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Toe-Clipping Dramatically Reduces Clinging Performance in a Pad-Bearing Lizard (*Anolis carolinensis*)

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ABSTRACT.—**Toe-clipping is a simple and widely used method for permanently marking small lizards. Although some studies have shown negligible effects of toe-clipping on locomotor performance and survival in terrestrial lizards, less is known about effects of toe-clipping on arboreal lizards. We evaluated effects of toe-clipping on clinging performance of the small arboreal, pad-bearing lizard *Anolis carolinensis*. We also examined how clinging ability scaled with size within this species. We measured clinging ability in the same lizards with unclipped toes and with one (two toes clipped total) and two toes (four toes clipped total) clipped per each forelimb. We found that clinging ability decreased dramatically even after clipping only two toes (about a 40% decline) and even more dramatically after clipping four toes (about a 60% decline). We also found that clinging ability scales isometrically with body size within unclipped anoles. Because clinging ability was measured on a smooth substrate and was not influenced directly by claws, toe-clipping appears to directly affect toepad function. Toe-clipping may affect clinging ability because of the severing of a tendon that plays a key role in toepad function. Thus, we suggest that researchers should be cautious before applying toe-clipping as a marking technique to pad-bearing lizards.**

The ability of arboreal lizards to effectively cling to surfaces can influence their ability to escape predators, find food, and defend territories (Hecht, 1952; Irschick et al., 1996; Zani, 2000; Autumn et al., 2000, 2002). Subdigital toepads have likely evolved independently several times within geckos and at least three times across all lizards, although structural differences exist among these toepad types (Ruibal and Ernst, 1979; Williams and Peterson, 1982). Previous studies have examined the functional anatomy of toepads (Ruibal and Ernst, 1979; Williams and Peterson, 1982; Peterson, 1983; Bels and Theys, 1989), gross toepad mechanics (Hora, 1923; Hiller, 1975; Losos, 1990; Irschick et al., 1996), and the properties of single setae (Autumn et al., 2000, 2002). Here, our goal is to evaluate the effects of toe clipping on clinging performance in the Green Anole (*Anolis carolinensis*) and to examine how clinging ability scales with size within a species.

Previous inter- and intraspecific scaling studies in lizards have examined such variables as maximum sprint speed, stride frequency, and stride length (e.g., Garland, 1985; Marsh, 1988), but few studies have rigorously examined how clinging ability scales with size within any arboreal pad-bearing lizards (but see Zani, 2000, 2001). Previous studies have compared the different clinging capabilities across widely divergent invertebrate and lizard species that possess adhesive structures (Autumn et al., 2000; Arzt et al., 2003; Gao and Yao, 2004). Some work has shown that clinging ability scales with positive allometry when comparing divergent groups (geckos, anoles, skinks) (Irschick et al. 1996) but isometrically when comparing different anole species (Elstrott and Irschick 2004). In these two examples, the expected slope for comparing clinging ability (y-variable) versus body mass (x-variable) was 0.67, if one assumes that clinging ability should be proportional to toepad area. Indeed, interspecific variation in toepad area among widely divergent pad-bearing species explains about 96% of the variance in clinging ability (Irschick et al. 1996). Here, we examine how clinging ability scales with body length (snout–vent length; SVL) within the Green Anole, which amounts to an expected slope of 2.0 (again assuming that clinging ability is proportional to toepad area).

The manner in which clinging ability scales with size is potentially important to arboreal lizards because it describes the relevant ratios of clinging force divided by size for large and small lizards. However, intra- and interspecific scaling may not be equivalent; hence, it is important to verify these results by examining different individuals within a species. Reptiles are good

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subjects for intraspecific scaling studies because they exhibit indeterminate growth that results in a large range in sizes of juveniles and adults (Maxwell et al., 2003). Furthermore neonate reptiles are born with fully developed motor skills and, because of often intense predation pressure, must perform adequately from the time of hatching (Carrier, 1996; Van Damme and Van Doren, 1999).

An unresolved issue is how functional capacities, such as clinging ability, are affected by marking techniques used for small reptiles. Indeed, a difficult problem for herpetologists is how to permanently mark small reptiles (i.e., <5 g) for use in mark-recapture studies. Techniques such as radio transmitters (e.g, Reinert, 1992) and passive integrated transponder (PIT) tags are difficult to place in such small reptiles. Ideally, permanent marks should allow individual identification and should not affect performance or behavior (Ferner, 1979). Thus, previous researchers have used toe-clipping as an inexpensive and practical way to permanently mark small reptiles. Indeed, by clipping variable numbers of toes on various feet, thousands of combinations are possible (e.g., Ferner, 1979; Dunham et al., 1994; Paulissen and Meyer, 2000). Some authors have found that toe-clipping does not affect maximum speed in various terrestrial lizard species (Huey et al., 1990 Dodd, 1993). However less research has examined how toe-clipping affects whole-organism performance capacities in arboreal lizards, although some work by Mahendra (1941) suggests that the removal of claws may affect the ability of geckos to effectively climb surfaces. We examined arboreal Green Anoles (*Anolis carolinensis*) to address two questions: (1) How does clinging ability scale with size within this species? (2) Does toe-clipping affect clinging ability? Therefore, we compared the clinging abilities of Green Anoles when their toes were unclipped and with two and four toes clipped.

Materials and Methods

All the lizards used for the experiments were collected on the Tulane University campus, located in New Orleans, Louisiana, between 9 February 2004 and 17 March 2004 and then transported to the laboratory where they were maintained in 38-liter glass aquaria, in groups of 2–3 lizards per aquarium. Lizards were fed vitamin-dusted crickets ad libitum and misted with water daily. We captured a total of 80 Green Anoles ranging in SVL from 29.9 mm to 70.6 mm. We sexed lizards based on the presence or absence of sexual characters. Juveniles were lizards that did not display obvious sexual characteristics (e.g., an enlarged dewlap). Adult females had a narrow tail base, and a reduced dewlap area and had an SVL $>$ 40 mm. Adult males had an SVL $>$ 45 mm and exhibited an enlarged dewlap area and an enlarged tail base. Lizards were weighed on a Denver Instrument Co. precision scale (accurate to 0.001 g) and then measured with digital calipers to obtain their SVL. Prior to clinging experiments, lizards were placed in an incubator (Percival Co.) at 30°C for at least 45 min, which is similar to the preferred body temperatures of Green Anoles (Irschick et al., in press.).

Clinging experiments were completed using a custom designed force plate ($30 \times 18 \times 1$ cm). The output of the strain gauges was sent to a K & N Scientific 12-Bridge, eight channel amplifier and subsequently A-D converted at 10 kHz (Instrunet, model 100B). Digital signals were read into a G4 Macintosh computer using Superscope II version 3.0 software. A transparency sheet was attached to the force plate because this surface promotes maximum clinging ability and has been used in other studies (Irschick et al., 1996; Elstrott and Irschick, 2004). For each trial, the lizard was removed from the incubator and placed with its front feet on the acetate sheet and dragged horizontally several times for 30 sec (as in Irschick et al. 1996; Elstrott and Irschick, 2004). After the completion of each clinging trail, the lizard was returned to its cage, and the five highest clinging values were recorded. We estimated the pulling speed to be approximately 5 cm s^{-1}, and only one investigator (Bloch) conducted these clinging trials to ensure consistency. Slight differences in the velocity of dragging do not affect force output (values of horizontal drag recorded by the computer), but rapid accelerations can potentially affect it (K. Autumn, unpubl. data). In other words, we made sure not to jerk or yank the lizards. We considered that a lizard was performing at its maximum capacity if its forelimbs were fully extended. Any trial that did not meet this criterion was discarded.

One potential confounding factor is the potential influence of claws in our measurement of clinging force. We have several reasons to believe that the claws do not contribute to our measure of adhesion using acetate sheets on a force platform. First, in many of the trials, including those in which anoles produced a strong clinging effort, the claws did not touch the acetate surface. Therefore, the clinging force from these individuals, for example, could not have occurred because of the claws. When the claws did touch the surface, they never engaged the surface but simply slid along the surface. Indeed, our own behavioral observations and force tracings show that the claws themselves do not engage the surface. A pattern of gripping with the claws should result in an irregular force tracing as the claws are engaged, become disengaged, and then engaged. By contrast, our force traces were uniformly smooth and, hence, on this basis alone would not suggest gripping. Finally, Irschick et al. (1996) used a similar design to examine clinging in 14 species of geckos, anoles, and skinks, all of which possess claws. Indeed, within that group, the anoles and skinks clearly have much more well-developed claws compared to the geckos, yet a regression between toepad area and clinging ability yielded an r-squared value of 0.96 (see fig. 3A of that paper). In other words, nearly all (excluding a reasonable 4% that could be attributed to inherent biological error) of the variance in clinging ability among these widely disparate groups can be attributed to variance in toepad area.

We only clipped toes on the forelimbs because we only examined the ability of the forelimbs to cling. We clipped toes using sharp scissors to remove the claw at the anterior base of the toe pad. Only the third and fourth toes (from a ventral perspective), which are the longest toes, were clipped. We first evaluated each lizard's clinging ability before clipping any toes (unclipped) and randomly clipped either the third or fourth toe (two toes clipped) on each forelimb and repeated the clinging trials. Finally we clipped the remaining toe (so that both the third and fourth toes on each forefoot were clipped for a total of four toes) and tested each lizard one more time (four toes clipped). This design allowed us to compare the clinging abilities of lizards with no toes clipped, two toes clipped, and four toes clipped. For each of these three treatments, we conducted two separate clinging trials on the same day, and the three different treatments were conducted on consecutive days. Lizards were allowed to rest for a minimum of two hours between each of the clinging trials.

To eliminate the possibility of time or exhaustion affecting our data, we conducted another experiment in which clinging ability was also examined on three consecutive days but without toe clipping. For this experiment, 20 different lizards were tested in the same manner (two trials a day for three consecutive days) without clipping any of their toes. We conducted an analysis of covariance (ANCOVA) to compare log-transformed clinging ability across each treatment (unclipped, two toes clipped and four toes clipped), using log-transformed SVL as a covariate. We also conducted an ANCOVA for the control data comparing log-transformed clinging ability for each day again using log-transformed SVL as a covariate. Finally, we tested for age/sex effects by conducting an ANCOVA on log-transformed clinging ability using age/sex class (males, females, and juveniles) as our treatment, and log-transformed SVL as the covariate.

Choosing the appropriate regression techniques for scaling analyses is a controversial issue (LaBarbera, 1989; Irschick et al., 1996). The most commonly used regression is the ordinary least square regression. However this technique may underestimate the slope if systematic error exists in the independent variable (in this case SVL; LaBarbera, 1989). One can correct for such bias by estimating the error in the independent variable and dividing the calculated least-squares regression slopes by the correlation coefficient from consecutive measurements of the independent variable (Moore and Ellers, 1993; Irschick et al., 1996). We estimated the error in our independent variable by measuring SVL twice in 29 lizards and dividing the calculated slope by the Pearson correlation coefficient (0.99) between those two measures (slope = 1.03, SE = 0.01). All data analyses were performed using SYSTAT (vers. 10, SPSS 2000).

Results

Our results showed that toe-clipping significantly decreased the clinging ability of Green Anoles (Fig 1). We pooled males, females, and juveniles together for all statistical analyses since age/sex class did not significantly affect clinging ability prior to toe-clipping (ANCOVA, slopes test: $F_{2,75} = 0.38, P > 0.05$; y-intercepts test: $F_{2,75} = 0.79$, $P > 0.25$). The ANCOVA test comparing clinging ability independently of SVL showed significant differences among the y-intercepts of the three experimental groups (Fig. 1A; slope tests: $F_{2,164} = 1.76$, $P > 0.10$; y-intercepts test: $F_{2,164} = 87.59$, $P < 0.001$). Therefore, clinging ability significantly decreases as additional toes were clipped, independently of SVL. Further, there was no significant decrease in clinging ability across different days when lizards were unclipped (Fig. 1B; ANCOVA, slopes test: $F_{2,59} = 0.12$, $P > 0.25$; y-intercepts test: $F_{2,59} = 1.05$, $P > 0.25$). Therefore, toe-clipping exerts a negative effect on clinging performance regardless of the potentially confounding effects of time.

Among all individuals (males, females, and juveniles combined), we obtained a slope of 1.95 (Fig. 2). The corrected slope (see Materials and Methods above) was 1.96 ± 0.23 (y = 1.96–7.27, $r = 0.71$). This slope did not differ significantly from the expected slope of 2.0 ($t = 0.17$; $P > 0.05$). Therefore, clinging ability scales isometrically with size within Green Anoles.

Discussion

The manner in which whole-organism performance capacity scales with size may have important implications for the ecology of arboreal lizards species (e.g., Emerson and Arnold, 1989). If large lizards cannot cling effectively, for example, then this might limit their ability to use certain microhabitats. Our data show that clinging ability scales with size within Green

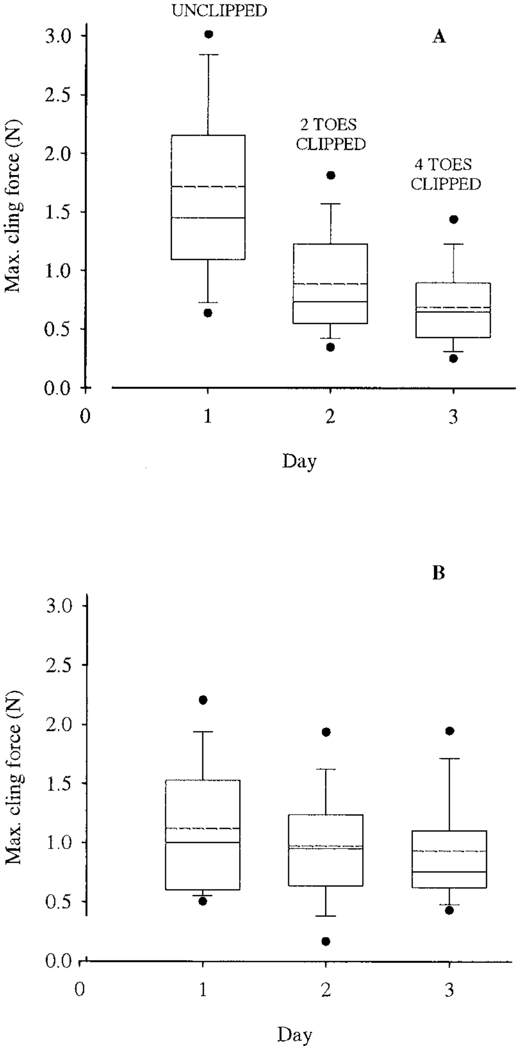

FIG. 1. (A) Effects of toe-clipping on the nonsize-adjusted clinging ability of lizards and (B) the time effect for the control group. The x-axis represents the day on the different treatments of the experiments (performed on three different days). The boxes indicate the 25th and 75th percentile and the wiskers indicate the 5th and 95th percentiles. The solid line inside the box represents the median and the dashed line marks the mean.

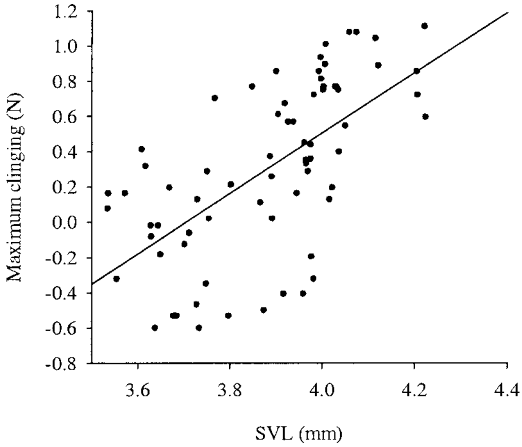

FIG. 2. A scatterplot of log-transformed clinging ability (y-axis) versus log-transformed SVL (x-axis) in *Anolis carolinensis*. Each point corresponds to an individual lizard. The regression is an ordinary least-square line. See text for statistics.

Anoles in accordance with a priori expectations of isometry (expected slope = 2.0, actual slope = 1.96). This result stands in contrast to the positive allometry of clinging ability when comparing across the three lizard groups that possess toepads (geckos, anoles, and skinks; Irschick et al., 1996) but is consistent with the isometry of clinging ability observed among anole species (Elstrott and Irschick, 2004).

Our results also show that toe-clipping dramatically reduces the ability of lizards to cling. Lizards suffered, on average, a 48% clinging decrement when we clipped two of their toes on the forelimbs and about a 60% clinging decrement when we clipped four of their toes on the forelimbs. We suggest that such a performance decrement could have profound effects on the ecology of pad-bearing lizards. Consider the fact that many lizards suffer high rates of toe loss (e.g., Hudson, 1996), suggesting that, if the same trend occurs with *A. carolinensis*, then some lizards will be much better clingers than others. Because of the importance of toe-pads for clinging, one might predict that arboreal lizards with large numbers of missing claws or toes might be at a strong selective disadvantage relative to lizards with a complete set of toes and claws (Hudson, 1996).

We suggest two explanations for why toe-clipping affects clinging ability. First, toe-clipping does not affect the actual function of the toepad but alters the behavioral manner by which lizards perceive the substrate. If lizards perceive whether a surface is slippery with their claws, they might be unable to determine how hard to push their toepad into the surface, for example. If true, we might expect that lizards would regain their clinging capabilities with time if lizards can behaviorally "adjust" to claw loss. A second explanation is that toe-clipping physically interferes with toepad function. We suggest that this second possibility is more likely, as toe-clipping apparently severs a key tendon linking the claw and the toepad (A. Russell, pers. comm., for details on the anatomy and function of toepads, see studies by Russell, 1975, 1981, 1986, 2002; Bauer and Russell, 1988). One way to assess this possibility would be to leave a small amount of claw uncut at the distal end of the toepad (and, hence, leave this tendon intact) and measure clinging force. Regardless, our results clearly suggest that toe-clipping by removing the entire claw (a standard practice) should be used cautiously, or not at all, for marking pad-bearing lizards, such as anoles and geckos. Finally, the dramatic effect of toe-clipping on clinging in green anoles may shed light on why pad-bearing lizards are so overbuilt in their clinging performance (Autumn et al. 2000, 2002; Irschick et al. 2003), since loss of even a few claws can dramatically reduce clinging performance. The overbuilt nature of toepads may, therefore, allow pad-bearing lizards to continue climbing even after the loss of several toes or claws, for example.

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Facial Lesions in Turtles, Observations on Prevalence, Reoccurrence, and Multiple Origins

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ABSTRACT.—We present the first report of aural abscesses in Ornate Box Turtles, *Terrapene ornata*, and of an epidermal inclusion cyst in Painted Turtles, *Chrysemys picta*. These observations were made in a mark-recapture study in eastern Iowa conducted over 25 yr. Detailed records of facial lesions in these species have been maintained for the last 12 yr. The prevalence of aural abscesses is lower than that reported for Eastern Box Turtles, *Terrapene carolina*. Aural abscesses tend to develop in Ornate Box Turtles at least 13 years old and are more common in females than males. The cysts sometimes spontaneously resolve but, in one instance, returned after being expelled in the field. In Ornate Box Turtles, they arise in the middle ear and displace the tympanum and its cartilaginous inner coat outward. Infection sometimes results in reactive bone formation with distortion of the cranium. A similar appearing lesion in a Painted Turtle arose in the skin covering the tympanum, and a second arose anterior to the ear. These were epidermal inclusion cysts and not abscessed.

Occasional cystic facial lesions were observed on Ornate Box Turtles (*Terrapene ornata*) during mark-recapture studies at Big Sand Mound in Muscatine and Louisa counties, Iowa. Although these were first noted in 1978, it was not until 1991 that careful searches for the cysts were conducted. The studies were usually conducted with triennial recapture studies along a 1-km drift fence (Christiansen and VanDeWalle, 2000), and several other species were examined for this condition as well. These included large numbers of Illinois Mud Turtles (*Kinosternon flavescens spooneri*),

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Painted Turtles (*Chrysemys picta bellii*), and a few False Map Turtles (*Graptemys pseudogeographica*), Red-Eared Turtles (*Trachemys scripta elegans*), Common Map Turtles (*Graptemys geographica*), and Common Snapping Turtles (*Chelydra serpentina*). Of these, only Ornate Box Turtles and Painted Turtles exhibited facial cystlike lesions.

We present our observed frequency of occurrence of facial abscesses in a wild Ornate Box Turtle population, the apparent durability of the lesion in this species, and the turtle's minimum age of lesion occurrence. We discuss the normal glandular epithelium in the ear where some cysts arise and origins of cysts lacking any trace of glandular cells. Although ours appears to be the first report of the condition in Ornate Box Turtles and Painted Turtles, Murray (1996), Dodd (2001, 2004), Tangredi and Evans (1997), and Holladay et al (2001) found similar lesions in Eastern Box Turtles (*Terrapene carolina*), and the latter two associated the pathology with hypovitaminosis A, possibly caused by organochlorine exposure.

Materials and Methods

This study was conducted approximately every three years from 1978 until 2003 when our data were summarized. The study area consisted of a two-mile long array of sand dunes adjacent to shallow ponds separated from the Mississippi River by a dike. The turtles were collected, marked, and released along a 1-km drift fence near edges of ponds. Flip-top-lid-covered and open pitfall traps were spaced along the fence approximately every 30 feet. From 1991 through 2003, we examined 689 Illinois Mud Turtles (*Kinosternon flavescens*), 460 Ornate Box Turtles (*Terrapene ornata*), and 212 Painted Turtles (*Chrysemys picta*). We examined less than 40 each of Red-Eared Turtles (*Trachemys scripta*), False Map Turtles (*Graptemys pseudogeographica*), Common Map Turtles, (*Graptemys geographica*) and Common Snapping Turtles (*Chelydra serpentina*). Each captured turtle was given a unique marking by notching marginal scutes.

Selected turtles were retained for histological examination. One of these was examined histologically at the Iowa State University Veterinary Laboratory. Three others were studied histologically by the authors. The contents of one abscess were expelled into a sterile container and examined for bacteria and fungi by the pathology laboratory of Iowa Lutheran Hospital. The mark-recapture studies and the euthanasia of turtles were done under permit SC255 0101 issued to the senior author by the Iowa Department of Natural Resources and with the approval of the Drake University Animal Care and Use Committee.

Turtle age was estimated by counts of plastral annulae (Legler 1960) adjusted when recaptures indicated fewer new annulae than the number of years that had passed since the earlier capture. This occurred in most turtles more than 15 years old, and it is possible that some of our 15-year-olds are much older than we indicate.

Abscesses of euthanized turtles were excised with a hacksaw when reactive bone growth was involved with the cut made outside the normal contours of the head. For comparison, the healthy ear and associated cranium on the opposite side was decalcified and sectioned in a plane that bisected the tympanum. All tissues were fixed in buffered 10% formalin, decalcified with Surgipath Decalcifier II^{TM}, when necessary, imbedded in paraffin, and sectioned at five micrometers. Slides were stained with hematoxylin and eosin. All specimens not released were retained in the Drake University Research Collection.

Results

Prevalence.—All the lesions observed over 25 years of study at Big Sand Mound involved Ornate Box Turtles with one exception. The exception is discussed here and was found in 2003 in a Painted Turtle. All lesions had a similar external appearance and were in approximately the region of the ear or anterior to it (Figs. 1 A and 2 A). The prevalence of the condition among 460 captures of Ornate Box Turtles from 1991 through 2003 was 16 (3.47%). Excluding the one turtle captured with a aural abscess twice and one juvenile (Table 1), only two individuals were adult males (two of 14 where sex was known, 14%), much less than the 12 of 14 (85.7%) that were female. Although observed incidence varied greatly from one year to the next, no trend toward increase or decrease could be detected over the 15 years of the study. Only one of 212 Painted Turtles (0.47%) examined over the same time period was found with a similar-appearing facial lesion, and that one was discovered in 2003. Lesions were absent in all of 689 mud turtles and in all of 10–40 Red-Eared Turtles, False Map Turtles, Common Map Turtles, and Common Snapping Turtles.

Lesions excised in Ornate Box Turtles in the field may reoccur (Table 1). In one of these (marked 1,1-3L1R), the lesion was observed in 1991 and the pea-sized cyst contents were expelled in the field through a small incision. A large, still soft lesion had reappeared when the turtle was recaptured three years later. This was biopsied at Iowa State University School of Veterinary Medicine.

The cysts we observed may spontaneously disappear. One of our oldest frequently recaptured *T. ornata* (marked 2L 9R) had a distinct swelling over or in the ear when first captured in 1978. At that time, the turtle had a carapace length

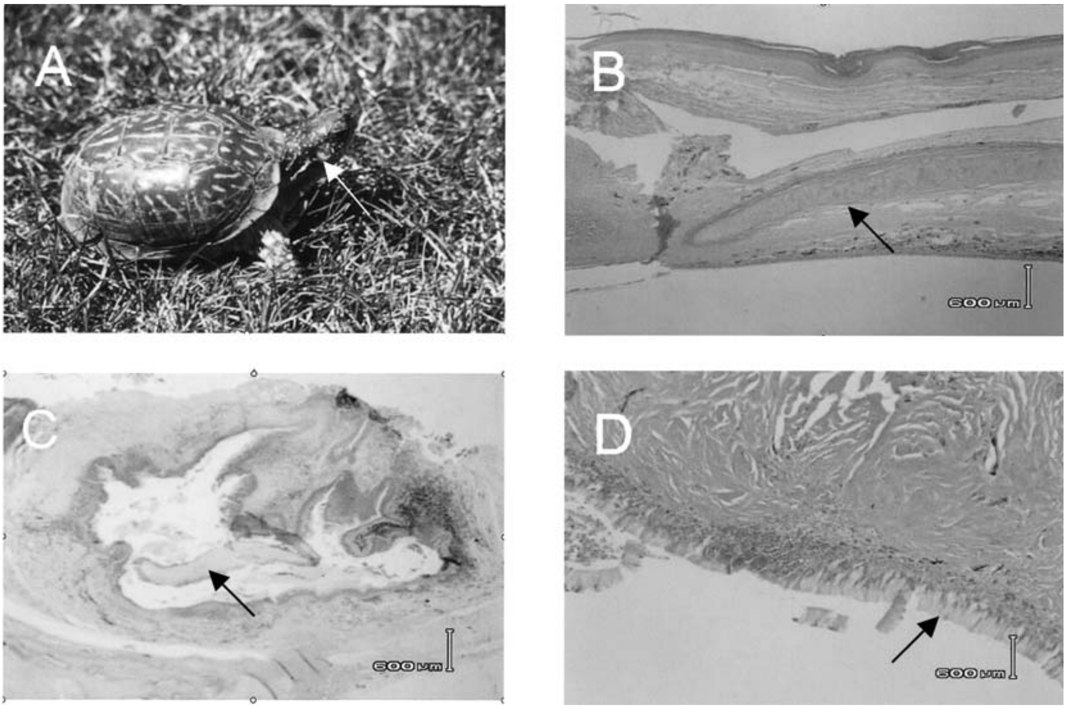

FIG. 1. Aural abscess of *Terrapene ornata*. (A) Typical aural abscess. (B) Normal ear on the side opposite the abscess; arrow indicates the cartilaginous extension of the extracollumella and tympanum separated from the skin by a space that appears to be filled with fluid. (C) The lesion outside the normal plain of the cranium showing the cartilage of the tympanum forced outward by the aural abscess. (D) Glandular cells of the infected ear. They are similar to those in the normal ear except for the absence of cilia.

of 114 mm. It was recaptured in 1982, 1994, 1997, and 2000 with no sign of the lesion. The turtle was estimated to be at least 15 years old in 1978, and it did not grow measurably after that time suggesting that the turtle was older than 15 yr. It was at least 37 years old at its last capture. A second (marked 1,6L 4R) was first captured in 1991 with no lesion. When captured in 1994, it had a small swelling posterior to the right side of the jaw over the ear. It was not captured in 1997 but was recaptured in 2000 and 2003 with no evidence of the lesion (Table 1). All lesions except one occurred in turtles that were 12–29 (or possibly much more) years old (mean at least 16.3).

Gross Nature of Lesions.—The lesions usually appeared as firm, single swellings immediately posterior to the angle of the jaw over the ear. All the Ornate Box Turtle lesions removed were in the middle ear cavity (Fig. 1); all other Ornate Box Turtle lesions appeared to involve the ear. Those found farther posteriorly along the neck were less firm and were usually posterior extensions of firm anterior abscesses involving the ear. They ranged in size from barely perceptible to masses so large that the head was deflected far to one side when the turtle withdrew it into the shell. In one instance (marked 1,1-0L3R, biopsied), the turtle was unable to withdraw the head sufficiently to be able to completely close the anterior portion of the plastron. In some instances, the lesion apparently persisted over a period of years with some presumably old lesions infected and with reactive bone formation, distorting the outline of the cranium. None of the turtles we observed with cysts exhibited lethargy, problems of orientation, or altered head position except for displacement of the head to one side as a result of the mass of the abscess when the head was withdrawn.

In 2003, a Painted Turtle was collected with two lesions posterior to and in line with the angle of the jaw on the right side of the head. The posterior cyst was under the thick skin covering the middle ear. Otherwise the two lesions appeared to be similar to those we had seen in Ornate Box Turtles except that the smaller anterior one had a cream-colored semisolid exudate extending from it (Fig. 2 A). Both were firm and freely movable, not involving the underlying bone. No inflammation was present nor was there evidence of reactive bone formation.

Histology.—Of the 16 Ornate Box Turtles found with facial lesions, three were used in detailed

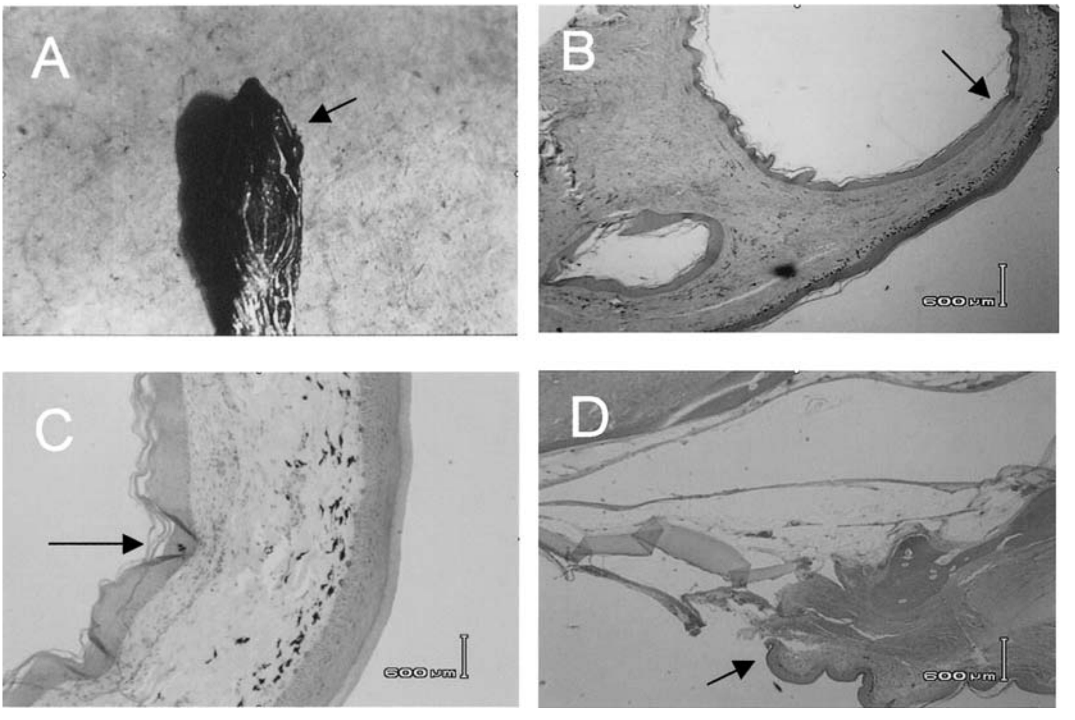

FIG. 2. Epidermal inclusion cyst overlying the ear of *Chrysemys picta*. (A) Large cyst over the ear and (arrow) smaller anterior cyst with exudate. (B) Section through both cysts; arrow shows area magnified in (C). (C) Exfoliating debris with no glandular cells, typical of epidermal inclusion cysts. (D) Section through ear after cyst removal showing the tympanum and cartilage distorted but still in place. Arrow indicates where skin was cut when cyst was removed.

histological examination. All showed evidence of inflammation and likely infection and were abscessed. The examination of all three was consistent in that the lesions involved the middle ear cavity and the tissue adjacent to the accumulated debris had numerous unciliated glandular cells (Fig. 1). The contents were usually round, hard, somewhat waxy in texture, and yellow. Abscess contents removed from a turtle and analyzed at Iowa State University Veterinary Pathology Laboratory consisted of keratin and squamous cell debris with colonies of bacteria and were described as of salivary gland origin. A cyst we excised in 1991 and examined for bacterial and fungal agents contained *Pasturella haemolytica*, alpha *Streptococcus* (not group D), nonfermenting gram-negative bacillus, and unidentified *Bacteroides* species.

To determine whether the ear normally contained glandular cells, we sectioned the normal ear on the side opposite the abscess in specimen JLC 6827 (Fig. 1 B). This showed abundant glandular and some ciliated epithelial cells. The tympanum was present under the skin and this was supported by a disc consisting mostly of cartilage but slightly ossified where it was contiguous with (and possibly part of) the extracolumella. The tympanum and its cartilaginous base were attached to the cranium by a short band of connective tissue.

The Ornate Box Turtle abscess in the following description was considered typical but was the most advanced for the three examined histologically (Fig. 1, C and D). The specimen (JLC 6827) and microscope slides have been submitted for cataloging into the Drake University research collection. The abscess was lined with pseudostratified columnar epithelium, often glandular and without cilia. Secondary changes were present with clusters of melanomacrophages and a few plasma cells with metaplastic epithelial changes manifested by pseudostratified epithelium adjacent to nonkeratinizing squamous epithelium. Cartilage and bone were present in the supporting abscess wall and the bony outer wall formed by the expanded extracolumella of the middle ear was displaced outward forming the outer edge of the abscess. To remove the lesion, it was necessary to saw through reactive bone growing out from the cranium at the abscess margins. The cut was made outside the flat plain of the cranium.

TABLE 1. Estimated ages of captures and recaptures of ornate box turtles with facial cysts. — = Turtle captured with no cyst apparent. Number after the symbol is the estimated age of the turtle based on plastral annulae and recapture records. Carapace length measurements are shown in (mm). C = Cyst apprent; NS = turtle Not Seen.

		Year of recapture study, turtle age, and (carapace length)					
Mark	Sex	1978	1991	1994	1997	2000	2003
2L9R	F	C 15 (114)	−28 (114)	−31 (115)	−34 (114)	−37 (114)	NS
1,8L1R	F	NS	C kept* 18 (107)	NS	NS	NS	NS
1,4L4R	F	NS	—	−16 (112)	C 17 (114)	−20 (114)	−23 (114)
11L12R	F	NS	C 13 (109)[a]	NS	NS	NS	NS
1,1-3L1R	M	NS	C excised[b] 15 (120)	C kept[c] 18 (120)	NS	NS	NS
1,6L4R	F	NS	−14 (111)	C 17 (111)	NS	−20 (112)	−23 (111)[c]
3,3-0L12R	F	NS	NS	C 13 (110)	NS	NS	NS
3,3-2L7R	Juv	NS	NS	C 5 (86)	NS	NS	NS
4,4-6L3R	F	NS	NS	NS	NS	C 20 (111)	NS
1,1-0L3R	F	NS	−18 (115)	NS	NS	NS	C kept 30 (114)[d]
4,4-8L7R	F	NS	NS	NS	NS	NS	C 18 (103)
3,3-5L1R	F	NS	NS	NS	NS	NS	C 12 (108)
4,4-1L0R	M	NS	NS	NS	NS		C 17 (97)
1,1-2L8R	F	NS	−7 (88)	NS	−13 (91)	NS	C 19 (103)
1,4L12R	F	NS	−17 (119)	NS	−23 (119)	−26 (119)	C 29 (120)

[a] Used in histological and bacterial studies.
[b] This lesion was opened in the field and the hard, yellow pea-sized cyst-contents removed; it had returned but was soft and inflamed in 1994 probably with infection. The turtle sent to ISU for biopsy and liver vitamin A analysis.
[c] These turtles often added one of fewer growth rings in three years; the lack of growth suggests that the age at the initial year is greater than listed.
[d] Tissues shown in Figure 1.

The Painted Turtle Cysts (JLC 6826) occurred outside the tympanum and the cartilaginous outer layer associated with the extracolumella of the ear. The ear, with the tympanum and its cartilaginous layer still in place but the skin and cyst removed, is shown in Figure 2B. The large cyst consisted of keratinous material without suggestion of infection or significant inflammation (Fig. 2C, and D). The ruptured smaller, anterior cyst had numerous granulocytes (PMNs) mixed with the keratin flakes. The lining was composed of normal squamous epithelium without either dendric melanocytes or melanomacrophages. The epithelial surfaces lacked a granular cell layer (Fig. 2C, D). Note the normal skin with these layers in the same section (Fig. 2D).

DISCUSSION

The prevalence of the lesions in our Ornate Box Turtle population has not changed significantly over the 13 years of our study. Our observation of cysts occurring in 3.47% of the Ornate Box Turtles we examined is slightly less than the 5.6% observed by Dodd (2001) in *Terrapene carolina bauri*.

The Ornate Box Turtle abscesses were lined with columnar, often glandular cells, consistent with the normal ear except for the lack of cilia. We judge the loss of cilia to be a metaplastic change. The potential origin from salivary gland as indicated by one analysis is ruled out in the turtles we excised by the apparent origin of the abscess under the tympanum and its cartilaginous base, displacing them outward. It is difficult to see how salivary gland could penetrate that ear covering although a salivary abscess could be expected between the skin and the tympanum, depressing the tympanum inward. It is possible that such a lesion could occur in box turtles and be difficult to distinguish from aural abscesses without excision.

The Painted Turtle cysts had the histological appearance of epidermal inclusion cysts and lacked evidence of chronic inflammation. The presence of a second cyst anterior to a cyst outside of the tympanum suggests that these cysts may arise from epidermal pockets similar to sebaceous cysts in mammals. Holladay et al. (2001) shows what appears to us to be such a cyst although they suggested that the histological picture they presented resulted from squamous metaplasia, clearly absent in our Painted Turtle specimen Figures 2D and F.

The abscesses are associated with infective agents, and all of those we observed in *T. ornata* showed extensive inflammation. The infection may be responsible for the reactive bone formation that we observed in Ornate Box Turtles and Jackson et al (1972) and Tangredi and Evans (1997) observed in Eastern Box Turtles. We did not identify the bacteria involved in the abscess described here, but an earlier inflamed Ornate Box turtle abscess yielded *Pasturella haemolytica*, alpha *Streptococcus*, nonfermenting gram-negative bacillus, and unidentified *Bacteroides* species. Several of these are potential pathogens, but none is common to the list of bacteria associated with cysts of *T. carolina* summarized by Dodd (in press). The lack of consistency in the bacteria involved further suggests to us that these bacteria may be transient infective agents that become involved as a result of the abscess or that many bacterial species may cause the lesion. A bacterial origin is possible, and Dodd (in press) found a correlation between increased abscess occurrence and an unusually cold, wet winter when the aural cavity might be subject to bacterially contaminated water, whereas the immune system may be suppressed by prolonged low temperatures in *Terrapene carolina bauri*.

One would expect infection spreading from an aural abscess to potentially involve the vestibular apparatus. We observed no problems in orientation or changes in extended head position in any of our turtles with abscesses. Dodd (in press) observed lethargy and prolonged basking in Florida Box Turtles with aural abscesses. Allard (1935) described swellings sometimes involving the eye as being "a rather common affliction." He reported observing lethargy in the early stages with the lesion usually resolving by fall. We too observed resolution of the cysts in at least two turtles but apparent persistence over three years in one. Allard suggested that the cysts are rarely seen in the fall. We cannot verify that ours do not recede in the fall and reoccur in spring because our sampling terminated by 15 July each year. It is logical that the abscesses could rupture and heal with little damage as Allard reported although this would seem more likely with the epidermal inclusion cyst we observed in *C. picta*. Dodd (2001, in press) reported emptying and cleaning cysts followed by normal recovery of the turtle. We have emptied only one without flushing it clean and treating it with antimicrobial agents, and that abscess recurred and three years later was badly infected.

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