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Brown anole (*Anolis sagrei*) adhesive forces remain unaffected by partial claw clipping

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Abstract. Morphological properties of animal locomotor systems should reflect costs and benefits associated with particular environments. Lizards that possess both claws and adhesive toe pads are specialized for environments that require movements in both the horizontal and vertical planes, and on both rough and smooth substrates. Although toe/claw clipping is a common technique for marking free-ranging lizards, this technique is disadvantageous to those lizards possessing adhesive toe pads. A previous study removed entire claws of *Anolis carolinensis* and observed a significant reduction in adhesive abilities, which was likely attributed to damage of underlying tendons that play critical roles in engaging the adhesive pads. Here, we report on the clinging ability of brown anoles (*Anolis sagrei*) with partial claw removal. We found that adhesive capacities were not affected by partial removal of the claw, suggesting that partial claw removal prevents damage to the underlying tendons and that it may be a safer alternative for short-term marking of adhesive pad-bearing lizards.

Keywords. Adhesion, *Anolis*, claw clipping, clinging force, toe clipping.

INTRODUCTION

Substrate characteristics of the natural environments of most lizards are usually heterogeneous and complex. Presumably, particular morphological properties of the locomotor systems of lizards reflect evolutionary responses to the costs and benefits associated with specializations for particular substrates. For example, several clades of lizards have evolved elaborate adhesive toe pads (while at the same time retaining claws) allowing them to cling to smooth substrates (Ruibal and Ernst, 1965; Williams and Peterson, 1982; Peterson, 1983). Claws perform well on rough or irregularly patterned substrates, whereas subdigital adhesive pads excel on smooth substrates (Irschick et al., 1996; Zani, 2000). Lizards that encounter both types of substrates may benefit by having both claws and toepads, as do species in the genus *Anolis*, allowing them to travel effectively on both smooth and rough surfaces (Irschick et al., 1996; Zani, 2000; Crandell et al., 2014).

Toe or claw clipping is a widely utilized technique for marking small reptiles and amphibians (Dunham et al., 1988). Although this may be a convenient marking technique for terrestrial species, few studies have investigated how toe or claw clipping affects adhesive toe pad function and whole organism performance in arboreal adhesive pad-bearing lizards. Bloch and Irschick (2005) examined the effects of complete claw clipping on clinging abilities of the adhesive pad-bearing lizard, *Anolis carolinensis*. After clipping toes at the distalmost portion of the adhesive toepad (effectively removing the entire claw; Fig. 1A), they found that claw removal significantly decreased clinging abilities. Several explanations were suggested including a change in the way the animal perceives its environment (a tactile response), and a morphological

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effect on the function of the toepad after the claw was removed (i.e., tendon damage; Bloch and Irschick, 2005). Although these mechanisms are not mutually exclusive, we wanted to test the relative importance of the possibility that clinging performance would suffer due to tendon damage as a result of claw clipping. While Bloch and Irschick (2005) did not directly state a mechanism of tendon damage, anoles (like most tetrapods) control flexion of the digits via flexor tendons attached to the musculus flexor digitorum longus. These flexor tendons span across the entire toe, including the distal phalanges (Abdala et al., 2009). When Bloch and Irschick (2005) removed the distalmost portion of the toe, they likely severed flexor tendons as well. Given this, it is possible that the destruction of those tendons may have resulted in decreased adhesion because the anoles were not able to properly engage their adhesive system. In our study, we used partial claw clipping to leave those tendons intact and measured maximum clinging ability on smooth substrates. Partial claw clipping reduces claw length and changes claw shape, but also decreases the interference between the adhesive pad and the substrate (Fig. 1B).

We hypothesized that partial claw clipping would not result in significantly reduced clinging abilities in *Anolis sagrei* on smooth substrates, likely because the less destructive marking technique would leave the underlying tendons preserved. The results of this study will not only increase our understanding of how claw clipping affects whole animal adhesive performance, but may also influence marking techniques utilized by researchers studying wild populations of adhesive pad-bearing lizards that also have claws.

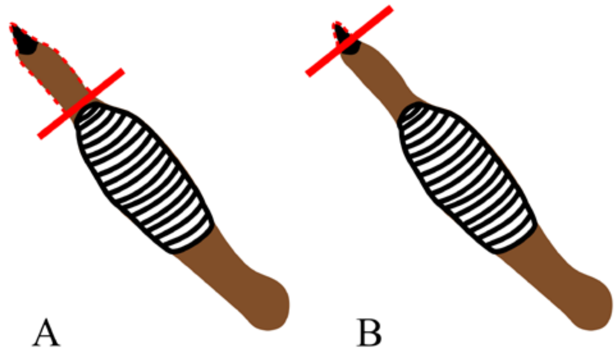

Fig. 1. Diagram of an anole toe indicating the differences in claw clipping locations between Bloch and Irschick (2005) and the present study. The clipping location is represented by the solid line and the removed portion of the claw is represented by the dashed line. Bloch and Irschick (2005) removed the entire claw at the distalmost portion of the adhesive toe pad (A), possibly compromising the underlying tendons that facilitate adhesive toe pad engagement. We partially removed the claw (B) in an effort to preserve the underlying tendons.

Table 1. Sample size, mass, and mean clinging forces of brown anoles (*Anolis sagrei*) used in experimental trials. All values reported as mean ± SE.

n	Mass (g)	Intact (N)	Clipped (N)	Acetate (N)	Glass (N)
19	4.55 ± 0.08	0.82 ± 0.12	0.60 ± 0.08	0.59 ± 0.09	0.84 ± 0.11

MATERIALS AND METHODS

Animals

A total of 19 captured brown anoles (*Anolis sagrei*) were used for experiments, including both adult males and females (Table 1). All lizards used for this study were collected at the Key West Botanical Gardens (Key West, FL) between 11 May 2008 and 16 May 2008, and then transported back to the Mote Marine Laboratory on Summerland Key, FL for clinging experiments. Anoles were released back to marked capture points in the gardens within 24 hours of capture.

Experimental procedures

Clinging experiments were completed using a custom-designed, motorized rig that measured adhesive force (Niewiarowski et al., 2008). The first set of trials were conducted on a sheet of glass covered with an acetate sheet (Bloch and Irschick, 2005). The two front feet of each anole were pulled three times in rapid succession along the substrate at a uniform speed of approximately 5 cm/s. For consistency, force measurements were done by a single individual. After the three pulls, the sheet of acetate was removed and the same anole was pulled along the sheet of glass three more times. Pulls on glass were used to test the possibility that claws (intact or clipped) contributed to clinging force by 'digging' into the acetate sheet (Bloch and Irschick, 2005). Maximum clinging force was recorded for each set of pulls and was defined as the force recorded at the point when both front feet of the anole were visibly slipping on the substrate. Subsequent to pulls with intact claws, claws on both front feet were partially clipped using a small pair of nail scissors. Unlike Bloch and Irschick (2005), claws were only clipped halfway to avoid potential damage to the underlying tendons (Fig. 1B). After clipping, clinging ability was tested identically to tests withint act claws. Smooth substrates were chosen to isolate the effect of partial claw clipping on the adhesive capability of *Anolis sagrei*. Rough or irregular substrates generally increase clinging ability of lizards with claws because claws can generate friction or mechanically interlock with surface asperities, thus possibly resulting in a bias towards higher clinging ability with claws left intact (Zani, 2000; Crandell et al., 2014). The order in which anoles were tested was randomized.

Statistical analysis

We used a mixed model analysis of variance (ANOVA) to compare maximum clinging ability as a function of substrate, claw removal, and their interaction. Maximum clinging ability was the dependent variable. Substrate type (glass or acrylic), claw state (intact or clipped), and their interaction were the independent variables. Individual anole was modeled as a random effect. Before statistical analysis, maximum clinging ability data were log-transformed in order to meet the assumptions of an ANOVA. All statistical analyses were completed using JMP Pro 12 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The mixed model ANOVA using an assumption of compound symmetry (Littell et al., 2000) fit the data nearly as well as the model relaxing the assumption of compound symmetry and leads to qualitatively similar conclusions, but we report the latter as it is more conservative. The effect of claw removal on maximum clinging ability of *Anolis sagrei* revealed that neither claw state ($F_{1,18} = 0.683$, $P = 0.419$) nor the substrate by claw state interaction ($F_{1,18} = 0.725$, $P = 0.406$) had a significant effect on maximum clinging ability (Fig. 2 and Table 2). However, maximum clinging ability was significantly affected by substrate type, with glass producing higher maximum clinging forces than acetate ($F_{1,18} = 4.435$, $P = 0.049$; Fig. 3 and Table 2).

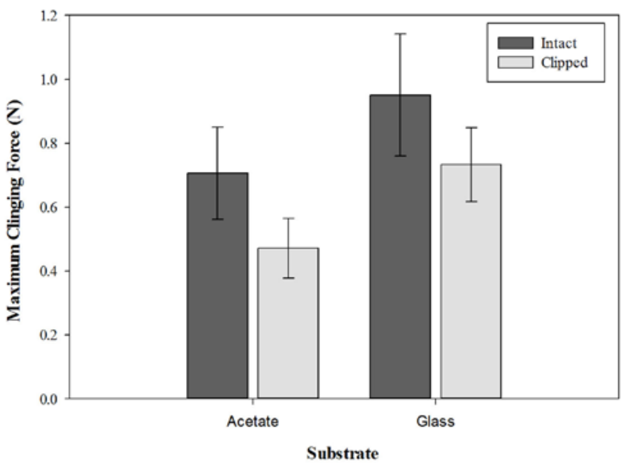

Fig. 2. Mean maximum clinging forces of *Anolis sagrei* on acetate and glass substrates with fully intact or partially clipped claws. A mixed model ANOVA indicates no significant difference in the mean maximum clinging forces of *A. sagrei* as a function of claw state ($F_{1,18} = 0.683$, $P = 0.419$) or the substrate by claw state interaction ($F_{1,18} = 0.725$, $P = 0.406$). Error bars represent ± 1 SE.

Table 2. Results of the mixed model analysis of variance (ANOVA). The table displays a significant difference in maximum clinging ability as a function of substrate (*$P < 0.05$). There is no significant difference in maximum clinging ability as a function of claw state or the interaction between substrate and claw state.

Effect	NumDF	DenDF	F	P
Substrate	1	18	4.435	0.049*
Claw state	1	18	0.683	0.419
Substrate * Claw state	1	18	0.725	0.406

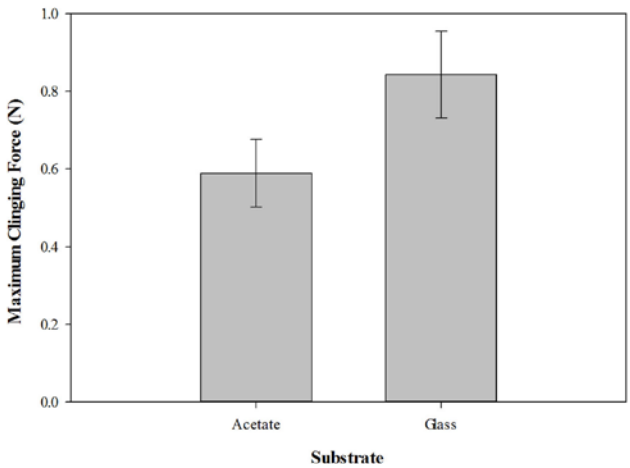

Fig. 3. Mean maximum clinging forces of *Anolis sagrei* on acetate and glass substrates. A mixed model ANOVA found that the mean maximum clinging force of *A. sagrei* was significantly higher on glass compared to acetate ($F_{1,18} = 4.435$, $P = 0.049$). Error bars represent ± 1 SE.

DISCUSSION

Bloch and Irschick (2005) reported a significant decrease in clinging forces when the claws of *Anolis carolinensis* were completely removed. Because several mechanisms were proposed to explain this effect, we examined the possibility that tendon damage may be responsible for the reduction in adhesion. To investigate this, we partially removed claws of *Anolis sagrei* and compared their clinging abilities to anoles with fully intact claws.

Our results demonstrated that partial claw clipping had no significant effect on maximum clinging force, supporting the hypothesis that entire claw removal may actually damage the underlying tendons (Bloch and Irschick, 2005). The main difference between our study and Bloch and Irschick (2005) is that claws were only partially clipped, not removed, thus the underlying tendons were left intact. All claws on both front feet were clipped in our work, as opposed to removing 2-4 claws on the front feet. Yet, our recorded clinging forces are similar to those observed by Bloch and Irschick (2005). Importantly, the difference in clinging forces between intact and partially clipped claws (~ 0.2 N or ~ 20 g) is about five times smaller in our study than what Bloch and Irschick (2005) found (~ 1 N or ~ 100 g). Although our samples sizes are smaller than Bloch and Irschick (2005), we used a mixed model ANOVA to account for the non-independence of observations attributable to individual lizards, and the small differences between treatments is likely to be biologically insignificant as well.

Do claws interfere with toe pads?

In lizards that possess both claws and subdigital adhesive pads, it has been demonstrated that increases in claw curvature, length, and height are correlated with increases in toe pad adhesive force on smooth substrates (Zani, 2000; Crandell et al., 2014). While morphological variation in claws appears to influence adhesion, the mere presence of claws should result in reduced adhesive capabilities. Clinging forces generated by adhesive toe pads are directly related to the numbers of individual setae that can make appropriate contact with the substrate (contact fraction) (Autumn et al., 2000; Autumn, 2006). Contact fraction depends on many factors, including pre-loads applied by the lizard, as well as the degree and nature of roughness of the substrate (Russell et al., 2007). On smooth substrates, it is intuitive to hypothesize that claws may result in decreased adhesive force because the toe pads may be more removed from the substrate resulting in decreased contact fraction. Thus, one would expect the partial removal of claws to result in an increase in adhesive force on smooth substrates. Surprisingly, our results reveal no significant difference in clinging ability between anoles with fully intact and partially clipped claws, at least on smooth substrates. Furthermore, Mahendra (1941) observed that the adhesive locomotion of *Hemidactylus flaviviridis* on smooth substrates appeared to be unaffected by claw removal. Thus, it may be that morphological or behavioral mechanisms minimize theoretical trade-offs between claws and toepads in their functional roles during adhesive locomotion.

Lizards that possess both claws and subdigital adhesive pads are likely able to effectively cling to both smooth and rough substrates (Irschick et al., 1996; Zani, 2000). While it appears that partial claw clipping does not result in a reduction in adhesive performance on smooth substrates, it is possible that claw removal results in lower clinging abilities on rough substrates, although this has yet to be tested empirically. Therefore, it is interesting to consider the ecological implications of losing one of the two clinging mechanisms discussed (adhesion via van der Waals interactions or clinging via claws). However, considering the heterogeneity of substrates in the natural habitat of *Anolis sagrei*, it is difficult to gauge which mechanism of clinging would be more costly to *A. sagrei* if it was lost via toe/claw removal. Regardless, partial claw clipping is the least invasive of the two marking techniques discussed and does not result in a reduction of adhesive performance.

Glass versus acetate

We recorded a significantly higher clinging force on glass than on acetate when claws were left intact. Two aspects of this result are relevant. First, it suggests that claws do not enhance adhesion by 'digging' into the acetate sheets (Bloch and Irschick, 2005). Second, and consistent with theory underlying the mechanics of van der Waals forces, substrates with higher surface energies should lead to higher clinging forces (glass is about 100 mJ/m^2 and acetate is about 30-40 mJ/m^2) (Israelachvili, 1992). A potential confounding factor for future adhesion studies could be which of these substrates is more appropriate to use and which is most similar to a smooth substrate encountered by adhesive pad-bearing lizards in their natural environments.

Marking techniques

A non-destructive, permanent marking technique would be ideal for working with reptiles, yet it is very challenging to achieve, particularly with animals that rely on adhesive functions during locomotion (Paulissen and Meyer, 2000). Bloch and Irschick (2005) addressed this problem with complete claw clipping as a permanent, relatively reliable solution, but with an undesired effect: a reduction in adhesive performance. When comparing permanence of a marking technique, complete claw clipping/removal is more effective than partial claw clipping. However, our work here suggests that partial claw clipping may serve as an excellent alternative during short-term mark and recapture studies because no significant change in adhesive capabilities was observed.

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