky and Farrell, 1996; Rabatsky, 2002). Specifically, our results show that rattles do not significantly influence growth rates (and thus, we argue, the foraging success by caudal luring) of juvenile *S. miliarius*. If rattles enhance luring, we would expect to have found evidence that juvenile *S. miliarius* (the

age group most dependent on luring; see Rabatsky and Farrell, 1996; Rabatsky, 2002) actually benefit from possessing rattles. The main assumption of the stimulating article by Schuett et al. (1984), that the rattle evolved to enhance luring, lacks strong support.

There is, however, another idea mentioned by Schuett et al. (1984) that deserves additional examination. These authors suggest that a proto-rattle may have protected a snake's tail from bites by caudally-lured prey. A proto-rattle might, as we have argued, also have functioned to protect the tail from bites by enemies during defensive encounters, enemies whose attention had been diverted away from the snake's head to its rapidly vibrating tail. Several findings provide at least tentative support to this "protect-the-tail-tip" idea. Tail injuries are often fatal, especially to small snakes (Willis et al., 1982). Species such as Rubber Boas (*Charina bottae*) use their tails to absorb bites from attacking female rodents, particularly female mice attempting to protect their pups from being eaten by the boa (Nussbaum and Hoyer, 1974; Hoyer and Stewart, 2000). The fused caudal vertebrae in *Charina* and other species have been suggested to be an adaptation that better absorbs such attacks (Greene, 1973). Rattlesnakes also have fused caudal vertebrae, variously referred to as the shaker or style (Zimmermann and Pope, 1948; Klauber, 1972) that underlie the matrix that produces the rattle. In staged encounters between Rock Squirrels (*Spermophilus variegatus*) and Western Diamond-backed Rattlesnakes (*C. atrox*), squirrels occasionally attack the snake (Owings et al., 2001), and often direct their attack at the snake's rattle and tail (M. Rowe, unpublished). It seems plausible that a bilobed proto-rattle might have provided protection from bites delivered by hungry prey or protective rodent parents, and that chains of interlocking rattle segments would be even more protective. *Sistrurus miliarius*, however, often feeds on small lizards and frogs, species that are unlikely to inflict much damage when they bite. Thus, the frequent loss of rattles in *S. miliarius* may reflect relaxed selection from the need to protect their tails from mammalian teeth.

The last alternative is that rattles evolved because they effectively warn enemies that the snake is venomous and should be avoided. The persuasive arguments of Klauber (1972) and Greene (1988) have led most biologists to favor this hypothesis. Results from this study do not, unfortunately, provide much help in distinguishing between the "aposematic" and "protect-your-tail" hypotheses for the evolution of rattles.

Our results may, however, suggest future investigations. Of particular interest here are the very low rattle loss rates of *C. pusillus* and *C. stejnegeri*. Both species have rattles that are not only absolutely small but, like *S. miliarius*, are disproportionately tiny even when scaled to the diminutive body sizes of both species (M. Rowe, unpublished). What selection pressures still exist in Mexico that favor rattle retention in these small-rattled species? Are White-nosed Coatis (*Nasua narica*) in Mexico more potent predators than Raccoons (*Procyon lotor*) in Florida? Do *C. lannomi*, *C. pusillus*, and *C. stejnegeri* use their tails to lure prey, perhaps robust species of lizards that have more damaging bites than the anurans and small lizards attracted by *S. miliarius*? A good deal of exciting comparative work must be conducted before we know whether "rattleless" *S. miliarius* have been released from the need to either warn away enemies or protect their tails from bites.

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