Sticky fingers: The functional morphology of the locomotor apparatus in geckos (Gekkota: Diplodactylidae)

Submitted by

Rishab Rajan Pillai

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Statement of the Contribution of Others

Supervision

My primary advisor Lin Schwarzkopf provided fundamental advice and guidance with all aspects of the thesis. My secondary advisor Will Edwards provided guidance and validated my statistical analysis. Will also assisted with suggesting and validating appropriate statistical analysis for my work. My first external advisor Jendrian Riedel helped with conceptualisation and discussion of all four chapters, especially with the morphological aspects included in this thesis. Eric Nordberg, my second external advisor, hosted me at the University of New England to conduct Scanning Electron Microscopy at the Centre for Animal Research and Teaching. Eric also validated my statistical analysis in Chapter 3 and Chapter 4.

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Abstract

Motile organisms need to move effectively within their habitat, to capture prey, avoid predators and acquire mates, thereby making effective movement a critical factor influencing Darwinian fitness. The structure of habitats can differ in many ways, therefore, habitat structure influences the evolution of morphology, which, in turn, fulfils different requirements for performance. To negotiate topographical challenges within their habitats, animals have evolved a range of morphological adaptations, like claws and hooves. However, some taxa have evolved more specialised adaptations, including hooks, suction pads, and adhesive pads comprising filamentous 'setae'. Adhesive pads have evolved in geckos, wherein they have enabled exploitation of inclined, vertical and inverted habitats, globally. Geckos are ecologically diverse, and on a broad scale, habitat structure do affect morphological traits like body size and limb lengths in this group. However, examinations of the fine-scale morphology of setae and how they vary in relation to habitat use are relatively few. The majority of studies understanding the function of the gekkotan adhesive system have been conducted on Tokay geckos (*Gekko gekko*) and these have informed the development of biomimetic, dry adhesives. Studies examining the structure and function of this system in an ecological context are lacking.

Australian diplodactylids are an ecologically diverse group of geckos, occupying a range of arboreal, saxicoline, and shrubby habitats. Microhabitats are likely to have different requirements for attachment, therefore, gecko morphology may differ in relation to these different needs for attachment. Their ecological diversity makes diplodactylid geckos an ideal study system in which to investigate the relationships between ecology, performance, and morphology.

I studied microhabitat use, and clinging ability, and investigated morphology of the adhesive system to determine if there were relationships between ecology, performance, and morphology. Firstly, I examined the influence of wear-and-tear from general use and damage on clinging ability, and if the system is repaired through the process of 'ecdysis' or 'skin-shedding' **(Chapter 1)**. To quantify the relationships between ecology and performance, I investigated if diplodactylid geckos preferred substrates with characteristics similar to those used in nature. Further, I examined if they performed better on preferred substrates **(Chapter 2)**. Many pad-bearing taxa retain claws, and diplodactylid geckos are one such group, however, their claws are smaller than those of other pad-bearing lizards. Therefore, I investigated if claws played a functional role in this group. Specifically, I used substrates that represented the fine-scale structure of their microhabitats in nature to test if claws provided functional advantages on certain substrates or at certain orientations **(Chapter 3)**. Lastly, I investigated microhabitat use, clinging ability, and morphology to understand the relationships between ecology, performance, and morphology in this ecologically diverse group **(Chapter 4)**.

I found that wear-and-tear from general use and damage to pads reduced clinging ability in diplodactylid geckos. However, this decline in clinging ability due to damage was repaired through 'ecdysis' or 'skin-shedding' **(Chapter 1)**. While investigating relationships between ecology and performance, I found that diplodactylid geckos preferred substrates with similar fine-scale characteristics to microhabitats in nature. Furthermore, geckos exerted greater shear force on substrates that they preferred **(Chapter 2)**. When I examined the functional role of claws in pad-bearing geckos, I found that despite claws being smaller in this group, they were critical to attachment ("holding on") and clinging ability (shear force generation) in this group and they fulfilled an additive role to adhesive pads **(Chapter 3)**. Lastly, when I examined the relationships between ecology, performance, and morphology, I found that performance did vary based on microhabitat use, and morphology reflected this, suggesting that ecology, morphology, and performance are related **(Chapter 4)**.

The form and function of the gekkotan attachment system have been understudied in diplodactylid geckos. My findings highlight key relationships between ecology, performance, and morphology in this group, and reveal considerable differences in performance and morphology when compared to other, commonly studied pad-bearing taxa. Firstly, we found that diplodactylid geckos exhibit different trajectories of clinging ability with increasing roughness, such that their clinging ability was highest on coarse surfaces, which apparently reduce attachment abilities in other gekkotans. Furthermore, we found that morphology, specifically the density of setae, was greater in diplodactylid geckos than in other groups, which may explain their capacity to effectively cling on rough surfaces previously thought to be beyond the capacity of attachment of the gekkotan adhesive system.

My findings support the need for further investigations of performance and morphology in an ecological context. Geckos are incredibly ecologically and morphologically diverse and there is great potential for further investigations in other groups in various habitats in other parts of the world.

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Chapter 1. General introduction

Motile animals need to move efficiently to avoid predators, capture prey and acquire mates (Aerts *et al.*, 2000; Bell *et al.*, 2009; Higham and Russell, 2010) thus, effective movement influences growth rates, survival and reproduction and associated Darwinian fitness (Arnold, 1983; Garland Jr. and Losos, 1994; Losos, Irschick and Schoener, 1994; Aerts *et al.*, 2000). Movement is likely to occur in diverse habitats with varying structural characteristics, such as vertical and inclined habitats; varying levels of 'openness' due to different canopy coverage; differences in ground cover, and surface characteristics of biotic and abiotic components of the habitat. The diversity of topographical features to which animals are exposed affects their movement capabilities, therefore, natural selection should act differentially on locomotor performance in response to varying habitat characteristics (Collins, Russell and Higham, 2015). Therefore, habitat structure influences the evolution of morphological adaptations (Moermond, 1979; Irschick *et al.*, 1996; Melville and Swain, 2000; Goodman, Miles and Schwarzkopf, 2008; Higham and Russell, 2010). As these adaptations are closely associated with movement, they predominantly influence the evolution of extremities (Tornier, 1899; Rosenberg and Rose, 1999; Nachtigall, 2013). In climbing taxa, the distal-most parts of the extremities making contact with the substrate of locomotion (i.e., digits in vertebrates or tarsi in insects) often evolve to possess systems that enable attachment via diverse mechanisms (Nachtigall, 2013).

Biological attachment systems

Claws fulfil various functional roles, like digging and burrowing (Kley and Kearney, 2007), digging and tearing (Coombs, 1983), grooming (Eckstein and Hart, 2000), defense (Coombs, 1983), and gripping (Turnbull *et al.*, 2023), prey capture and handling (Thomson and Motani, 2021a), however, one of the most widespread functions of claws is climbing. Claws are the most common adaptation for climbing in vertebrates, and are responsible for the exploitation of niches that were not previously accessible (Cartmill, 1985). The association of claw morphology with climbing can be seen in several taxa (Thomson and Motani, 2021a), and claws aid climbing in multiple ways (Gorb, 2008; Chan and Carlson, 2019). Claws enable attachment to a range of surfaces through mechanical interlocking (Crandell *et al.*, 2014; Garner, Lopez and Niewiarowski, 2017) when surface asperities are larger than the diameter of the claws. When surface asperities are smaller than the claw tip, claws generate friction (Song *et al.*, 2016). The morphology of claws has been linked to differences in habitat type (D'Amore *et al.*, 2018; Baeckens, Goeyers and Van Damme, 2020), and claws provide a functional advantage to organisms that possess them (Cartmill, 1985; Maddin *et al.*, 2009).

In addition to claws, several taxa have evolved attachment systems in the form of adhesive pads (Alberch, 1981; Green, 1981; Williams and Peterson, 1982; Stork, 1983; Gorb and Beutel, 2001; Beutel and Gorb, 2006; Gamble *et al.*, 2012; Wolff, Jonas O and Gorb, no date). Adhesive pads occur in invertebrates and vertebrates, however, they have evolved multiple times in vertebrates (Gamble *et*

al., 2012; Russell and Gamble, 2019). Adhesive pads can be diverse, and some attach using wet adhesion, whereas some use dry adhesion. Examples of wet adhesion, in which attachment occurs through a combination of suction and glandular secretions, are often associated with soft pads and can be seen in ants, frogs, bats, mouse lemurs, tarsiers and feathertail gliders (Rosenberg and Rose, 1999). On the other hand, dry adhesion is often associated with hair-like or filamentous pads, seen in stick insects, dock beetles and jumping spiders, however, such structures have most notably involved in lizards, specifically, geckos and anoles (Ruibal and Ernst, 1965) and few species of scincids (Williams and Peterson, 1982). Geckos and anoles are the largest groups to evolve highly developed attachment pads that are so efficient that they can support forces greater than their body mass (Russell and Garner, 2023).

The Gekkotan attachment system

In geckos, adhesive pads comprise fields of hierarchically arranged microfibrillar outgrowths termed ‘setae’ (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Autumn, 2002). Each seta may culminate in just one tip (in anoles) or expand into several tips (geckos, Garner and Russell, 2021). Whether singular or branched, each tip culminates in triangular structures termed ‘spatulae’ (Ruibal and Ernst, 1965; Autumn *et al.*, 2000; Autumn, 2002). The ventral surface of the toepads of geckos and anoles possess millions of such filaments, that make contact with surfaces upon which these animals move. Certain physical properties of these filaments could differentially affect the amount of force that they generate. For example, longer setae have lower bending stiffness or greater elasticity, which permits greater setal loading parallel to the surface, forming a strong interaction with the surface to generate shear force (Persson and Tosatti, 2001; Persson, 2002). Alternatively, high densities of setae could cause clumping, therefore, setae may be shorter to avoid this. In such cases, splitting of tips into multiple branches increases individual points of contact and consequent shear force generation (Arzt, Gorb and Spolenak, 2003a; Spolenak *et al.*, 2005).

Current understanding of the system

Mahendra (1941) and Dellit (1934) were among the first studies to make observations on a gecko's ability to scale vertical and inverted surfaces. Since then, this system has received a lot of attention, firstly in studies that described the morphology (Maderson, 1964; Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Russell, 1975), leading to studies that were essential in shaping our understanding of how these structures functioned. Autumn et al. (2000) was the key study that provided evidence for van der Waals forces being the primary mechanism of adhesion (Autumn *et al.*, 2000; Autumn, 2002). Since then, several studies have also suggested roles for additional mechanisms of attachment, such as capillary interactions (Huber *et al.*, 2005; Puthoff *et al.*, 2010; Mitchell *et al.*, 2020), acid-base interactions (Singla *et al.*, 2021) and possibly, electrostatic interactions (Izadi, Stewart and Penlidis, 2014). A detailed understanding of the gekkotan attachment system was critical to further studies that explored the biomimetic potential of this dry adhesive mechanism.

The need for highly standardised experimental setups and incentive to develop innovative biomimetics consequently led to several studies of attachment being conducted on artificial surfaces (Hiller, 1968; Irschick *et al.*, 1996; Autumn *et al.*, 2000; Zani, 2000; Persson, 2002, 2003; Campolo, Jones and Fearing, 2003; Meine *et al.*, 2004; Huber *et al.*, 2005; Vanhooydonck, Andronescu, *et al.*, 2005). These studies were critical in highlighting the potential of the attachment system for effective attachment on smooth surfaces and found that geckos generated forces several magnitudes greater than that required to support their body weight (Irschick *et al.*, 1996; Autumn *et al.*, 2006). Additionally, studies on smooth, fine-grained and rough surfaces reported a linear decline in attachment capabilities as surface roughness increased (Cole, Jones and Harris, 2005; Vanhooydonck, Andronescu, *et al.*, 2005; Naylor and Higham, 2019; Cobos and Higham, 2022). Although these studies formed the foundation of our understanding of this system, there was a gap in our understanding of the function of the adhesive system on surfaces used by geckos in nature (Johnson, Russell and Delannoy, 2009; Niewiarowski, Stark and Dhinojwala, 2016; Higham *et al.*, 2019), as habitat characteristics could likely influence the evolution of morphology in this system. Furthermore, despite an impressive range of morphological variation at the spatula, seta, scansor and toe levels in geckos (Peattie, 2001; Autumn and Gravish, 2008; Gamble *et al.*, 2012), which may be correlated with occupancy of different structural habitats (Collins, Russell and Higham, 2015), relatively few studies examined the attachment systems of geckos in an ecological context (Higham and Russell, 2010; Russell and Johnson, 2014; Collins, Russell and Higham, 2015; Higham, Russell and Niklas, 2017; Garner *et al.*, 2022).

Australian diplodactylids as a model system

Australia supports the highest diversity of lizards compared to any other country (>750 species) of which 89% are endemic (Vidan *et al.*, 2019; Uetz, Freed and Hošek, 2020). Among these, diplodactylid geckos are one group that exhibits high levels of ecological and morphological diversity (Brennan and Oliver, 2017). Ecological diversity in the form of varied microhabitat use and associated differences in habitat structure could require varied performance capabilities to navigate the topographical features of different habitats. In other groups, the morphology of the adhesive system has been tenuously linked to habitat use (Collins, Russell and Higham, 2015; Garner *et al.*, 2022), which makes diplodactylids a good system in which to investigate the associated diversity in morphology. In the context of performance, we know that those diplodactylid geckos examined in this regard so far show non-linear variation in shear force generation with increasing surface roughness (Pillai *et al.*, 2020b), opposite to the linear decline in shear force generation with increasing surface roughness in non-diplodactylid gecko species examined (Cole, Jones and Harris, 2005; Vanhooydonck, Andronescu, *et al.*, 2005; Naylor and Higham, 2019; Cobos and Higham, 2022).

Rationale

Studies on attachment systems have predominantly focused on artificial surfaces, with minimal incorporation of ecological considerations, while relatively few studies used natural substrates (Schwarz et. al. 2021). Furthermore, these studies have focused predominantly on the gekkonid Tokay geckos (*Gekko gecko*, Russell, 1975; Hansen and Autumn, 2005; Niewiarowski et al., 2008; Pugno and Lepore, 2008; Puthoff et al., 2010; Gillies et al., 2013; Bauer, 2019; Mitchell et al., 2020; Garner et al., 2021; Russell and Garner, 2021; Cobos and Higham, 2022), with few studies examining other groups (Genus *Rhoptropus*, Russell and Johnson, 2007, 2014; Johnson, Russell and Delannoy, 2009; Higham and Russell, 2010; Collins, Russell and Higham, 2015). Australian diplodactylids occupy different microhabitats in nature, and very little is known about the morphology of the adhesive system. If differences in habitat structure require different performance capabilities for successful navigation of topographical differences, this relationship should be reflected in the evolution of features that enable effective performance. Therefore, quantifying differences in clinging ability on different substrates in relation to microhabitat use, while investigating the morphology of the adhesive system on macro- and micro-scales provides us with an opportunity to understand the relationship between ecology, morphology and performance.

Attachment in gekkotans has been quantified using various approaches. Some studies have focused on whole-animal performance (Niewiarowski *et al.*, 2012), while some measured performance generated by a single digit (Crandell *et al.*, 2014), single limb (Naylor and Higham, 2019) or a single seta (Autumn *et al.*, 2000). Furthermore, the function of claws have usually been neglected in pad-bearing taxa (Crandell *et al.*, 2014). Given attachment is functionally relevant to climbing, many studies have measured attachment on vertical surfaces (Garner *et al.*, 2020). Despite suggestions that setae have evolved to enable climbing on both vertical and inclined surfaces, there are relatively fewer studies measuring attachment on inclines (Russell and Higham, 2009; Birn-Jeffery and Higham, 2016; Higham *et al.*, 2021). Therefore, it is necessary to examine attachment on different orientations and substrates, while trying to determine functional differences and potential advantages on certain surfaces.

Thesis chapter outline

My thesis aims to analyse the relationship between the attachment system, morphology and ecology in Australian diplodactylid geckos. To address this relationship, my thesis is separated into four stand-alone publications, each addressing certain aspects of the relationship between ecology, morphology and performance, specifically clinging ability.

In Chapter 2, I investigated clinging ability in relation to mechanical damage and wear-and-tear caused by surface interactions, and the potential for the physiological process of ecdysis to mitigate the negative consequences of this interaction. I addressed these questions in three species of

diplodactylid geckos, namely, *Oedura castelnaui* (Thominot, 1889), *Oedura monilis* De Vis, 1888 and *Strophurus krisalys* Sadlier, O'Meally and Shea, 2005. I tested the clinging ability at repeated intervals after a shedding event to replicate damage caused by repeated use of the attachment system. Additionally, I examined if wear and tear from general use reduced shedding. In both cases, I analysed the role of ecdysis in repairing the reduction of shear force generation caused by multiple surface interactions. I examined how this reduction in performance affected geckos' ability to support their body weight by examining safety factors. This chapter is published in the Journal of Experimental Biology (Pillai, R.R., Riedel, J. and Schwarzkopf, L., 2023. The role of ecdysis in repair of an attachment system: a case study using geckos. Journal of Experimental Biology, 226(10), p.jeb245286).

In Chapter 3, I examined the relationship between ecology and performance, by investigating microhabitat choice and clinging ability. I used observations of microhabitats used in nature and their fine-scale habitat structure (surface roughness) to inform my trials testing microhabitat choice and clinging ability in the laboratory in three diplodactylids, namely arboreal *Oedura castelnaui*, rock-dwelling *Oedura coggeri* (Bustard, 1966) and generalist *Oedura monilis*. Using surfaces that were comparable to those geckos used in nature, I tested if clinging ability was higher on surfaces that geckos preferred. This chapter is published in Frontiers of Zoology (Pillai, R., Nordberg, E., Riedel, J. and Schwarzkopf, L., 2020. Geckos cling best to, and prefer to use, rough surfaces. Frontiers in Zoology, 17, pp.1-12).

In Chapter 4, I examined the functional role of claws in diplodactylid geckos that possess adhesive pads. Diplodactylid geckos have reduced claws and well-developed pads, therefore, it was crucial to understand if claws still contributed to function, and how they did it, that is, did they function independently to pads, or were they additive? Did they provide an advantage on certain substrates or surface orientations? I tested two aspects of function that contribute to moving on vertical and inclined surfaces, firstly the probability of attachment (avoiding falling) and secondly, the shear force generated. Both aspects were tested with claws intact and clipped, and tests were conducted on three substrate types that represented the spectrum of surfaces encountered in nature. Tests with and without claws, on three substrates were repeated on inclines (45°) and vertical surfaces (90°). This chapter is submitted for peer review in Proceedings of the Royal Society B.

Lastly, in Chapter 5, I examine the relationship between morphology, ecology and performance by investigating the differences in morphology in relation to habitat use, and how morphology affected performance in 17 species of climbing diplodactylids classified as arboreal, shrub-dwelling, rock-dwelling, or climbing generalists. I investigated morphology on a macro-scale by quantifying toepad area, and on a micro-scale I measured setal length and setal density using scanning

electron microscopy. I identified differences in morphology based on microhabitat use and analysed how morphology affected the generation of shear force.

Chapter 2. The role of ecdysis in repair of an attachment system: a case study using geckos

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Abstract

Skin provides functions such as protection and prevention of water loss. In some taxa, the outer surface of skin has been modified to form structures that enable attachment to various surfaces. Constant interaction with surfaces is likely to cause damage to these attachment systems and reduce function. It seems logical that when skin is shed via ecdysis, its effectiveness will increase, through repair of damage or other rejuvenating mechanisms. We address two questions using three diplodactylid geckos as model species. (1) Does repeated mechanical damage affect clinging ability in geckos to the point that they cannot support their own body weight? (2) Does use without induced damage reduce effectiveness of the attachment system, and if so, does ecdysis restore clinging ability? We found that repeated damage reduced clinging ability in all three species, although at different rates. Additionally, use reduced clinging ability over time when no apparent damage was incurred. Clinging ability increased after ecdysis in all three species, both when damage was specially induced, and when it was not. After normal use without induced damage, the increase in clinging ability after ecdysis was statistically significant in two of three species. Our findings show that use decreases clinging ability, and mechanical damage also affects geckos' capacity to exert shear forces consistently. Thus, ecdysis improves clinging ability both in scenarios where damage is induced and more generally. In addition to the physiological functions provided by skin, our study highlights an important function of ecdysis in a speciose vertebrate group.

KEY WORDS: Attachment, Damage, diplodactylidae, Ecdysis, Integument, Geckos, Safety factors

Introduction

Effective movement within the environment is essential for prey capture, predator avoidance and mate acquisition (Alexander, 2003; Betz and Kölsch, 2004). Movement in taxa such as spiders (*Aphonopelma seemanni* and *Cupiennius salei*; Niederegger & Gorb, 2006), dock beetles (*Gastrophysa viridula*; Bullock & Federle, 2011), stick insects (*Carausius morosus*; Labonte et al., 2019), cockroaches (*Nauphoeta cinerea*; Clemente et al., 2009) and geckos (*Gekko gecko* and *Hemidactylus garnotii*, Autumn and Peattie, 2002) is dependent upon their attachment mechanisms, which are associated with modifications of the outer epidermal generation (OEG). These structures

differ morphologically among taxa and the mechanisms of attachment vary. Despite the morphological differences, the function of these attachment systems is dependent on interactions with substrates upon which movement occurs (Pillai *et al.*, 2020b, 2020a; Burack, Gorb and Büscher, 2022). During these interactions potential damage and contamination might occur, reducing the effectiveness and function. Processes such as self-cleaning could potentially reduce the effect of contamination (Hansen and Autumn, 2005; Clemente *et al.*, 2010); however, the process of 'ecdysis' or 'skin-shedding' repairs damage and wounds that could potentially restore function (Lai-Fook, 1968; Weis, 1976; Vafopoulou, 2009; Pellett and O'Brien, 2019)

The removal and replacement of the OEG is a trait common across terrestrial vertebrates (Maderson, 1966): in a process known as 'shedding', 'sloughing' or 'ecdysis', the old OEG is removed after a new one is formed from the underlying living tissue. Some clades shed their OEG constantly, for example, the mammalian epidermis is produced and replaced continuously through movement of new cells into the cornified layers (Maderson, 1965, 1967). In contrast, squamate reptiles shed periodically, losing the entire outer epidermal generation at once. The process of shedding may have evolved to keep pace with somatic growth and to rid the surface of parasites and other pollutants (Böhme and Fischer, 2000). Shedding also likely repairs external damage caused by the environment (Fushida *et al.*, 2020).

Squamates have a multilayered and complex epidermis: the stratum germinativum is the deepest epidermal layer, preceding its derivatives, the inner epidermal generation (IEG) and the OEG, which consist of three layers each (Maderson, 1964). The outermost part of the top layer of the OEG, termed Oberhäutchen, gives rise to microscopic derivates called microornamentation or, more precisely, epidermal outgrowths (Irish, Williams and Seling, 1988; Maderson *et al.*, 1998; Garner and Russell, 2021) One example of epidermal outgrowths are the hairlike structures, termed 'setae' (Garner and Russell, 2021), which are responsible for adhesion of the toepads of geckos (family Gekkota; Ernst & Ruibal, 1966), some iguanians (Anolidae, Ruibal & Ernst, 1965) and few species of scincids (Williams and Peterson, 1982). The adhesive pads consist of modified scale rows, lamellae and scansors, bearing millions of setae, which are critical for effective locomotion and have enabled these groups to adhere to vertical and inverted substrates (Russell and Johnson, 2007, 2014; Gamble *et al.*, 2012). Setae enable attachment through a combination of van der Waals forces (Autumn *et al.*, 2000; Autumn, 2002), capillary interactions (Huber *et al.*, 2005; Puthoff *et al.*, 2010; Mitchell *et al.*, 2020), acid–base interactions (Singla *et al.*, 2021) and possibly, electrostatic interactions (Izadi, Stewart and Penlidis, 2014).

Early studies of gecko adhesion found that adhesive capabilities decreased with increased time since shedding. These studies suggested that clumping of setae caused by fouling, rather than damage, may have caused the decrease (Dellit, 1934; Hiller, 1968). On the other hand, a decline in adhesion

over time since shedding is difficult to reconcile with more recent work suggesting that, on a micro-scale, individual seta are resistant to, or undergo little wear with repeated use (Gravish *et al.*, 2010; Autumn, Niewiarowski and Puthoff, 2014), apparently suffering no reduction in adhesion and friction. It is possible that fouling and clumping, or damage to entire lamellae or pads at the macro-scale, rather than of individual setae, caused the reduced performance observed in early studies (Dellit, 1934; Hiller, 1968). If so, it is likely that shedding and consequent rejuvenation of the adhesive system restores attachment capabilities.

Several studies have investigated the role of ecdysis in wound repair (Lai-Fook, 1968; Weis, 1976; Vafopoulou, 2009; Pellett and O'Brien, 2019); however, the role of ecdysis in repairing damage and rejuvenating the function of attachment systems has rarely been investigated. Hiller (1968) suggested that ecdysis might restore the attachment system of geckos after exposure to contamination and damage. Attachment of geckos is the culmination of contact on the macro (lamellae and scansors), micro (setal fields) and nano (spatulae) levels that could be exposed to damage through use and interaction with substrates. Therefore, using geckos as a model system, we examine how ecdysis affects performance when the attachment system is used normally, and when damage is induced. Specifically, we: (i) quantified the effect of mechanical damage on clinging ability of pad-bearing diplodactylid geckos, including quantifying safety factors for clinging, and (ii) tested whether ecdysis improves clinging ability when the attachment system is used for a period of time between shedding events. We hypothesised that both damage and general use would reduce clinging ability, and that ecdysis would repair and rejuvenate setal fields, leading to an improvement in clinging in both scenarios.

Materials and methods

Study species and husbandry

We examined clinging performance in three scansorial diplodactylid gecko species, *Oedura castelnaui* ,(Thominot, 1889) (N=10 in experiment 1 and N=9, in experiment 2, 4 males and 6 females), *Oedura monilis* , De Vis, 1888 (N=11, 5 males and 6 females) and *Strophurus krisalys* Sadlier et al., 2005 (N=6, 2 males and 4 females), all of which have leaf-like terminal toepads but different toepad areas relative to body size (Pillai *et al.*, 2020b, 2020a). Geckos were housed at James Cook University, Townsville, Australia, in constant temperature rooms with a 12 h:12 h light: dark inverted photoperiod. Each gecko was housed individually in a plastic enclosure lined with paper towel, containing a ceramic tile hide, and provided with water ad libitum. Geckos were fed four large domestic crickets (*Acheta domestica*) once a week. All enclosures were placed on heat mats at 32°C, running along one end of the enclosure, to provide a thermal gradient. Information on the geographic

origin of the animals and capture methods are provided elsewhere (Pillai *et al.*, 2020a; Riedel, Nordberg and Schwarzkopf, 2020).

Fieldwork to collect and house animals was undertaken under permits 'To Take, Use, Keep or Interfere with Cultural or Natural Resources (Scientific purposes)', Nature Conservation (administration) Regulation 2006 WITK18258517 and Scientific Purposes Permit 'Taking a protected animal for scientific purposes' WA0005590. Clinging ability experiments were undertaken under James Cook University Ethics Permit A2691.

Measuring toepad area

Surface area of the adhesive toepads may influence clinging performance (Irschick et al., 1996; Russell and Johnson, 2007, 2014; Peattie, 2009); therefore, surface area of adhesive regions was measured prior to testing, and total surface area, not excluding damaged areas, was included in all statistical analysis. The palmar and plantar surfaces of all individuals of the three species were photographed through glass against a uniform dark background with a scale in each image. To adjust the contrast and highlight the adhesive regions (both lamellae and scansors), we used Lightroom CC (Adobe). We used the incorporated scale bar to calibrate measurements in ImageJ (Schneider, Rasband and Kevin, 2012; Gillies and Fearing, 2014). The 'thresholding' feature was then used to select the toepads by manipulating the saturation of the photo, as they contrasted strongly with the rest of the image. Measurements were taken for all five toes on the right hand (manus) and right foot (pes) of all geckos, and doubled to calculate total adhesive area for each gecko.

Shedding intervals

From 23 March 2020 to 31 December 2020, we monitored geckos daily for shedding. Geckos were marked using a non-toxic Sharpie™ pen, which does not affect the rate of skin shedding in these gecko species (Fushida *et al.*, 2020). When a shedding event was detected after the mark disappeared, the date, temperature and humidity of the enclosure were recorded. Shedding intervals (number of days) were variable within and among species, and are provided in (Figure 0.1).

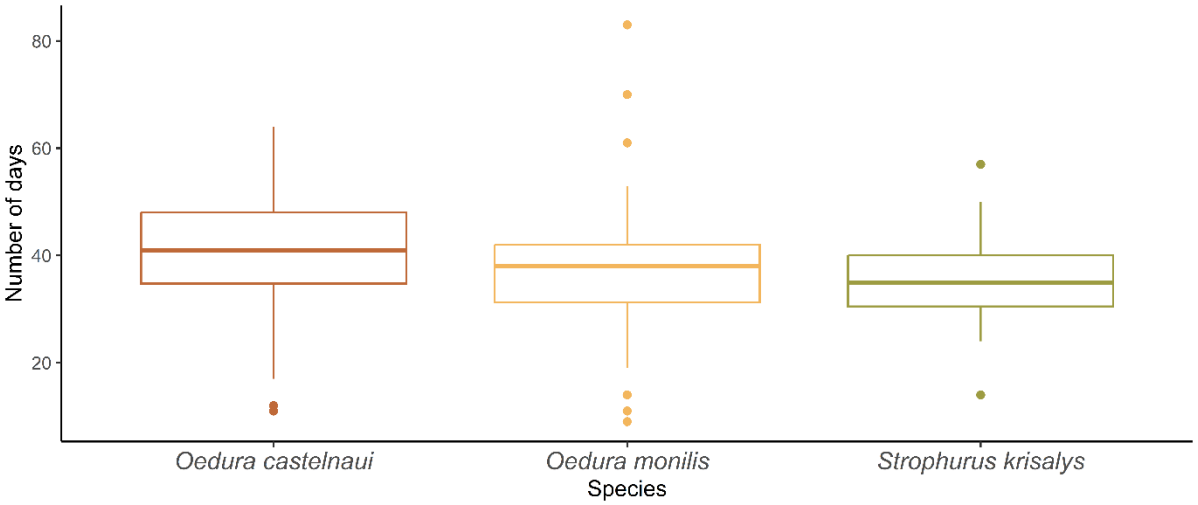

Figure 0.1. Shedding intervals of diplodactylid geckos. Shedding intervals of three species of geckos. *Oedura castlenaui* (N=10), *O. monilis* (N=11) and *Strophurus krisalys* (N=7). Shedding intervals did not vary among species. The boxes represent interquartile ranges (IQR), the bold line represents medians, and the whiskers extend to the largest or smallest value within 1.5* IQR from the first and third quartiles, respectively. Colour palette using r-package colRoz, Kong J, Wu N (2019). _colRoz: A colour palette for the land down under_. R package version 0.2.2, https://jacintak.github.io/project/colRoz

Damage and ecdysis (experiment 1)

Five days after a shedding event, we tested each gecko three times to quantify the maximum shear force generated on glass. Then, to cause accumulated damage, clinging ability was tested on glass again at ten-day intervals until the next shedding event. All three species were tested four times within a shedding cycle. We artificially induced damage to the entire system comprising of both scansors and lamellae as we could not distinguish scansors from lamellae with certainty owing to lack of information about the internal morphology of diplodactylid gecko toes (Russell and Bauer, 1988; Russell, Stark and Higham, 2019). We recorded the maximum force generated by the whole gecko (four limbs) using a force gauge (Extech 475040, Extech Equipment Pty Ltd) attached to a fishing line (diameter 0.5 mm) looped around the inguinal region of the gecko (Niewiarowski *et al.*, 2012). Each individual was tested three times to constitute one trial. To ensure that the attachment system of the gecko was engaged completely, each gecko was allowed to take one step with each of the four limbs. Once contact was made with all four limbs, geckos were pulled backwards horizontally at an angle of zero degrees and a constant velocity of ~0.5 cm s−1 (a ruler was placed beside the gecko and used in conjunction with a stopwatch). The test surface was cleaned using Kimwipes™ and reverse osmosis water, and airdried for 15 min between individuals. Only one investigator (R.R.P.) conducted clinging performance trials to reduce experimenter-specific effects (Pillai *et al.*, 2020b, 2020a).

We repeatedly tested shear force on glass to induce damage. Glass provides a greater attachment area for the setal fields than naturally rough surfaces and permits greater clinging ability due to the attachment system adhering firmly as a result of high levels of contact (Pillai *et al.*, 2020b). This high level of adhesion can lead to damage, removing lamellae by partially rupturing, or entirely removing scansors or lamellae (Autumn *et al.*, 2006). Visible damage to lamellae or scansors was recorded for each gecko.

Use and ecdysis (experiment 2)

Following experiment 1, geckos underwent one shedding event before being used in experiment 2. This time, we quantified the role of ecdysis in renewing clinging ability, when no damage was inflicted. The setal fields were subjected only to normal substrate interactions on paper towel, the ceramic shelter or plastic container walls while walking or running within their enclosure. Thus, after we completed trials investigating the role of damage on shear force, geckos were rested until the following shedding event. Then, to determine if clinging ability was restored by shedding, we measured shear force generated prior to, but as close to shedding as possible. Because the timing of shedding is variable among individuals and species, we could not predict exactly when shedding would occur, and we did not want to test individuals repeatedly for this experiment, so we tested them close to when we thought they might shed (1–45 days before, Figure 0.2). Then, each gecko was tested exactly 5 days after shedding (except one individual, which was tested 7 days after), to determine if shear force increased after shedding. Clinging ability was recorded using the same methods described above. As before, only one investigator (R.R.P.) conducted clinging performance trials to reduce experimenter-specific effects. A timeline of the tests we performed on shear force to investigate the effect of damage (experiment 1), and to investigate the effect of ecdysis following use (experiment 2) is shown in Figure 2.3

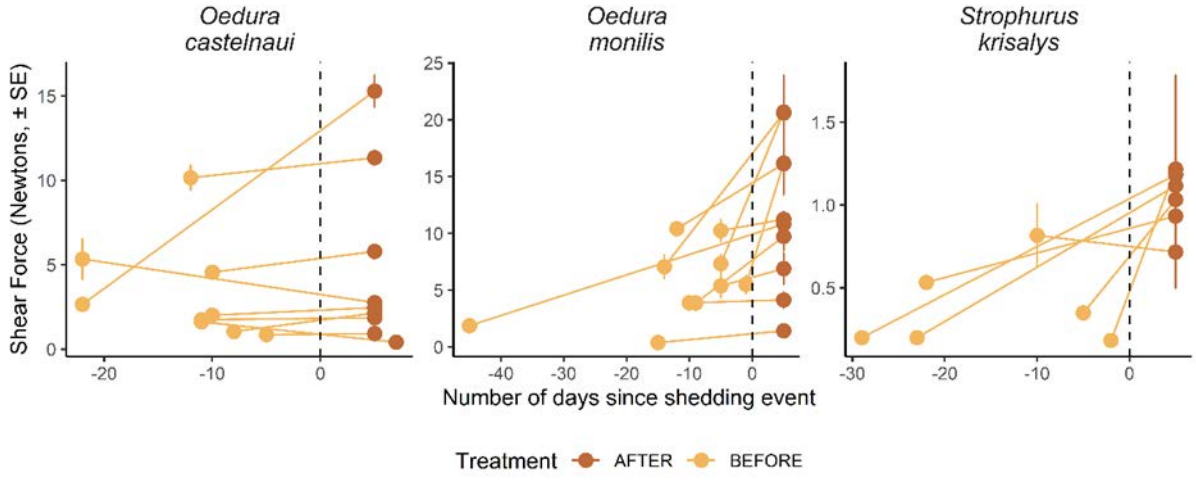

Figure 0.2. Shear force after ecdysis in individual diplodactylid geckos. Absolute shear force (Newtons, 1-43 days) before a shedding event, and five days after shedding, from the top model

(lowest AICc) that included the interaction between number of days before shedding, when maximum shear force was measured, species, mass and toepad area. Note: Y axis scales have been adjusted to reflect differences in magnitude of clinging ability exerted by each species. *Oedura castlenaui* (N=9), *O. monilis* (N=11) and *Strophurus krisalys* (N=7). Colour palette using r-package colRoz, Kong J, Wu N (2019). _colRoz: A colour palette for the land down under. R package version 0.2.2, https://jacintak.github.io/project/colRoz

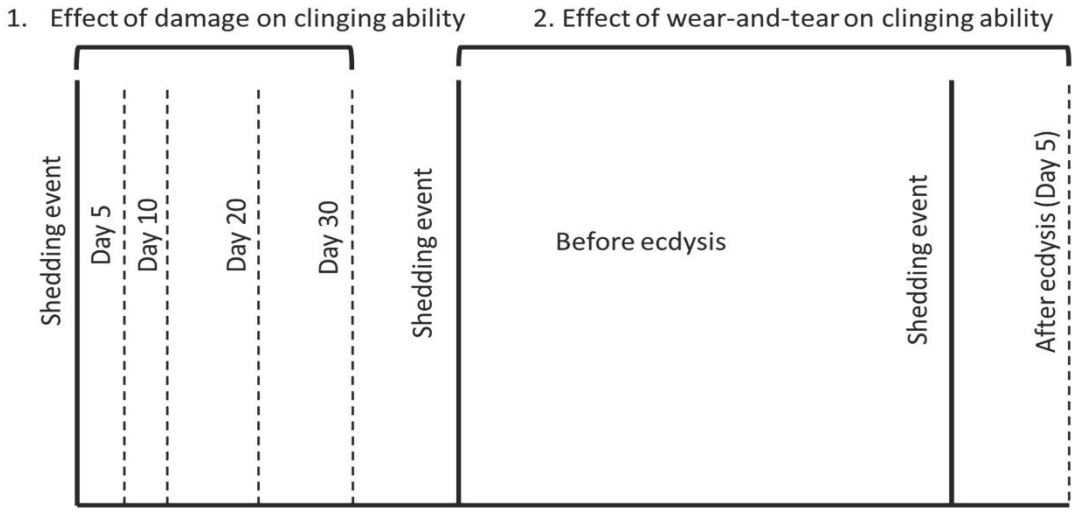

Figure 0.3. Experimental timeline of the study. Effect of mechanical damage (Experiment 1) on clinging ability. To induce repeated damage, we tested animals at 5-day and then at 10-day intervals following shedding. To allow damage to the attachment apparatus to repair and rejuvenate, geckos were allowed to complete a shedding event prior to testing of the influence of time and ecdysis only on clinging ability (Experiment 2). Geckos were tested once before (1-43 days) shedding and once after (5 days, one individual was tested 7 days after). *Oedura castlenaui* (N=10 in experiment 1, N=9 in experiment 2), *O. monilis* (N=11) and *Strophurus krisalys* (N=6).

Safety factors

We calculated safety factors under both scenarios, damage (experiment 1) and general use (experiment 2). Safety factors represent the margin of security-mitigating risks, in this case, the amount of force that can be applied in excess of that required to support an organism's body mass (Russell and Johnson, 2014) and reflect the capacity of a system to perform beyond expected or normal limits (Niewiarowski, Stark and Dhinojwala, 2016). For example, when all spatulae make contact with a substrate (under laboratory conditions), attachment forces are several times greater than what is needed to support a gecko's body weight (Autumn *et al.*, 2000, 2006; Higham, Russell and Niklas, 2017). This gave us an indication of safety factors under a scenario with substantial

mechanical damage and also after normal use for locomotion. We calculated safety factors for experiment 1 and experiment 2, by dividing the shear force imparted by each gecko by its mass, to examine if the damage we inflicted affected attachment to the point where geckos could not support their own body weight. We recorded shear force in Newtons, which was converted to gram force mm−1 by multiplying shear force values by 101.97, which accounted for acceleration due to gravity (Russell and Johnson, 2014).

Statistical analysis

All statistical analyses were conducted in R v. 4.0.3 (https://www.rproject. org/). Linear mixed effects models (LME models) were implemented with the package lme4 (https://cran.r-project.org/ package=lme4; Bates et al., 2015), while model comparison was conducted using the package MuMin (https://cran.r-project.org/ package=MuMIn). ANOVAs on all models were run using the package car (https://cran.r-project.org/package=car; Fox & Weisberg, 2019) and post hoc comparisons were implemented with the emmeans package (https://cran.r-project.org/ package=emmeans). Toepad area was not significantly different between males and females; therefore, we pooled sexes for all statistical analyses

Damage and clinging ability (experiment 1)

To quantify the effect of mechanical damage on clinging ability, we used log-transformed shear force per individual as the response variable in LME models as the distribution was right-skewed. Our initial set of models included 'days since shedding' and 'species' as individual fixed effects. These models included either 'mass' and 'toepad area' together, or just mass, or just toepad area. Each of these latter variables were included as either an offset or a scaling factor, as the units of measurements were in grams (mass) and mm^2 (toepad area), producing six models. Additionally, two models included an interaction between 'species' and 'days since shedding', and both 'mass' and 'toepad area', either as offsets or scaled. As each gecko was tested multiple times in each trial at each interval, we added individual gecko IDs as random effects in each model to account for effects of repeated measures on individuals. This process gave us eight candidate models, which we compared with each other and the null model using the Akaike's information criterion (AIC; Table 0.1). Models with the lowest AICc values were considered the best models explaining the effect of damage on shear force. The relative importance of the fixed terms included in the top models were determined using a type III ANOVA and post hoc comparisons were made using estimated least squares means. Geckos may not exhibit peak attachment capabilities immediately following shedding (Hiller, 1968); therefore, we used the maximum measure of attachment (rather than the first measure of attachment) to compare to the last (post-damage) measure of attachment to quantify the loss of clinging ability caused by damage.

Table 0.1. Candidate models included in selection using Akaike's information criterion to analyse the effect of mechanical damage on clinging ability. Models are arranged based on increasing AICc values and top model is in bold. df = degrees of freedom.

Fixed effects	df	AICc
Days since shedding*species + scale (mass) + scale (toepad area)	**22**	**972.20**
Days since shedding + species + scale (mass)	12	993.84
Days since shedding + species + scale (mass) + scale (toepad area)	13	995.97
Days since shedding + species + scale (toepad area)	12	996.25
Days since shedding + species + offset (mass)	11	1042.57
Null model	3	1135.19
Days since shedding + species + offset (toepad area)	11	1950.46
Days since shedding + species + offset (toepad area) + offset (mass)	11	1968.99

General use and ecdysis (experiment 2)

To investigate how ecdysis influenced performance after use without inducing damage, we created four specific candidate linear mixed effects (LME) models to both investigate and correct for the effects of mass and toepad area. Natural-log-transformed measures of shear force per individual before and after the shedding event (experiment 2) were used as response variables in all candidate models to normalize a right-skewed distribution. Two models included the three-way interaction among ‘species’, ‘treatment’ (before or after) and ‘days before shedding’, while two models included these variables as fixed effects only, with no interactions. All models included mass and toepad area, either as scaled fixed effects or as offsets, in two models each (Table 0.2). The same gecko was tested three times before and three times after ecdysis, so individual gecko IDs were included as random effects in all candidate models. The models with the lowest AICc were considered the most likely to accurately predict the effect of ecdysis on shear force and were further analyzed using a type III ANOVA to understand the relative importance of fixed effects, following which, differences among variables were examined using estimated least squared means post hoc comparisons.

Table 0.2.Candidate models included in selection using Akaike's information criterion to analyse the role ecdysis in restoring clinging ability after general use. Models are arranged based on increasing AICc values and top model is in bold. df = degrees of freedom.

Fixed effects	df	AICc
Species: Treatment: Days + scale(mass) + scale (toepad area)	**13**	**288.14**
Treatment + Species + Days + scale (mass) + scale (toepad area)	10	303.73
Species: Treatment: Days + offset(mass) + offset (toepad area)	11	454.68
Species + Treatment + Days + offset(mass) + offset (toepad area)	8	467.6922

Clinging ability after damage and after general use

We used a linear mixed-effects model to compare the impact of damage and general use on declines in shear force. Shear force was the response variable, with 'species' and 'test period (shear force after damage and after general use) as fixed effects. We also included an interaction between 'species' and 'test period' in our model. As each individual was tested multiple times during each trial, individual gecko IDs were included as random effects in the model.

Safety factors

Safety factors for each individual were calculated from experiment 1 and experiment 2. We conducted separate analyses to test the effect of damage (experiment 1) and general use (experiment 2). Each analysis consisted of four candidate LME models. To analyze the effect of damage, we used safety factors calculated from shear force exerted at four intervals (5, 10, 20 and 30 days). Models included the interaction between 'days since shedding' and 'species', and also 'species' and 'days since shedding' additively as fixed effects. Additionally, we tested models including only 'species' or only 'days since shedding' as fixed effects. The same set of fixed effects was used in candidate models to investigate safety factors before and after ecdysis, with the response variables being safety factors before and after shedding. In model sets of candidate models, response variables were log-transformed to normalize a right-skewed distribution. In both experiments, safety factor values were calculated for multiple measures per individual, so individual gecko IDs were included as random effects. The relative importance of the fixed terms included in the top model were determined using a type III

ANOVA, and post hoc comparisons were made using estimated least squares means in the package emmeans (Table 0.3).

Table 0.3.Candidate models included in selection using Akaike's information criterion to analyse the changes in (A) safety factors associated with reductions in clinging ability due to damage and (B) safety factors associated with role of ecdysis in restoring clinging ability after general use.

Models are arranged based on increasing AICc values and top model is in bold. df = degrees of freedom.

a. Damage

Fixed effects	df	AICc
Species: Days since shedding	**14**	**887.76**
Species + Days since shedding	8	912.02
Days since shedding	6	941.17
Species	5	989.98

b. General use

Fixed effects	df	AICc
Species: Treatment (Shear force before and After shedding)	**8**	**270.03**
Species + Treatment (Shear force before and After shedding)	6	290.34
Treatment (Shear force before and After shedding)	4	302.66
Species	5	338.30

Results

Damage and clinging ability (experiment 1)

The best model predicting the effect of damage on shear force included an interaction between 'days since shedding' and 'species', and 'mass' and 'toepad area' as scaled fixed effects (R2 conditional=0.77). Slopes of plots of clinging ability over time after repeated testing were negative, indicating that clinging ability always declined as damage increased. The slope of these relationships (or the rate at which clinging ability declined with damage) differed among species, indicated by the significant interaction between 'days since shedding' and 'species' ($P<0.01$, $\chi2=46.61$, ANOVA, Type III Wald $\chi2$ tests). Mass ($P=0.21$) and toepad area ($P=0.63$) had no significant effect on shear force (ANOVA, Type III Wald $\chi2$ tests). After damage, clinging ability decreased significantly in *O. castelnaui* (N=10, test day 5 versus 30, $P<0.01$, estimated marginal least square means post hoc comparisons) and *S. krisalys* N=7, test day 5 versus 30, $P<0.01$, estimated marginal least square means post hoc comparisons). In *O. monilis*, the decrease in clinging ability was marginally significant (N=11, test day 5 versus 30, $P=0.05$, estimated marginal least square means post hoc comparisons). After ecdysis, clinging ability improved and all species exhibited peak clinging ability on the first day they were tested (5 days after shedding), except for *O. monilis*, which generated the highest clinging ability in the test 10 days after shedding. Clinging ability reduced by 1.90 N between day 5 and day 30 in *O. castelnaui* (N=10), by 2.70 N between day 10 and day 30 in *O. monilis* (N=11) and by 0.52 N between day 5 and day 30 in *S. krisalys* (N=7; Figure 0.4).

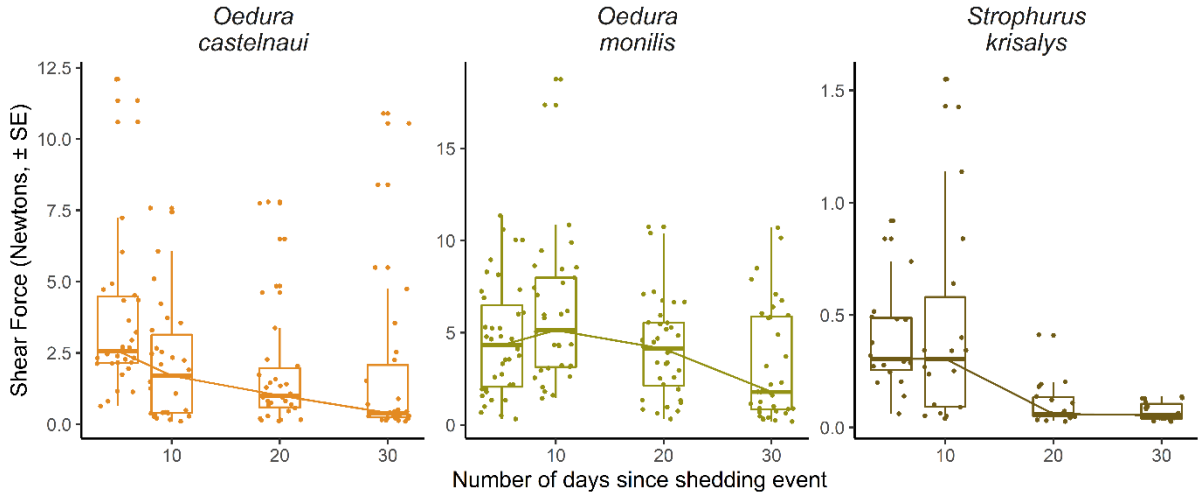

Figure 0.4. The effect of mechanical damage on clinging ability. Differences in absolute shear force (Newton, median ± SE) with repeated damage. Following repeated damage, *Oedura castelnaui* exerted 1.94 Newton less shear force 30 days after shedding compared to 5 days after shedding (N =10, $P < 0.01$, estimated marginal least square means post hoc comparisons) ; *Oedura monilis* exerted 2.7 Newton less shear force between day 10 and day 30 since shedding (N = 11, P = 0.05, estimated

marginal least square means post hoc comparisons), and clinging ability decreased by 0.52 between day 5 and day 30 in *Strophurus krisalys* (N=6, P < 0.01, estimated marginal least square means post hoc comparisons). Colour palette using r-package colRoz, Kong, J. and Wu, N. (2019). colRoz: A colour palette for the land down under. R package version 0.2.2,<https://jacintak.github.io/project/colRoz>

Macroscopic damage to toepads

Macroscopic damage to toepads was obvious in 11/29 geckos during experiment 1. Toepads were entirely removed, leaving parts of the mesos layer exposed. No such damage was ever observed on geckos within 5 days of shedding. Damage on the micro- and nanoscale may have occurred, but could not be observed without destructive microscopy (Figure 0.5).

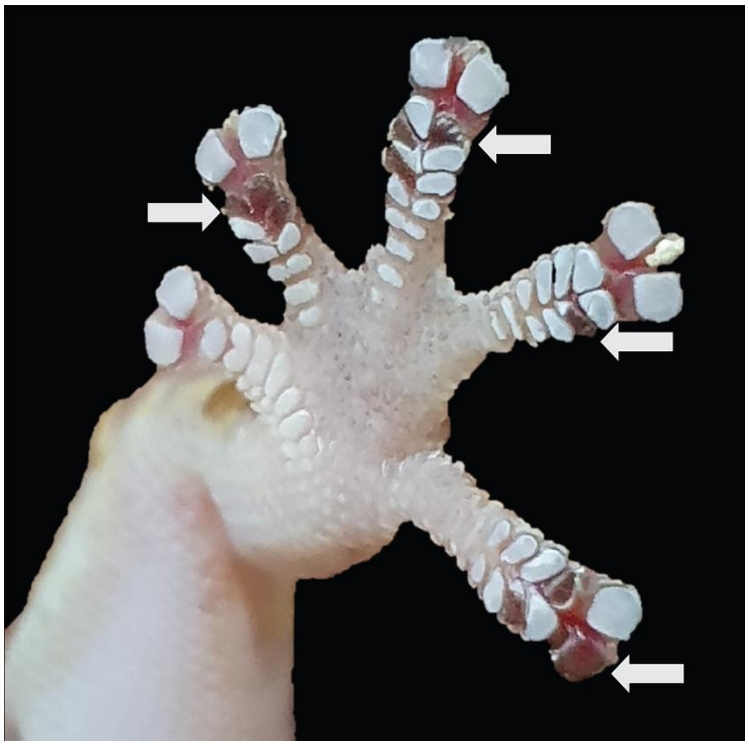

Figure 0.5. Lamellae damage on a macro-scale. (Photo taken on Galaxy S10 at 2x, Samsung ®). White arrows indicate regions where partial or entire lamellae have been separated from the likely mesos layer.

General use and ecdysis (experiment 2)

The change in shear force before versus after shedding, when no 'extra' damage was induced, was best predicted (lowest AICc) by a model including an interaction among species, treatment and days before shedding, which also included toepad area and mass as scaled, fixed terms (R^2 conditional=0.89). The significant three-way interaction indicated that the slopes of the change in shear force before and after shedding were different among species (or that the amount of improvement induced by ecdysis varied among species), and also varied with the number of days

before shedding the measurement was taken. The interaction among species, treatment and days before shedding (P<0.01, $\chi 2$ =34.60, ANOVA, Type III Wald $\chi 2$ tests), mass (P<0.01, $\chi 2$=4.3, ANOVA, Type III Wald $\chi 2$ tests) and toepad area (P=0.06, $\chi 2$=3.59, linear mixed-effects model, ANOVA, Type III Wald $\chi 2$ tests) were significant variables in the best model (Table 0.2). Maximum shear force was significantly higher 5 days following shedding compared with just before it in most species; however, the magnitude of increases in clinging ability varied among species Generally, with no extra induced damage, mean clinging ability still increased following shedding in all species, and the increase was statistically significant in *O. monilis* (N=11, P<0.01, estimated marginal least square means post hoc comparisons) and *S. krisalys* (N=7, P <0.01, estimated marginal least square means post hoc comparisons). The increase in clinging ability following ecdysis was not statistically significant in *O. castelnaui* (N=9, P=0.3, estimated marginal least square means post hoc comparisons). Following ecdysis, clinging ability increased by 5.23 N in *O. monilis* and by 5.67 N in *S. krisalys* (Figure 0.6). The magnitude of increase in clinging ability were stronger in some species than others and depended on the number of days since shedding (Figure 0.2).

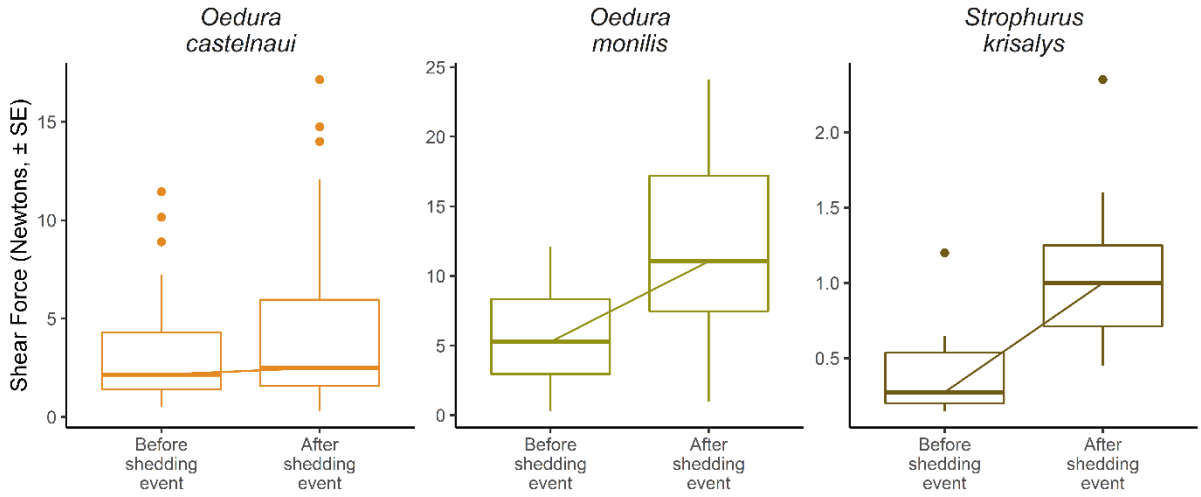

Figure 0.6. The role of ecdysis in restoring clinging ability after general use. Absolute shear force (Newtons, median ± SE) before and after a shedding event. Following ecdysis, clinging ability increased by 5.23 Newton in *Oedura monilis* (N=11, P < 0.01, estimated marginal least square means post hoc comparisons), and by 5.67 in *Strophurus krisalys* (N = 6, P < 0.01, estimated marginal least square means post hoc comparisons). Clinging ability increased in *Oedura castelnaui* (N=9), however, this increase was not statistically significant. Note: Y axis scales have been adjusted to reflect differences in magnitude of clinging ability exerted by each species. Colour palette using r-package colRoz, Kong, J. and Wu, N. (2019). colRoz: A colour palette for the land down under. R package version 0.2.2, <https://jacintak.github.io/project/colRoz>

Clinging ability after damage and after general use

Damage caused a greater reduction in shear force compared with general use. The interaction between 'species' and 'test period' significantly affected the shear force exerted after damage and with general use (P<0.05, $\chi 2$=6.88, linear mixed-effects model, ANOVA, Type III Wald $\chi 2$ tests). Decline caused by damage was 1.52N greater than that cause by general use in *O. castelnaui* (P<0.01), 2.80 N in *O. monilis (*P<0.01) and 0.26 N in *S. krisalys* (P<0.01, Figure 0.7).

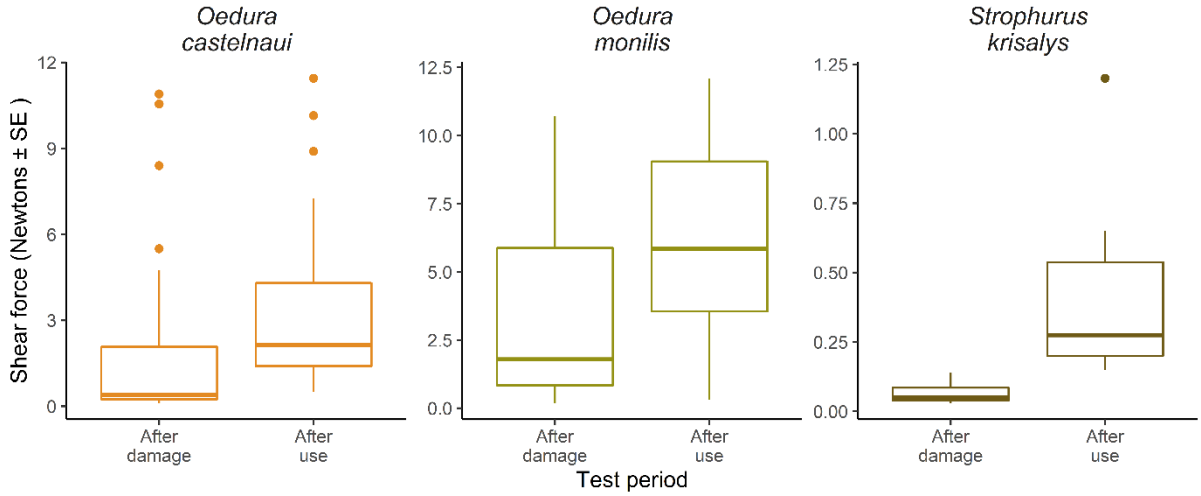

Figure 0.7.Clinging ability after damage, and after general use. Clinging ability (Newton, median ± SE) after induction of damage (30days after a shedding event). Decline caused by damage was 1.52 Newtons greater than that cause by general use in *O. castelnaui* (N=10 in experiment 1 (damage), N=9 in experiment 2 (general use), P < 0.01), 2.8 Newtons in *O. monilis* (N=11 , P < 0.01) and 0.26 in *S. krisalys* (N=6 ,P < 0.01).

Safety factors

The best model (lowest AICc) predicting the differences in safety factors in relation to increasing mechanical damage (experiment 1) included the interaction between 'species' and 'days since shedding' (R2 conditional=0.61, linear mixed-effects model, ANOVA, Type III Wald $\chi 2$ tests) consistent with the best model predicting the influence of damage on clinging ability (above). Safety factors decreased significantly in *O. castelnaui* between day 5 and day 30 (P<0.05), and between day 10 and day 30 in *O. monilis* (P<0.01). The decrease in safety factors between day 5 and day 30 was not statistically significant in *S. krisalys* (P=1). We concluded that the slope of the decline in safety factors with damage differed among species because the best model predicting the decline in safety factors with increasing damage included an interaction between 'species' and 'days since shedding event'. Safety factors reduced by 12.54 in *O. castlenaui* between day 5 and day 30 after damage, 9.87 in *O. monilis* between day 10 and day 30 after damage. Safety factors decreased by 4.77 in *S. krisalys*,

but this decline was not statistically significant (Figure 0.8). The best model predicting the change in safety factors before and after ecdysis (experiment 2) included the interaction between 'species' and 'days since shedding' ($P<0.01$, $\chi2 = 27.36$, ANOVA, Type III Wald $\chi2$ tests) and 'species' by itself ($P<0.01$, $\chi2 = 24.95$, ANOVA, Type III Wald $\chi2$ tests). Safety factors were significantly lower by 36.54 prior to ecdysis in *O. monilis* ($P<0.01$) and by 9.27 *S. krisalys* ($P<0.01$); however, they did not change significantly in *O. castelnaui* ($P=0.84$, Figure 0.8).

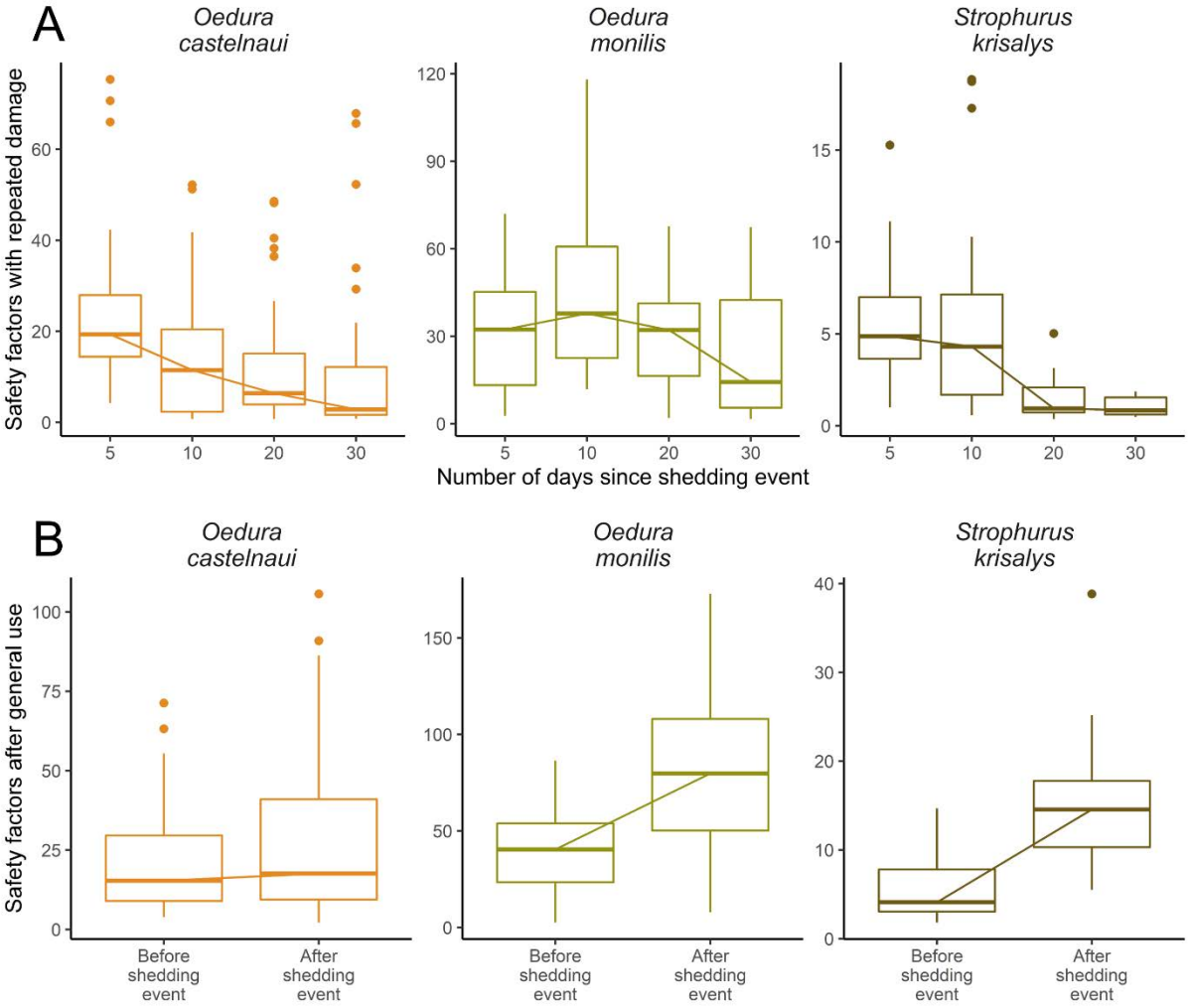

Figure 0.8. Safety factors. (A) Safety factors (median ± SE) associated with absolute shear force in relation to repeated damage. *Oedura castlenaui* (N=10), *O. monilis* (N=11) and *Strophurus krisalys* (N=6). (B) Safety factors (± SE) associated with absolute shear force with general use and after ecdysis. *Oedura castlenaui* (N=10), *O. monilis* (N=11) and *Strophurus krisalys* (N=6). Colour palette using r-package colRoz, Kong, J. and Wu, N. (2019). colRoz: A colour palette for the land down under. R package version 0.2.2, https://jacintak.github.io/project/colRoz

Discussion

We show that macro-scale damage in the form of ruptured scansors or lamellae, most likely combined with additional damage on the micro- and nano-scale, lead to a decline in clinging ability in

all three species we examined, with an associated reduction in safety factors. After ecdysis, clinging ability increased in all three species, although not significantly in *O. castelnaui*. Even without exposure to 'extra' damage at consistent intervals, shear force was lower prior to ecdysis. Possibly, just walking and contacting substrates causes mechanical damage reducing shear force. Alternatively, clumping or fouling of setae may be occurring (Hiller, 1968), or perhaps all three mechanisms reduce the effectiveness of setae over time. Thus, we conclude that both damage and general use affected attachment capabilities in these geckos. It was clear from our studies that the periodic process of ecdysis restored attachment capabilities and significantly increased safety factors.

Damage and clinging ability (experiment 1)

While some early studies suggested that declines in performance with time since shedding may be caused by damage and clumping of setae caused by accumulation of contaminants (Hiller, 1968), other studies have found little evidence for declines in performance at the level of individual setae (Gravish *et al.*, 2010; Puthoff *et al.*, 2013; Autumn, Niewiarowski and Puthoff, 2014). In our study, especially when we induced damage, we attribute the majority of the decline in performance to physical damage on the macro-scale, possibly with some breakage of individual setae or entire fields (nano- and micro-scale, respectively). When we did not induce damage, the reduction in clinging ability is difficult to explain. There may be damage at the micro-scale or clumping and fouling of the setae may have occurred. Evidence exists that setae have self-cleaning mechanisms (Hansen and Autumn, 2005), and because our geckos were housed on paper towel in plastic enclosures, the opportunity for microdamage and fouling seem low, but we cannot dismiss them as factors causing the decline. More detailed studies of the role of natural micro-scale damage and fouling are required, to clarify their roles in loss of clinging ability over time, especially for geckos using natural substrates.

There are six stages in the shedding cycle of geckos, which comprise a very long stage 1, followed by a rapid series of shorter stages just before shedding (Maderson, 1966). Depending on the stage of shedding, attachment could vary. In our study, the declines in shear force we quantified very likely occurred entirely in stage 1, the resting phase. We observed no consistent patterns of decline in shear force in relation to shedding phases, for example, there were no sudden drops late in testing as other shedding phases may have been reached. Future studies should investigate clinging ability, without the effect of damage, in the different shedding phases, to understand possible influences of the shedding cycle on clinging ability.

With damage, decreasing shear force caused reductions in safety factors, the extent of which was different among species. In our study, safety factors declined in *O. castelnaui* (24.42 to 11.88) and *O. monilis* (31.98 to 22.11) with increasing mechanical damage. Safety factors were consistently low in *S. krisalys*, which reflects the low shear forces throughout the entire experiment, and dropped close to 1 (5.77 to 1.03). Safety factors below 1 indicate failure of the adhesion system (Higham, Russell

and Niklas, 2017); therefore, repeated damage influenced adhesive capacity to a level close to where geckos could not support their body weight.

Use and ecdysis (experiment 2)

Following experiment 1, geckos were allowed to shed before being tested for experiment 2. This gave us a measure of the renewal provided by ecdysis, without beginning from a (possibly) artificially low point after extensive damage. The opportunity for damage due to strong attachment was minimal in the second experiment; however, the attachment system was subjected to interactions with substrates within the enclosure. Although there was no induced damage, and potentially minimal other factors reducing clinging ability, which we could not quantify in our study, clinging ability was still lower before, and increased after ecdysis in this experiment. Hsu et al., (2012) found 'gecko footprints' of phospholipid residue left behind on surfaces close to a shedding event. They suggested that these lipid molecules may be a sacrificial layer, that protects the degradation of the β-keratin spatulae. Furthermore, the quantity of these lipids on setae is likely to affect the ductility of these structures. Therefore, the concentration of these lipids in setae prior to and after ecdysis might cause the differences in clinging ability we observed before and after a shedding event. Furthermore, the declines in clinging ability due to 'use only' were lower than the declines caused by 'damage' in all three species (Figure 0.7).

We could not predict exactly when geckos would shed and as we were avoiding repeated testing, the number of days before ecdysis varied in experiment 2. Interestingly, our geckos' ability to attach varied in relation to the time of testing: there was a greater decrease in clinging ability of geckos as they approached ecdysis. If damage, clumping or fouling (or likely all three) accumulate over time, for those individuals tested immediately before ecdysis (as little as 1 day before), clinging ability should have been closer to its lowest value, whereas those tested earlier (as many as 43 days before) had higher clinging ability. Thus, the time of testing in relation to ecdysis influenced measures of attachment, suggesting that performance declines close to shedding, consistent with our first experiment. Another possibility is that the process of ecdysis itself lowers performance, perhaps by increasing the likelihood or severity of damage late in the cycle (Watson, 1971) or by reducing clinging ability by directly reducing setal performance. This kind of effect may be similar to other negative effects of shedding, such as clouded eyes in snakes (Brown, 1956) and soft shells in crabs (Watson, 1971) before ecdysis. It would be interesting to determine if use of certain orientations or angles of attachment, boldness or other behaviors requiring good clinging performance change as ecdysis approaches in geckos.

In experiment 2, when the attachment system was exposed only to substrate interactions through locomotion, safety factors did not increase significantly after ecdysis in *O. castelnaui*; however, they increased following ecdysis in *O. monilis* and *S. krisalys*. Clinging ability before and

after ecdysis was highly variable (three out of nine individuals exhibited lower clinging ability following ecdysis) in *O. castelnaui*, potentially because of the differences in the level of interactions with substrates upon which they moved. Our findings indicate that even in a scenario where damage was not excessive, ecdysis did improve clinging ability and associated safety factors in *O. monilis* and *S. krisalys*. Even though safety factors were lower prior to ecdysis, they were still greater than 1, indicating that animals could still support their body weight despite declining performance over time. However, when damage was induced (experiment 1), safety factors fell to critically low levels in *S. krisalys*, which exhibited lower initial safety factors. This effect of damage indicate the attachment system's capacity to compensate for abrasion from general use if damage is not extensive; however, in instances when damage is extensive, safety factors can be lowered to a point where geckos cannot support their body weight using setae, which may indicate a role for claws.

Attachment mechanisms and their underlying morphology have been the focus of several studies over the past two decades (Federle, 2006; Niederegger and Gorb, 2006; van Casteren and Codd, 2010; Bennemann, Scholz and Baumgartner, 2011; Hagey *et al.*, 2014). Other studies have investigated differences in performance in relation to varying substrate characteristics (Langowski *et al.*, 2019; O'Donnell and Deban, 2020; Palecek, Schoenfuss and Blob, 2020; Stark and Yanoviak, 2020; Song *et al.*, 2021). Interactions with these substrates are likely to cause wear, damage or contamination, influencing performance (Hiller, 1968; Dellit, 1934). Here, we found that induced damage and general substrate interaction reduce performance in geckos, which is restored through ecdysis. Other attachment systems, for example, in cockroaches, spiders and stick insects are likely to undergo damage or wear as well, but the effect of damage on these attachment systems has received little attention. Self-cleaning can restore attachment in dock beetles (*Gastrophysa viridula*) and stick insects (*Carausius morosus*) (Clemente *et al.*, 2010); however, to our knowledge, improvement in performance after use and damage has rarely been investigated. The role of ecdysis to repair damage in general, such as lost limbs or open wounds are well studied in invertebrates (Lai-Fook, 1968; Weis, 1976; Vafopoulou, 2009; Pellett and O'Brien, 2019), as are the underlying endocrine processes that enable ecdysis (Park *et al.*, 2003; Žitňan *et al.*, 2003; Zhu *et al.*, 2019) but further studies could investigate the effect of ecdysis on performance in other attachment systems.

Chapter 3. Geckos cling best to, and prefer to use, rough surfaces

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Abstract

Background: Fitness is strongly related to locomotor performance, which can determine success in foraging, mating, and other critical activities. Locomotor performance on different substrates is likely to require different abilities, so we expect alignment between species' locomotor performance and the habitats they use in nature. In addition, we expect behaviour to enhance performance, such that animals will use substrates on which they perform well.

Methods: We examined the associations between habitat selection and performance in three species of *Oedura* geckos, including two specialists, (one arboreal, and one saxicolous), and one generalist species, which used both rocks and trees. First, we described their microhabitat use in nature (tree and rock type) for these species, examined the surface roughnesses they encountered, and selected materials with comparable surface microtopographies (roughness measured as peak-to-valley heights) to use as substrates in lab experiments quantifying behavioural substrate preferences and clinging performance.

Results: The three *Oedura* species occupied different ecological niches and used different microhabitats in nature, and the two specialist species used a narrower range of surface roughnesses compared to the generalist. In the lab, *Oedura* geckos preferred substrates (coarse sandpaper) with roughness characteristics similar to substrates they use in nature. Further, all three species exhibited greater clinging performance on preferred (coarse sandpaper) substrates, although the generalist used fine substrates in nature and had good performance capabilities on fine substrates as well.

Conclusion: We found a relationship between habitat use and performance, such that geckos selected microhabitats on which their performance was high. In addition, our findings highlight the extensive variation in surface roughnesses that occur in nature, both among and within microhabitats.

Keywords: Adaptation, Attachment, *Oedura*, Performance, Peak-to-valley height, Substrates, Shear force

Introduction

Habitat use has been a critical variable included in ecological niche studies for several decades (Wainwright and Reily, 1994). A common functional requirement for successful niche use is effective locomotion within the environment (particularly on specific microhabitats, for example on trees or rocks). Locomotory ability influences an animal's success at capturing prey, avoiding predators, and acquiring mates (Aerts et al., 2000), thereby influencing growth rates, survival, reproduction, and consequently, Darwinian fitness (Aerts et al., 2000; Garland Jr. & Losos, 1994; Higham & Russell, 2010; Irschick et al., 1996; Irschick & Losos, 1999; Losos, 1990; Turchin, 1989). If variation in performance in relation to substrate microhabitat use is adaptive, species should use substrates that enhance locomotor capabilities to increase fitness in nature (Robinson, Wilson and Shea, 1996; Vanhooydonck, Damme and Aerts, 2002; Russell and Johnson, 2014). Given that species exploit a range of topographical features within their environment we expect natural selection to act on locomotor performance in relation to the specific challenges encountered (Vanhooydonck, Damme and Aerts, 2002; Kohlsdorf *et al.*, 2004; Collins, Russell and Higham, 2015). Examples of relationships between performance and habitat use exist in a range of habitats, including marine (Fulton, Bellwood and Wainwright, 2001), aerial (Norberg and Rayner, 1987), arboreal (e.g., *Anolis*, Brandt et al., 2015; Robinson et al., 1996; Vanhooydonck et al., 2005), and terrestrial (e.g., *Tropidurus* sp. (Melville and Swain, 2000; Kohlsdorf *et al.*, 2004); Lacertids, (Vanhooydonck, Van Damme and Aerts, 2002); and skinks, (Melville and Swain, 2000; Goodman, Miles and Schwarzkopf, 2008) environments. In general, this work suggests the presence of trade-offs, in which adaptations that optimize performance in certain habitats may not be beneficial in others (Irschick and Losos, 1999). For example, traits like foraging performance (Barkae *et al.*, 2012), running (Goodman, Miles and Schwarzkopf, 2008), sprinting (Melville and Swain, 2000; Goodman, Miles and Schwarzkopf, 2008), and clinging (Goodman, Miles and Schwarzkopf, 2008; Tulli, Abdala and Cruz, 2011) are a function of morphological adaptations that evolve in the context of habitat. These relationships between habitat use and performance, may mean animals only use certain subsets of the entire range of microhabitats available, in which their performance is high, or may, at least, avoid microhabitats in which they do not perform well. Therefore, it is essential to incorporate habitat selection behaviour into studies investigating the relationship between habitat structure and performance (Irschick and Losos, 1999).

Both the general nature of the habitat (e.g., open versus closed habitats Clemente, Thompson, et al., 2009; Melville & Swain, 2000; Vanhooydonck & Van Damme, 2003) and specific structural components like incline (Irschick and Jayne, 1999; Astley and Jayne, 2007), and perch characteristics (Irschick and Losos, 1999; Mattingly and Jayne, 2004; Irschick *et al.*, 2005; Calsbeek and Irschick, 2007) influence performance capabilities. Locomotor performance shapes habitat use (Fulton, Bellwood and Wainwright, 2001), such that species that can move well on a range of substrate widths, for example, occupy a broader range of habitats than species specialized to narrow range of substrates

(Irschick and Losos, 1999; Calsbeek and Irschick, 2007). Further, rocky habitats appear to select for increased jumping capacity and lower absolute sprint speeds (Kohlsdorf *et al.*, 2004), increased limb lengths (Goodman, Miles and Schwarzkopf, 2008), and resistance to mechanical traction (Tulli, Abdala and Cruz, 2011), compared to sandy (Thompson, 1990; Tulli, Abdala and Cruz, 2011; Brandt, Galvani and Kohlsdorf, 2015) and other habitats (Goodman, Miles and Schwarzkopf, 2008; Tulli, Abdala and Cruz, 2011). Recently, some studies have investigated performance in relation to structural components like surface textures and microtopographies (Clemente *et al.*, 2009; J. M R Bullock and Federle, 2011; Ditsche, Wainwright and Summers, 2014; England *et al.*, 2016). Microtopographical characteristics like surface roughness (Clemente *et al.*, 2009; J. M R Bullock and Federle, 2011; Ditsche, Wainwright and Summers, 2014; Crawford *et al.*, 2016; England *et al.*, 2016; Langowski *et al.*, 2019), fouling (Ditsche, Wainwright and Summers, 2014) and periodical wrinkles (Voigt *et al.*, 2012) affect attachment capabilities of various taxa, which influence performance due to differences in the area available for contact with the attachment apparatus (Fuller and Tabor, 1975; Vanhooydonck, Andronescu, *et al.*, 2005; Russell and Johnson, 2014). Attachment capabilities of adhesive systems are generally higher on smooth surfaces that provide a greater area for attachment as compared to rough surfaces (Crawford *et al.*, 2016; England *et al.*, 2016). Studies examining the range of morphologies and capabilities on natural surfaces remain rare in some systems, but are critical to understanding selective forces.

Geckos (Gekkota) are a diverse and widely distributed lizard clade comprising of approximately 2008 species of geckos belonging to over 100 genera (Uetz, Freed and Hošek, 2020). Geckos occupy a variety of habitats (Russell, 2002; Gamble *et al.*, 2012; Hagey *et al.*, 2014; Meiri, 2019; Riedel, Nordberg and Schwarzkopf, 2020), and are well known for their specialized adhesive toepads and climbing abilities (Hagey *et al.*, 2014; Russell and Gamble, 2019). The development of adhesive toepads has enabled geckos to use inverted and inclined surfaces on rocks and vegetation (Glossip and Losos, 1997; Gamble *et al.*, 2012; Hagey, Harte, *et al.*, 2017). Adhesion occurs through finely tuned, hierarchically arranged adhesive. toe pads (Hansen and Autumn, 2005; Peattie and Full, 2007; Hagey *et al.*, 2014; Stark, Palecek, *et al.*, 2015), characterized by subdigital scansors that carry highly organized fields of microfibrillar setae. Each seta is branched, and branches terminate in broadened tips called spatulae (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Stark, Ohlemacher, *et al.*, 2015). Friction is achieved as van der Waals forces generating a normal force between the spatulae and the substrate (Autumn, 2002; Hagey *et al.*, 2014). Substrate surface topology influences the area available for attachment of the setae, and high surface area increases the magnitude of force generated (Ernst and Ruibal, 1966). Thus, the adhesive system of geckos was thought to perform better on smooth and uniform surfaces (Russell and Johnson, 2014). Recently, however, some studies have found that geckos are capable of attachment on rough and undulant substrates, previously thought to provide limited purchase for attachment (Russell and Johnson, 2014; Pillai *et al.*, 2020b). Clearly,

further study of gecko performance on rough and undulant surfaces, similar to those geckos encounter in nature, is required (Robinson, Wilson and Shea, 1996; Spolenak *et al.*, 2005; Russell and Johnson, 2014; Nordberg and Schwarzkopf, 2019a; Russell and Gamble, 2019; Pillai *et al.*, 2020b).

Studying functional relationships between performance and texture (microtopography) is important for understanding the biomechanics of gecko adhesion, but to understand the evolutionary, adaptive, and ecological aspects of surface texture and its relationship with fitness, we need to understand how geckos use surfaces they encounter in the wild. We investigated microhabitat choice in the context of locomotory performance, both in the laboratory and in nature, in three sympatric species of the Australian Diplodactylid gecko genus *Oedura*: one saxicoline or rock-dwelling species, one arboreal species, and one generalist species that uses both rocks and trees (this study, Meiri, 2019; Russell & Gamble, 2019). We quantified habitat choice of these geckos in the field, and measured roughness (as peak-to-valley heights, a two-dimensional measure of surface roughness), of surfaces used by these geckos in nature. We then brought individuals of all three species into the laboratory, and examined their microhabitat choice using artificial substrates that spanned the surface roughnesses we measured in nature. Finally, we measured shear force as a measure of performance on these different artificial substrates, to determine if there was a link between substrate choice and performance (Fuller and Tabor, 1975; Tulli, Abdala and Cruz, 2011; Riedel, Nordberg and Schwarzkopf, 2020). We hypothesized that geckos select substrates, and would perform better on surfaces more similar to those they use in nature. We also hypothesized that, if gecko performance was closely adapted to the substrates they used, the generalist species may perform better (on average) on a variety of microhabitats, whereas specialists may be more likely to exhibit better performance on specific habitats.

Methods

We assessed microhabitat use in *Oedura* geckos by compiling observations from individuals sighted or captured in nature (124 observations). To assess surface roughness used by these geckos, and to determine the roughnesses of the surfaces used in the lab in relation to natural surfaces, we measured the peak-to-valley heights (μm) of the surfaces geckos used, and also measured the two artificial surfaces (sandpaper) we used to assess microhabitat choice (N = 10 for the three *Oedura* species) and clinging performance (*O. castelnaui*, N = 10; *O. coggeri*, N = 11, and *O. monilis*, N = 11) in the laboratory. From the geckos observed in nature, a subset of individuals of each species were collected for laboratory trials of microhabitat choice and clinging performance.

Study species

We studied three closely related (Stark, Palecek, *et al.*, 2015), and otherwise morphologically similar (Hoskin and Higgie, 2008), species of velvet gecko (genus *Oedura*) with well-developed toepads, namely, northern velvet geckos (*Oedura castelnaui*), northern spotted velvet geckos (*Oedura*

coggeri) and ocellated velvet geckos (*Oedura monilis*, Autumn, 2002; Meiri, 2019; Nordberg & Schwarzkopf, 2019; Zani, 2000).

Microhabitat use in nature

To assess microhabitats used by these geckos, we compiled observations from 2015 to 2020. We recorded microhabitat categories used by geckos (tree species and rock types). We included observations from different times of the year and from a range of localities (Supplementary material S4). We used a total of 124 observations of three species (*O. castelnaui*, N = 67; *O. monilis*, N = 40; and *O. coggeri*, N = 17) and calculated the percent of observations for each species on each microhabitat type.

Microhabitat roughness and selection of test substrates

All three species occurred in open eucalyptus woodlands, but used different microhabitats. We measured the peak-to-valley heights of substrates used by our geckos using a surface profile gauge (Landtek Srt-6223, Accuracy: ± 5 μm; Resolution: 0.1 μm/1 μm). Ten randomly selected points on each substrate were measured to quantify the variation in surface roughness encountered by these geckos in nature. We compared the roughness of all substrates with non-parametric tests (substrate roughnesses were not normally distributed) using a Kruskal-Wallis Test, followed by Wilcoxon Signed Rank tests for post hoc comparisons of substrate roughness.

For our laboratory assessments of microhabitat choice and clinging performance, we wanted substrates with a quantifiable range of roughnesses similar to the ranges found in nature, but with uniform surface chemistry, so we used sandpaper exhibiting peak-to-valley heights representing the highest and lowest measures of peak-to- valley heights used by our geckos in nature.

Field collection

To use in laboratory trials, adult geckos of each species were collected by hand during spotlighting surveys in northeast Queensland, Australia between June–August 2016. The arboreal species (*O. castelnaui*; 4:6 male to female) were collected from eucalyptus open woodland habitats on the James Cook University, Townville campus. The generalist species (*O. monilis*; 5:6) and the saxicolous species (*O. coggeri*; 4:7) were collected from open woodlands and rocky outcrops at three sites at Hidden Valley (Supplementary material 5). Geckos were returned to the laboratory at James Cook University in cloth bags.

Following collection, geckos were housed in controlled temperature rooms (mean ± SE: 25 °C ± 1.5) at the James Cook University, Townsville Campus, and exposed to a 12-h light-dark cycle (0600–1800 L; 1800–0600 D). Geckos were housed individually in plastic enclosures (30 × 15 × 9 cm), with a ceramic tile shelter and water ad libidum. To allow geckos to thermoregulate, all enclosures were placed on racks, with heat sources that reached 33 °C during the day running under

one end, to form a thermal gradient within each enclosure. Geckos were fed live crickets (*Acheta domestica*) dusted with vitamin and calcium powder supplements (Reptivite™), twice weekly.

Morphometrics

Snout-vent-length (SVL) and mass were measured on the each species (*O. castelnaui*, N = 10, *O. coggeri*, N =11, *O. monilis*, N = 11) that were collected and housed in the laboratory, using a ruler and a digital scale (SVL in mm ± 0.1 and mass in g ± 0.05). Surface area of the adhesive toepads may influence clinging performance (Irschick *et al.*, 1996; Russell and Johnson, 2007, 2014; Peattie, 2009; Schneider, Rasband and Kevin, 2012). To measure the surface-area of toepads, the subdigital (ventral) aspect of the hands and feet of all individuals collected and housed at James Cook University were photographed through glass against a uniform dark background with a scale in each image. Lightroom CC (Adobe, 2017) was used to adjust the contrast of images to ensure that the emphasis was on the adhesive lamellae. The thresholding feature in ImageJ (Schneider, Rasband and Kevin, 2012; Gillies and Fearing, 2014) was then used to select the toepads by saturation, as they contrasted strongly with the rest of the image. Measurements were calibrated using the scale incorporated in every image. Measurements were taken for all five toes on the right hand (manus) and right foot (pes) of all geckos and doubled to calculate total adhesive area for each gecko (Table 0.1).

Table 0.1 Mean morphometric measurements (mean ± SD) of the three *Oedura* species.

Species	n	SVL (cm)	Mass (g)	Toepad area (mm)
Arboreal (*O. castelnaui*)	10	73.43 ± 10.94	12.28 ± 4.79	50.50 ± 10.77
Generalist (*O. monilis*)	11	82.64 ± 5.77	11.35 ± 1.87	79.35 ± 15.35
Saxicolous (*O. coggeri*)	11	70.52 ± 8.98	7.86 ± 0.96	55.10 ± 9.56

Assessment of microhabitat choice in the laboratory

Enclosure experiments are a powerful tool to study substrate choice in geckos, as variables like substrate availability can be precisely controlled (Vanhooydonck, Van Damme and Aerts, 2000; Higham *et al.*, 2019). Substrate selection testing arenas (plastic containers, 70 × 32 × 12 cm; total area – 2448 cm2) were lined on all inner surfaces with equal areas (1224 cm2 each) of P40 grit (coarse) and P400 grit (fine) aluminium oxide sandpaper (Active Abrasives Pty Ltd., Australia; Supplementary material 6). We investigated substrate choice in the laboratory using the 10 individuals of each species, each sampled once. The front of the testing arenas were covered with transparent plastic film (Clorox Australia Pty Ltd., New South Wales, Australia) sprayed with canola oil (Pascoe's, Western Australia, Australia) to allow us to observe the geckos in the arena through the transparent film, while simultaneously keeping geckos from walking on the non-sandpaper surface. No food or shelter was available in the testing arenas and light and heat were uniform within the arenas. Geckos were

randomly selected and introduced individually to the centre of the arena on the vertical area facing the observer. To reduce the influence of external variables on geckos' substrate choice, the orientation of substrates (left-hand side or right-hand side) was randomly selected before each trial. Substrate choice was video recorded between 18:00 and 21:00 under infrared lights using a tripod-mounted DCR-SR55 Sony Handycam (Sony Corporation, Tokyo, Japan) in 'NightShot' mode. Two to three separate arenas containing one gecko each were recorded simultaneously in each two-hour video. Before use, enclosures were sprayed with 80% ethanol, cleaned, and allowed to air dry completely to remove any scent from previous geckos. No observers were present in the room during trials.

Videos were reviewed (VideoLan and VLC Authors, 2017) and the substrate upon which the geckos were observed was recorded every minute for 90 min. To eliminate possible behavioural effects of introduction to the testing chamber, the first 15 min of each video were discarded. Instances when geckos were in the centre (i.e., the body spanned both substrates) or when geckos attempted to climb the front (non-sandpaper surface) were excluded from analysis (< 5% of all observations). Having touched the oily surface seemed not to influence gecko behaviour, locomotion or substrate choice. Proportion of time spent on each substrate (count of observations on each substrate divided by total number of observations on both substrates) were calculated for each individual and used as a measure of substrate choice for each individual gecko. Toepads have evolved as a mechanism for climbing (Collette, 1962; Zani, 2000; Goodman, Miles and Schwarzkopf, 2008) and the adhesive apparatus may not be deployed during locomotion on horizontal surfaces (Autumn *et al.*, 2006; Russell and Higham, 2009), therefore, observations on horizontal surfaces were excluded from analysis (46.02% of 2137 observations). To ensure that excluding horizontal observations did not affect our conclusions, we repeated our analysis including both vertical and horizontal observations, which did not affect our conclusions.

To investigate microhabitat choice among gecko species, we used generalized linear mixed-effect models (GLMMs) fit to a binomial distribution using the package lme4 (Bates *et al.*, 2015). We used the proportion of time spent on each substrate by each individual gecko as the response variable. Individual gecko IDs were included as a random factor, to account for variation among individuals in the number of observations on vertical substrates. As binomial responses (choice of coarse or fine sandpaper) were expressed as proportions, total observations of each individual on each substrate were included as 'weights' in all candidate models. Four candidate models were constructed with fixed effects as (1) species and substrate, (2) species only, (3) substrate only and (4) species * substrate interaction (Table 0.2). We identified a best fit model using Akaike's information criterion (ΔAICc < 2) using the R package *AICcmodavg* (Mazerolle, 2019). Further, we conducted post hoc pairwise comparisons using the package emmeans (Lenth, 2020) on the variables included in the best fit model(s). All analyses were carried out in the R program environment (RStudio, 2019).

Table 0.2. Four candidate models used to analyse the proportion of time spent on coarse and fine sandpaper in three *Oedura* geckos, *O. castelnaui*, *O. monilis* and *O. coggeri*.

Model	Response variable	Random effect	Fixed effect
1	number of observations on each substrate	gecko id	substrate + species
2	number of observations on each substrate	gecko id	species
3	number of observations on each substrate	gecko id	substrate
4	number of observations on each substrate	gecko id	species*substrate

Shear force as a measure of clinging performance

Gecko toepads are likely most adhesive after shedding, when lamellae, setae, and spatulae are intact and undamaged (Hiller, 1968), therefore, clinging performance experiments were conducted within 3 days of shedding. Geckos were tested on coarse and fine sandpaper in a randomized order. We recorded the maximum shear force generated by a gecko's toepads as outputs of the maximum force observed over the trial by attaching a force gauge (Extech 475,040; Extech equipment Pty Ltd., Australia) to the inguinal region of the gecko using a harness of fishing line with a diameter of 0.5 mm (Jarvis Walker Pty Ltd., Dandenong, Australia). Each gecko was allowed to take one step with each of the four feet on the testing substrate, thereby ensuring that the natural attachment system of the gecko was engaged (Niewiarowski *et al.*, 2008; Collins, Russell and Higham, 2015; Stark and Mitchell, 2019). Once each gecko made contact with all four feet, they were pulled horizontally backwards, at an angle of 0°, ensuring constant velocity (~ 0.5 cm per sec using a 30 cm-ruler and stopwatch), for 15 cm. Only one investigator (RP) conducted clinging performance trials to ensure consistency (Tulli, Abdala and Cruz, 2011). Each trial was repeated three times (N = 24) and five times (N = 8) on each substrate (Cole, Jones and Harris, 2005; Russell and Johnson, 2007; Stark *et al.*, 2015, Supplementary material 4).

Clinging ability among species was compared using a set of 12 linear mixed-effects models in the R package lme4 (Bates *et al.*, 2015), which were compared using Akaike's information criterion

(AICc, Niewiarowski et al., 2016). Measures of shear force (three measures for 24 individuals and five measures for eight individuals) on each substrate were included as the response variable in all models. Substrate and species were included individually, additively, and as interactions in the candidate models (Table 5). Body size and toepad area are correlated (Collette, 1962; Irschick *et al.*, 1996), with larger toepads more likely to have a greater area of setal fields that produce increased shear forces (Irschick *et al.*, 1996; Losos and Irschick, 1996; Webster, Johnson and Russell, 2009; Gorb, 2013; Russell and Johnson, 2014). Hence, the attachment force generated by the adhesive system to a substrate should increase proportionally with an increase in toepad area and with mass (Song et al., 2021, Supplementary material 7). The three *Oedura* species we studied had different body sizes and toepad areas, therefore, to control for the influence of these variables on shear forces, we included toepad area and mass as fixed effects individually and additively. To account for inter-individual variation arising from repeated measures, we included individual gecko ID as a random factor in all candidate models. Shear force, mass and toepad area were log transformed prior to analyses (Table 0.3). Model selection was conducted using AICc for 12 candidate models using the R package AICcmodavg (Mazerolle, 2019) to identify the model of best fit (ΔAICc < 2). Models with ΔAICc < 2, were averaged using the 'model.avg' function in the package *MuMIn* (Barton, 2019) and the relative importance of each variable in the averaged model was calculated. We conducted post hoc analyses on the best fit models (<2ΔAICc) to identify differences within the fixed effects using the R package emmeans (Lenth, 2020). Three measures of shear force, which exceeded three standard deviations from the mean of other measures, were identified as outliers and excluded from our analyses (*O. castelnaui* on coarse sandpaper: 1.23 N and 1.36 N; *O. castelnaui* on fine sandpaper: 1.76 N).

Table 0.3. Twelve candidate linear mixed-effects models used to analyse shear forces exerted by the three *Oedura* geckos, *O. castelnaui*, *O. monilis* and *O. coggeri*.

Model	Response variable	Random effect	Fixed effects
1	log(shear force)	gecko ID	Substrate + log(toepad area)
2	log(shear force)	gecko ID	Substrate + log(mass)
3	log(shear force)	gecko ID	Substrate + log(mass) + log(toepad area)
4	log(shear force)	gecko ID	Substrate + Species + log(mass) + log(toepad area)
5	log(shear force)	gecko ID	Substrate + Species + log(mass)
6	log(shear force)	gecko ID	Substrate + Species + log (toepad area)

7	log(shear force)	gecko ID	Species + log(mass) + log(toepad area)
8	log(shear force)	gecko ID	Species + log(toepad area)
9	log(shear force)	gecko ID	Species + log(mass)
10	log(shear force)	gecko ID	Substrate*Species + log(toepad area)
11	log(shear force)	gecko ID	Substrate*Species + log(mass) + log(toepad area)
12	log(shear force)	gecko ID	Substrate*Species + log(mass)

Results

Microhabitat use in nature

The habitat use we observed corresponded well to habitat use reported in the literature for these species, according to which northern velvet geckos (*O. castelnaui*) use arboreal habitats (Nordberg and Schwarzkopf, 2019a, 2019b; Riedel, Nordberg and Schwarzkopf, 2020); spotted velvet geckos (*O. coggeri*) use saxicoline habitats, (Pillai *et al.*, 2020b; Riedel, Nordberg and Schwarzkopf, 2020); and ocellated velvet geckos (*O. monilis*) use both arboreal and saxicoline habitats (Hagey, Uyeda, *et al.*, 2017; Wilson and Swan, 2017; Riedel, Nordberg and Schwarzkopf, 2020). We found *Oedura castelnaui* (N = 67) exclusively on arboreal microhabitats and used dead trees and silver-leaf ironbark trees (*Eucalyptus melanophloia*) approximately equally. *Oedura monilis* (N = 40) were found in approximately equal proportions on both arboreal and saxicoline habitats, arboreal habitats included dead trees, *E. melanophloia*, cabbage gums (*E. platyphylla*), paperbark trees (*E. similis*), and saxicoline habitats were comprised of granite. *Oedura coggeri* (N = 17) were found exclusively on granite (Figure 0.1). Furthermore, the generalist species (*O. monilis*) encountered a wider range of surface roughness compared to the arboreal and saxicolous species (Figure 0.2A, Supplementary material 1).

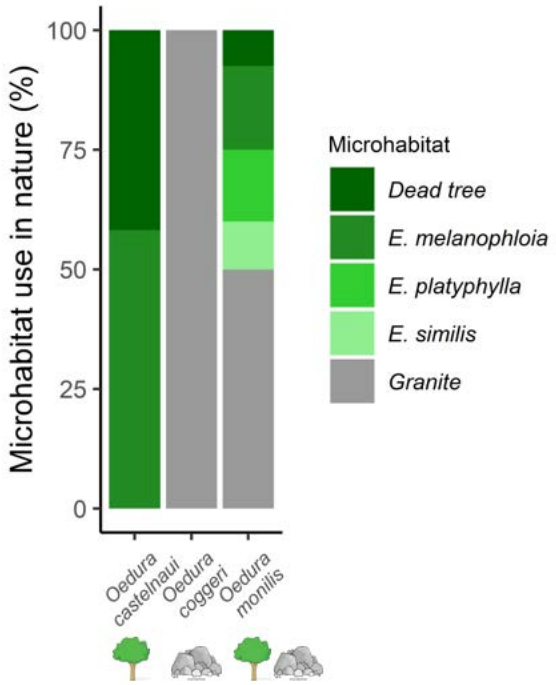


Figure 0.1.Microhabitat use in *Oedura* geckos (%). Substrates were dead trees, silver-leaved ironbark (*E melanophloia*), Cabbage Gum (*E platyphylla*), Queensland yellowjacket paperbark (*E. similis*) (shades of green) and granite (grey). Amounts shown are the percentage of observations

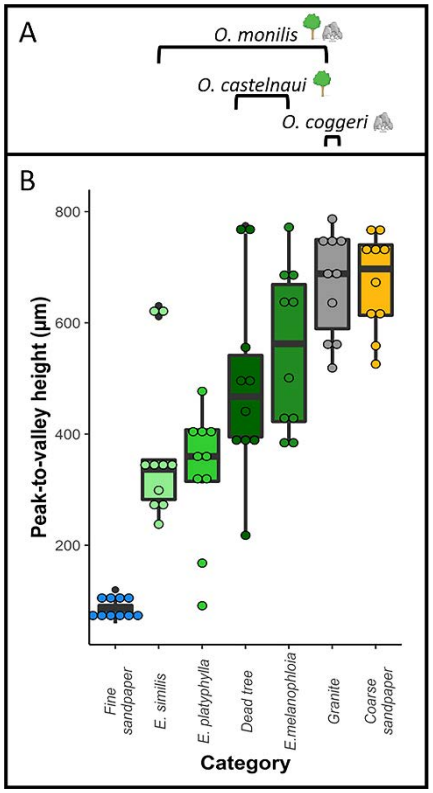


Figure 0.2. A) Black brackets encompass substrates used by three *Oedura* geckos: saxicoline, *O. coggeri* (granite), arboreal *O. castelnaui* (dead trees and *Eucalyptus melanophloia*) and *O. monilis* (*E. similis*, *E. platyphylla*, dead trees, *E. melanophloia* and granite). B). Peak-to-valley heights of natural and test substrates (μm). Natural substrates include *E. similis*, *E. platyphylla*, dead trees, *E. melanophloia* (shades of green) and granite (grey). Test substrates consisted of coarse sandpaper (P40 grit; blue) and fine sandpaper (P400 grit; orange).

Microhabitat roughness and selection of test substrates

Peak-to-valley heights are a two-dimensional measure of surface roughness used to compare surface microtopographies in this study. Natural substrates that formed the microhabitats used by *Oedura* geckos exhibited. significantly different peak-to-valley heights (Kruskal-Wallis rank sum test, $\chi 2 = 48.64$, df = 6, p-value < 0.01). Paperbark trees (*E. similis*, Mean peak-to-valley height ± SE = 370.20 ± 27.77 μm) exhibited similar peak-to-valley heights to cabbage gums (*E. platyphylla*, 331.74 ± 37.32 μm, Wilcoxon rank sum test, P = 0.77) and dead trees (491.50 ± 54.29 μm, Wilcoxon rank sum test, P = 0.07). *Eucalyptus similis* exhibited significantly lower peak-to-valley heights compared to silver-leaf ironbark trees (*E. melanophloia*, 554.60 ± 45.83 μm, Wilcoxon rank sum test, P < 0.01) and granite (668.90 ± 30.00 μm, Wilcoxon rank sum test, P < 0.01). Granite exhibited similar peak-to-valley heights to ironbark trees (*E. melanophloia*, Wilcoxon rank sum test, P = 0.09), however, exhibited significantly higher peak-to-valley heights than dead trees (Wilcoxon rank sum test, P < 0.05), cabbage gums (*E. platyphylla*, Wilcoxon rank sum test, P < 0.01) and paperbarks (*E. similis*, Wilcoxon rank sum test, P < 0.01; Figure 0.2B).

Among our test substrates, peak-to-valley heights of fine sandpaper (P400 grit, 85.76 ± 5.36 μm) were similar in roughness to the very smoothest tree bark we measured, and were significantly different from all natural substrates (Wilcoxon rank sum test, P < 0.01), and to the coarse sandpaper (P40 grit, 672 ± 27.77 μm, Wilcoxon rank sum test; P < 0.001) used in our study. Coarse sandpaper used in our study had peak-to-valley heights not significantly different from silver-leaf ironbark (*E. melanophloia*, Wilcoxon rank sum test, P = 0.12) and granite (Wilcoxon rank sum test, P = 1.00; Figure 0.2B).

Assessment of microhabitat choice in the laboratory

The best model (ΔAICc < 2) predicting microhabitat choice (in the laboratory) included only 'substrate' as a fixed effect and individual gecko ID as a random factor (marginal $R^2 = 0.57$, conditional $R^2 = 0.57$, Table 0.2 and Figure 0.3), indicating that substrate choice did not vary significantly among species. Repeating analyses including use of both vertical and horizontal surfaces did not affect our conclusions about microhabitat choice in the laboratory (marginal $R^2 = 0.05$, conditional $R^2 = 0.06$, Supplementary material 2 and 3).

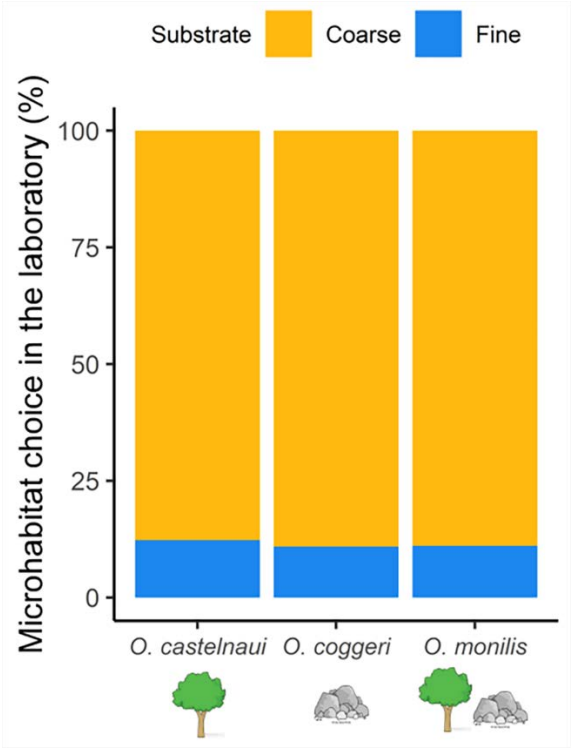


Figure 0.3. Microhabitat choice of *Oedura* geckos in the laboratory (%). Mean percent of observations (n = 10 individuals of each species), amounts shown are the mean proportion of observations (range = 0 to 91) on each substrate for three *Oedura* species. Substrates were coarse sandpaper (P40 grit; orange) and fine sandpaper (P400 grit; blue)

All three gecko species spent a significantly more time on coarse sandpaper (mean proportion of observations ± SE = 0.88 ± 0.008) compared to fine sandpaper (0.11 ± 0.008; Figure 0.3). The coarse sandpaper had peak-to-valley heights similar to ironbark (*E. melanophloia*) and granite, whereas the fine sandpaper they used less had peak-to-valley heights similar to the lowest measurement for any substrate we made in nature (cabbage gum *E. platyphylla* extremes; Figure 0.2).

Shear force as a measure of clinging performance

Both models with a ΔAIC < 2 included a species-by substrate interaction, and mass, while one of these two models also included toepad area (Table 0.4). Post hoc analysis on the models including the variables of high relative importance showed that shear force exerted by the arboreal species (*O. castelnaui*, N = 10) was greater on coarse sandpaper (mean ± SE, 0.59 ± 0.07 N) compared to fine sandpaper (0.39 ± 0.04 N; estimated marginal least square means post hoc comparison: df = 223, t = 4.43, P < 0.001, Figure 0.4). Similarly, the saxicolous species (*O. coggeri*, N = 11) also exerted greater shear force on coarse sandpaper (0.70 ± 0.07 N) compared to fine sandpaper (0.37 ± 0.04 N; estimated marginal least square means post hoc comparison: df = 223, t = 6.83, P < 0.001). Clinging performance was not significantly different on coarse sandpaper (0.69 ± 0.07 N) compared to fine sandpaper (0.52 ± 0.06 N) in the generalist species, *O. monilis* (N = 11, estimated marginal least

square means post hoc comparison: df = 223, t = 2.70, P = 0.08; sandpaper and Supplementary material 3).

Table 0.4. Models included in selection using Akaike's information criterion to analyse clinging performance in *Oedura* geckos, *O. castelnaui*, *O. monilis* and *O. coggeri.* Models are arranged in increasing order of ΔAIC values and top models are in bold. Abbreviation: df, degrees of freedom. Abbreviation: df degrees of freedom

Fixed effects	ΔAIC	df	Weight	Residual Deviance
Substrate*Species + log(mass) + log(toepad area)	**0**	**10**	**0.509**	**269.6**
Substrate*Species + log(mass)	**1.2**	**9**	**0.273**	**269.6**
Substrate + log(mass) + log(toepad area)	3.5	8	0.090	273.8
Substrate + Species + log(mass) + log(toepad area)	3.7	6	0.079	278.0
Substrate + Species + log(mass)	4.7	7	0.049	277.0
Substrate*Species + log(toepad area)	19.5	9	<0.001	287.8
Substrate + Species + log(toepad area)	22.1	7	<0.001	294.5
Substrate + Species + log(toepad area) + log(mass)	27.2	5	<0.001	303.6
Substrate + log(toepad area)	33.4	5	<0.001	309.7
Species + log(mass) + log(toepad area)	55.5	7	<0.001	327.8
Species + log(mass)	56.3	6	<0.001	330.6
Species + log(toepad area)	68.1	6	<0.001	342.4

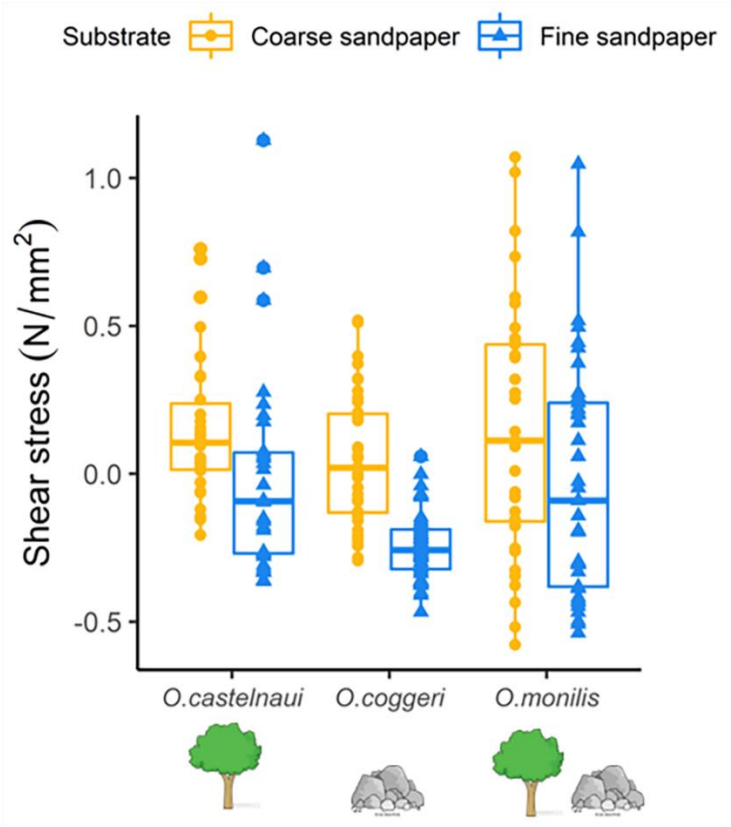


Figure 0.4. Clinging performance of *Oedura* geckos (N/mm2); three measures per substrate for 24 individuals and five measures per substrate for eight individuals on test substrates. Measures shown are shear stress (residuals of regressing absolute force against toepad area) by *O. castelnaui* (N = 10), *O. coggeri* (N = 11) and *O. monilis* (N = 11) on coarse sandpaper (P40 grit; orange) and fine sandpaper (P400 grit; blue). Dots represent each shear force measure (Newtons) for each individual on coarse sandpaper. Triangles represent shear force measures (Newtons) for each individual on fine sandpaper.

Discussion

Our measurements of habitat use were consistent with literature reports for these species (Hagey, Uyeda, *et al.*, 2017; Nordberg and Schwarzkopf, 2019b; Pillai *et al.*, 2020b; Riedel, Nordberg and Schwarzkopf, 2020), but we report the range of roughnesses used by these geckos in nature (Figure 0.1). In nature, the saxicoline species used the narrowest range of roughnesses, the arboreal species a slightly wider range, and the generalist the widest range (Figure 0.2 and Supplementary material 1). The coarse sandpaper (P40grit; 672 ± 27.77 µm) we used as a test substrate in our experiments, exhibited peak-to-valley heights similar to the roughest surfaces used by these geckos in nature, silver-leaf ironbark (*E. melanophloia*; 554 ± 45.83 µm) and granite (668.90 ± 30.00 µm), whereas the fine sandpaper (P400; 85.76 ± 5.36 µm) exhibited peak-to-valley heights comparable to the least rough substrates used in nature (some measures of cabbage gum bark, *E. platyphylla*; 331.74

± 37.32 μm; Figure 0.2). Consistent with the roughness of many of the substrates they used in nature, all three species preferred coarse sandpaper in laboratory assessments of microhabitat choice (Figure 0.3). In addition, clinging performance in the arboreal and saxicolous species was greater on coarse than on fine sandpaper, and thus the microhabitats they selected in the laboratory were those on which clinging performance was high. Interestingly, the generalist species also preferred coarse sandpaper, although its clinging performance was only slightly, and not significantly, higher on coarse sandpaper (Figure 0.4). Thus, although the generalist species in our study preferred rougher substrates in the laboratory, their ability to cling did not vary significantly between test substrates, suggesting that their adhesive system is capable of competent attachment to a wider range of substrates than the microhabitat specialists. Thus, our findings were consistent with the hypothesis that species should select substrates on which they perform well. In addition, we provide important considerations for studies investigating gecko attachment systems in relation to ecology and specifically, surface roughness.

Microhabitat roughness and selection of test substrates

Locomotory capabilities are a consequence of mechanical interactions between animals and the substratum, and the attachment capability of geckos is one such surface interaction (Autumn *et al.*, 2000; Spolenak *et al.*, 2005; Gillies and Fearing, 2014). Over the past few decades, studies investigating locomotor performance have mostly focused on very smooth substrates (Hagey *et al.*, 2014; Russell and Johnson, 2014; Niewiarowski, Stark and Dhinojwala, 2016; Higham *et al.*, 2019; Pillai *et al.*, 2020b), however, recently some studies have used naturally rough and ecologically relevant substrates (Vanhooydonck, Andronescu, *et al.*, 2005; Russell and Higham, 2009; Russell and Johnson, 2014; Stark, Ohlemacher, *et al.*, 2015; Higham *et al.*, 2019). We used artificial surfaces (sandpaper) for uniformity, but selected roughnesses (measured as peak-to-valley heights) similar to those of natural substrates (among the roughest and least rough) used by our geckos in nature. Our findings highlight the variation that exists within and among substrates used by geckos in nature, and were consistent with previous studies showing there was high variation in rock surface roughnesses used by geckos (Russell and Johnson, 2014; Pillai *et al.*, 2020b). Our three gecko species faced broadly similar challenges, at least in terms of roughness. The granite (668.9 ± 67.88 μm) used by our saxicolous species had average roughness similar to the roughest trees (*E. melanophloia*; 554.60 ± 45.83 μm; Wilcoxon rank sum test, $P = 0.09$) frequently used by the arboreal and generalist species in our study, although on average, species using arboreal environments experienced a range of roughnesses, as did the generalist, which experienced the widest range. Thus, classifying species only as ‘arboreal’ or ‘saxicoline’ might not be informative to predict required performance, instead, perhaps more details of surface microtopography should be examined.

The need to incorporate an ecological component into gecko performance studies, especially surface roughness, has been highlighted recently (Niewiarowski, Stark and Dhinojwala, 2016; Russell,

Stark and Higham, 2019). Although peak-to-valley heights are one important measure expressing characteristics of surface microtopography, they do not consider other surface characteristics, such as shape and spacing of asperities, which also could influence shear forces generated by geckos (Spolenak *et al.*, 2005; Gillies and Fearing, 2014; Higham *et al.*, 2019). Future studies should incorporate other, potentially more informative, three-dimensional measurements of roughness produced from multiple two-dimensional profiles (Russell and Johnson, 2014; Higham *et al.*, 2019; Kumar *et al.*, 2019). Furthermore, fabricating particular surface characteristics, while standardising all other properties, would allow us to accurately assess performance exclusively in the context of surface microtopography (Russell and Johnson, 2014; Kumar *et al.*, 2019).

Assessment of microhabitat choice in the laboratory

One ecological factor influencing locomotor performance is microhabitat use (Vanhooydonck, Andronescu, *et al.*, 2005), as different substrates will impose different requirements for performance (Vanhooydonck, Van Damme and Aerts, 2002), making habitat selection a critical determinant of the relationship between performance and fitness (Irschick and Losos, 1999). Laboratory comparisons of functional capabilities may be meaningful only if comparable habitats are used in the field (Irschick and Losos, 1999). *Oedura* geckos preferred coarse sandpaper(P40grit; 672 ± 27.77 μm) in our study, which had microtopography in some ways similar to granite (668.90 ± 30.00 μm; Wilcoxon rank sum test, P = 1.00) and ironbark (*E. melanophloia*; 554 ± 45.83 μm; Wilcoxon rank sum test, P = 0.12) used in nature (Figure 0.2 and Figure 0.3), suggesting our study was at least moderately relevant to substrate choice in nature (Irschick and Losos, 1999), although further comparisons, for example, using samples of natural materials, would be informative.

A range of factors, such as temperature (Vasconcelos, Santos and Carretero, 2012; Nordberg and Schwarzkopf, 2019a), substrate colour and height , ambient light (Williams & McBrayer, 2007; Zozaya et al., 2015), competition (Vanhooydonck and Van Damme, 2003; Nordberg and Schwarzkopf, 2019b) and refuge availability (Williams & McBrayer, 2007) influence microhabitat selection in geckos. We excluded the influence of these confounding factors by performing trials in constant temperature rooms, matching substrate colour, using infrared light, and not providing any refuge in the testing arenas (Supplementary material 6). An advantage of our experimental design was that surface microtopography was the only characteristic that varied between the sandpaper types used in this study, but we were forced to use artificial substrates to achieve this level of control. Using natural substrates also has advantages, but introduces a range of variables other than surface microtopography into habitat choice. Experiments using both approaches will help us better understand the relationship between performance and habitat choice in these animals.

Shear force as a measure of clinging performance

In general, habitat specialists use a narrow spectrum of available habitats, and are expected to perform better in these specific habitats compared to generalists (Huey and Hertz, 1984; Futuyma and Moreno, 1988; Robinson, Wilson and Shea, 1996; Futuyma, 2001; Barkae *et al.*, 2012). For specialists, broader habitat use is thought to entail a trade-off, in which they cannot perform as well in other habitats compared to those in which they specialise (Huey and Hertz, 1984; Barkae *et al.*, 2012), whereas generalists are thought to have lower overall performance, but to perform better in various habitats (the 'jack of all trades is master of none' concept). Thus, we expected the generalist, *O. monilis*, to have the capacity to perform well on a range of microhabitats, and, for the same reasons, we did not expect better performance on either substrate type (Higham *et al.*, 2019). Consistent with this, clinging performance of *O. monilis* was highly variable and was not significantly different between coarse and fine sandpaper (Figure 0.4). Surprisingly, the performance of *O. monilis* was not worse than the specialists on the rougher sandpaper. Possibly, morphological adaptations in some geckos can permit attachment to a wide variety of unpredictable surfaces, instead of being specially adapted to specific substrate types (Russell and Johnson, 2014). If all gecko attachment systems have evolved to attach to the range of substrates they may encounter in nature, then we would not expect increased clinging performance on subsets of microhabitats, but we did observe this in the rock and tree specialists. On the other hand, perhaps the need to attach to a range of different surfaces simply selects for high performance on that range of substrates, without incurring trade-offs.

The attachment mechanisms of geckos are certainly efficient on very smooth artificial substrates (e.g., glass, acetate, Plexiglass and polishing film), and can diminish on rough or undulant natural surfaces (Niewiarowski *et al.*, 2012). This may be because smooth surfaces generally provide more surface area available for contact (Vanhooydonck, Andronescu, *et al.*, 2005; Russell and Johnson, 2014). Preliminary experiments in our laboratory, blocking setal fields (pers obs.), indicate that our observations of high performance on coarse and undulant substrates is a function of both claws and setal fields, claws alone impart significantly lower shear forces. Given that these geckos have both claws and setae, we expect clinging performance to be high on substrates that provide purchase for both components to attach (Naylor and Higham, 2019; Pillai *et al.*, 2020b; Song *et al.*, 2020). Furthermore, specific morphology of both claws and toes can increase performance on rough substrates (Zani, 2000). All three *Oedura* species exhibited good clinging performance on rough substrates, therefore, future studies should examine both claw and toe morphology in relation to substrates they encounter and use in nature.

Conclusion

Our study examined whether clinging performance, in the context of substrate roughness, is related to microhabitat preference. We found that surface roughness of natural substrates was highly variable, highlighting complexity even within specific habitat categories (i.e., 'trees'). Therefore, future studies

should ensure classifications of gecko habitats provide sufficient detail to describe surface microtopographies encountered by geckos in nature. All three *Oedura* geckos preferred coarse sandpaper, upon which they performed best, and which was similar in roughness to the substrates they encountered and used in nature. The shear forces imparted by the generalist were high on both substrates, consistent with their use of both coarse- and fine-grained surfaces in nature, and they preferred coarse sandpaper in the lab. Our findings revealed an association between habitat preference and performance, and provide further evidence for the capacity of the gecko adhesive system to accommodate a wide range of surface roughness

Chapter 4. What's the point? The functional role of claws in pad-bearing taxa (Gekkota: Diplodactylidae)

Under review at the Proceedings of Royal Society B: Rishab Pillai, Jendrian Riedel, Wytamma Wirth, Slade Allen-Ankins, Eric Nordberg, Will Edwards and Lin Schwarzkopf

Abstract

Adhesive pads enable various taxa to exploit vertical and inverted surfaces. Despite having specialised pads for climbing, many taxa that use adhesive filaments or 'setae', also retain claws. The relative contributions of claws and setae-covered pads to climbing are understudied, particularly in vertebrates. Studies suggest setae cling well to smooth surfaces, and claws to rough surfaces, implying a potential trade-off between the roles of these mechanisms on different surfaces. We investigated the relative contributions of setae and claws in diplodactylid geckos, firstly by measuring attachment on a range of substrates, and at different orientations, with and without claws. On inclines (45°), geckos attached to all substrates, independent of claw presence. At 90°, the probability of attachment with claws intact was highest on coarse surfaces and declined on intermediate- and fine-grained substrates. The same pattern occurred when claws were removed, although attachment was always lower without claws than with them. Secondly, shear force (Newtons [only on inclines]) exerted in maintaining attachment declined with surface roughness, and was always higher in geckos with claws than without. Thus, in this clade, setae fulfil a similar and additive functional role to claws, rather than enhancing attachment on finer-grained surfaces as observed in other taxa.

Keywords: *Oedura*, *Strophurus*, function, attachment, shear force, substrates, orientations

Introduction

The environment in which motile animals live exerts selective pressures that lead to the evolution of morphological traits that, in turn, enable effective locomotion in that environment. For example, organisms that climb trees and other vertical structures need to overcome challenges associated with inclined and vertical habitats, specifically interacting with the surface structure of such habitats to counter the effects of gravity (Cartmill, 1985). Climbing requires working against gravity to support the body's mass, and can be achieved in various ways. For example, hooks (Essah *et al.*, 2020), prehensile tails (Molnar *et al.*, 2017), grasping extremities (Toussaint *et al.*, 2020), patches of adhesive skin (Barnes, Oines and Smith, 2006), and claws (Thomson and Motani, 2021b) all improve vertical force generation, which is critical for climbing (Cartmill, 1985).

Movement on non-horizontal surfaces is a culmination of attachment (holding on) and clinging ability (force generation). Adhesive pads that enable attachment and shear force generation are found in cockroaches (Clemente *et al.*, 2009), spiders (Niederegger and Gorb, 2006), dock beetles (James M. R. Bullock and Federle, 2011b, 2011a), stick insects (Labonte *et al.*, 2019), geckos

(Autumn, 2002), some iguanians (Anolidae, Ruibal and Ernst, 1965) and few species of scincids (Williams and Peterson, 1982). These pads can be smooth or filamentous (Beutel and Gorb, 2006; Bullock, Drechsler and Federle, 2008) and the mechanism for attachment differs between structural forms. Smooth pads generate attachment via secretion of fluids (Gorb, 1998; Eisner and Aneshansley, 2000; Betz, 2002; Federle, 2002; Langer, Ruppersberg and Gorb, 2004) that result in capillary and viscous forces, while filamentous pads consist of fields of adhesive microfibrillar outgrowths, each culminating in a single or branched tip that makes contact with the surface.

Geckos are one example of an organism with filamentous pads that comprise fields of microfibrillar outgrowths, called setae (Autumn, 2002), that increase attachment capabilities. Attachment occurs through friction, van der Waals forces (12, 22), capillary interactions (Huber *et al.*, 2005; Puthoff *et al.*, 2010; Mitchell *et al.*, 2020), acid–base interactions (Singla *et al.*, 2021), and possibly, electrostatic interactions (Izadi, Stewart and Penlidis, 2014). In geckos, setae are arranged as fields in different configurations on modified scales called scansors or lamellae (Gamble *et al.*, 2012), which have fields of microfibrillar setae (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966). These fields consist of millions of tips that culminate in structures called spatulae that must make direct contact with substrate to enable attachment. Because smooth surfaces lack asperities (i.e. microscopic peaks and valleys), they therefore represent areas presenting extensive surface area for contact with setal tips. As such, some studies have suggested that setae cling best on smooth surfaces (Mahendra, 1941; Irschick *et al.*, 1996; Autumn *et al.*, 2000, 2006; Huber *et al.*, 2007; Naylor and Higham, 2019).

Claws, which are also present in many pad-bearing species, also interact with substrates to allow attachment for climbing, and vertebrates with claws occupy larger portions of the structural niche space than those without (Cartmill, 1985; Crandell *et al.*, 2014). Claws interact closely with surfaces to enable successful attachment. Attachment occurs in multiple ways, through mechanical interlocking when surface irregularities are larger than the claw tip, or, when surface irregularities are smaller than the claw tip, attachment can result from friction (Dai, Gorb and Schwarz, 2002). Further, if the radius of the asperities are larger than the claw radius, attachment capabilities decrease. Conversely, claws generate strong forces when asperities have smaller radii than claws (Song *et al.*, 2016). Generally, mechanical interlocking is stronger than frictional attachment, with a lower likelihood of failure (Crandell *et al.*, 2014). As claws interact with asperities in multiple ways, it is likely that claws function best on rough surfaces (Scholz *et al.*, 2010; James M. R. Bullock and Federle, 2011a; Burack, Gorb and Büscher, 2022; Winand, Gorb and Büscher, 2023).

Interestingly, taxa with adhesive pads also often have claws, which leads to the question: why have both? Is their function complementary or additive? Studies of species with both setae and claws have focused predominantly on invertebrates (Burack, Gorb and Büscher, 2022; Winand, Gorb and Büscher, 2023), however, relatively few have investigated the relationship between claws and setae in

vertebrates (Naylor and Higham, 2019). In particular, these studies have complementarity: or more specifically, if they function to reinforce one another on all surfaces, or whether there is a trade-off between them whereby one clinging mechanism works well on some types of surfaces, while the other is better on others. Pad-bearing organisms exhibit significant diversity in the microhabitats they occupy, and some surfaces have a greater surface area available for attachment compared to others. Where setal attachment may be compromised or reduced, existence of claws could exert a compensatory function. In this respect, differential, complimentary contributions of claws and setae associated with specific substrate orientations and roughnesses (peak-to-valley heights), could enable attachment to a wide range of surfaces.

Current understanding suggests that claws perform well on rough or irregular substrates (Scholz *et al.*, 2010; James M. R. Bullock and Federle, 2011a; Burack, Schwarz et. al. 2021, Gorb and Büscher, 2022; Winand, Gorb and Büscher, 2023), whereas subdigital adhesive pads excel on smooth substrates (Irschick *et al.*, 1996; Zani, 2000). This implies that the two systems complement each other. For example, (Naylor and Higham, 2019) showed that in turnip-tailed geckos (*Thecadactylus rapicauda)*, claws contributed most to shear force generation on intermediate to rough surfaces (smooth leaves, wood, and P60 sandpaper), however, contributed little on smooth surfaces (acrylic), where setae were highly effective. Similarly, (Pillai *et al.*, 2020b) hypothesised that in diplodactylid geckos, setae and claws complement each other on coarse surfaces, whereas, on extremely smooth surfaces, setae are more effective, due to high contact with the surface area available (Vanhooydonck, Andronescu, *et al.*, 2005; Russell and Johnson, 2007). Thus, we suggest that to understand this multicomponent system, it is important to investigate the functional role of each component individually, in different contexts.

Diplodactylid geckos are some of the most ecologically and morphologically diverse gecko radiations globally (Meiri, 2019). They use a wide range of microhabitats, with varied orientations and surface characteristics (Pillai *et al.*, 2020a; Riedel, Nordberg and Schwarzkopf, 2020), and attach to these surfaces using well-developed leaf-like toepads. In addition to toepads, they also have claws, making diplodactylid geckos an ideal group to examine the relative function of claws and setae in relation to the ecology of climbing. Studies investigating the attachment abilities of claws and setae have mostly been done in the laboratory, and most have examined surfaces do not represent those found in nature (Higham *et al.*, 2019). While replicating the surface characteristics of natural surfaces is challenging, using surfaces comparable to those found in nature is essential to increase the ecological relevance of these studies (Niewiarowski, Stark and Dhinojwala, 2016; Higham *et al.*, 2019), since these are the selective environments in which they have evolved. Using rough to fine-grained surfaces, we examined the functional role of claws *versus* setae in Australian diplodactylids. We measured both attachment (the probability of holding on) and the shear force imparted on different surfaces, and at different orientations. Most importantly, we manipulated morphology to allow direct

assessment and comparison of the relative roles of setae and claws. Specifically, we investigated: 1) What is the function of claws in attachment to different surface types?, 2) What is the function of setae on the same surfaces?, and 3) What shear forces are imparted by these structures on different surfaces?

Methods

Study species and microhabitat use in nature

We selected adults of six species of diplodactylid geckos, that occupied different microhabitats in nature. *Oedura castelnaui* (N = 9) were collected exclusively from arboreal habitats. *Oedura coggeri* (N = 8) were collected exclusively from saxicolous habitats, whereas *O. cincta* (N = 10) and *O. monilis* (N = 11) were scansorial generalists collected either from trees, rocks or fallen logs. *Strophurus krisalys* (N = 6) and *S. ciliaris* (N = 5) were collected from shrubs from six locations in Queensland, Australia. Following collection, geckos were housed in controlled temperature rooms (mean ± SE: 25 °C ± 1.5) at the university campus, and exposed to a 12-h light-dark cycle (0600–1800 L; 1800–0600 D). Geckos were housed individually in plastic enclosures (30 × 15 × 9 cm), with a ceramic tile shelter and with access to water *ad libidum*. To allow geckos to thermoregulate, all enclosures were placed on racks, with heat sources running under one end that reached 33°C during the day, to form a thermal gradient within each enclosure. Geckos were fed wood cockroaches (*Nauphoeta cinerea*) dusted with vitamin and calcium powder supplements (Reptivite™) twice weekly.

All individuals were collected by hand during spotlighting surveys in Queensland, Australia. Upon capture, substrate type (tree, rock, or other) was scored, and surface roughness (peak-to-valley height, μm) was measured using a surface roughness gauge (Landtek Srt-6223, Accuracy: ± 5 μm; Resolution: 0.1 μm/1 μm). Five randomly selected points were measured within 30cm of capture to quantify the variation in surface roughness encountered by these geckos in nature. A mean of these five measures were used in further comparisons.

Comparisons with other pad-bearing lizards

Australian diplodactylid geckos have small claws compared to many other lizard species. To investigate whether this reflected functional capabilities we compared claw length and height in the diplodactylid genera used in this study (*Oedura* and *Strophurus*) to four species of *Cyrtodactylus* (*C. zebraicus, C. annulatus, C. tiomanensis, C, consobrinus,* Riedel et. al. 2024) and 12 species of *Anolis* (*A. auratus*, *A. biporcatus*, *A. capito*, *A. cupreous*, *A. frenatus*, *A. humilis*, *A. lemurinus*, *A. limifrons*, *A. lionotus*, *A. pentaprion*, *A. poecilopus*, *A. polylepis*, Crandell et. al, 2014) using published data. Before comparing measurements, we adjusted for body size (snout-vent-length) using the package *GroupStruct* (Chan and Grismer, 2022). To compare the differences in relative claw length and height, we used one linear model each, with size-adjusted claw measurement as the response variable and genus as the explanatory factor.

Function

We measured two functional traits: attachment (probability of holding on to a substrate versus falling off); and clinging ability (shear force generated on inclines, from which geckos never fell). To quantify attachment, we recorded a gecko's ability to attach (i.e., not fall off) or failure to do so. Shear force was measured (in Newtons) using an ATI Nano 17 force transducer attached to the underside of a 3D-printed test surface mounted level with an aluminium platform that could be adjusted for orientation (45° and 90°). Each gecko was allowed to take one step with each of the four feet on the testing substrate, thereby ensuring that the natural attachment system of the gecko was engaged (Niewiarowski *et al.*, 2008; Collins, Russell and Higham, 2015; Stark and Mitchell, 2019). Once each gecko made contact with all four feet, they were pulled horizontally backwards using a harness of fishing line with a diameter of 0.5 mm (Jarvis Walker Pty Ltd., Dandenong, Australia) attached to the inguinal region of the gecko (Pillai *et al.*, 2020b, 2020a; Pillai, Riedel and Schwarzkopf, 2023).

We tested performance in both functional traits with geckos placed on surfaces covered with three sandpaper types; P40 (coarse), P60 (intermediate) and P80 (fine) aluminium oxide sandpaper (Active Abrasives Pty Ltd., Australia). These provide a range of "roughness" that represented the spectrum of roughness' geckos encountered in nature (Supplementary material 8). All individuals were tested at 45° and 90° on the three sandpaper types. Each individual was tested three times on each substrate and each orientation, resulting in 18 trials per individual. 'Shear force' data were collected automatically for five seconds resulting in 3,163 measures of shear force on three axes (X, Y and Z) per trial. If the task is difficult, one would expect to see variations among individuals that are strongly associated with differences in their morphology, physiology, and fitness. On the other hand, if the task is not physically demanding or if the animals are not pushed to their limits, the connections between performance and fitness may be weak or absent. we used only the maximum value from each trial in further analysis (Irschick et. al. 2008). Therefore, we used only maximum values of shear force in our analysis (Song *et al.*, 2021; Winand, Gorb and Büscher, 2023). The transducer was zeroed before every trial. Since we were interested in the shear force applied in the main direction in which the attachment system of geckos acts (distal to proximal plane), we extracted the maximum values from the X-axis dimension for further analysis. 'Attachment' performance was recorded as the dichotomous variable 'attached or held on' or 'failed to attach or fell off'.

To measure the role of claws in attachment, all individuals were tested with their claws intact within five days of shedding their skin. Immediately upon completion of these trials, the tips of all claws were removed by clipping using a micro-scissor, avoiding any damage to the underlying tissue (Garner, Lopez and Niewiarowski, 2017) and minimising eventual damage to setal fields. Following claw removal, geckos were rested until they shed their skin allowing any damage caused to the setal fields during tests or clipping of claws to be rejuvenated by shedding (Pillai, Riedel and Schwarzkopf,

2023). Once the shedding process was complete, trials without claws were repeated within five days of the shedding event.

Statistical analysis

To estimate the predicted probabilities of failure to attach to each surface with and without claws, we used a binomial logistic generalised linear mixed model in the package *glmmTMB* (Brooks *et al.*, 2017). Attachment (ability to hold on or fall) was the binary categorical response variable. The presence or absence of claws, maximum value of shear force from three trials, microhabitat of origin for the geckos (arboreal, saxicolous, generalist), orientation (45° or 90°), and substrate (P40, P60, P80) were explanatory variables in our models. As each individual was tested multiple times on different substrates and orientations, individual gecko IDs were included as a random effect to control for individual variation. Significant terms were identified using the 'Anova()' function in the package *car* (Fox and Weisberg, 2019), and predictions visualised using the 'ggemmeans()' function in the package *emmeans* (Lenth, 2020). We then calculated the relative contribution of claws by subtracting the probability of attachment with claws from the probability of attachment after claws were clipped.

To analyse the effect of claws on shear force, we used a linear mixed effects model that included shear force exerted with and without claws as the response variable. Data from inclined surfaces (45°) only were included to ensure shear force was measured from orientations at which geckos successfully attached in every instance (see results). Because we were specifically examining the role of claws, and we anticipated this effect could differ on different substrates, an interaction between substrate and claw (presence/absence) was included. To examine any potential differences in performance arising from microhabitat associations, 'microhabitat of origin' was included as a fixed effect. Again, individual gecko IDs were included as a random factor. The response variable (shear force in Newton) was natural log-transformed after examination of standard residual diagnostics. Significant terms in the model were identified using the 'Anova()' function in the package *car* (Fox and Weisberg, 2019). We used emmeans to run *post hoc* tests to compare how claws affected shear force within species that used a certain microhabitat. Additionally, we compared shear force with claws among species that used different microhabitats. All analyses were carried out in R using RStudio Version 4.3.1 (The R Foundation for Statistical Computing, 2024).

Results

Comparisons with other pad-bearing lizards

Size-adjusted claw length was significantly different among genera ($F = 34.82$, $P < 0.01$). *Oedura* (t ratio = 9.67, $P < 0.01$) and *Strophurus* (t ratio = 8.05, $P < 0.01$) geckos used in our study had shorter claws than *Cyrtodactylus* geckos. Similarly, *Oedura* (t ratio = 3.12, $P < 0.05$) and *Strophurus* (t ratio = 2.78, $P < 0.05$) geckos had smaller claws compared to *Anolis* lizards. Similarly, size-adjusted claw height also differed among genera ($P < 0.01$). *Oedura* geckos had lower claw height than

Cyrtodactylus geckos (P < 0.01), however, claw height was not significantly different from those of *Anolis* lizards (P > 0.05), whereas *Strophurus* had shorter claw height than both *Cyrtodactylus* geckos (P < 0.05) and *Anolis* lizards (P < 0.01, Figure 0.1).

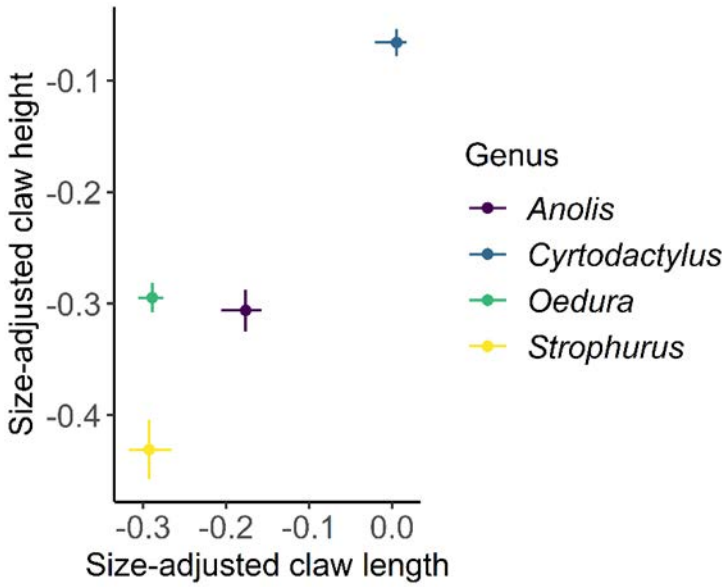


Figure 0.1. Comparison of Diplodactylid (*Oedura*, green; and *Strophurus*, yellow points ± SE) claw morphology with other pad-bearing lizards (*Anolis*, purple; and *Cyrtodactylus*, blue ± SE). Size-adjusted claw length was significantly different among genera. *Cyrtodactylus* and *Anolis* lizards had taller claws compared to *Oedura* geckos. *Oedura* and *Strophurus* geckos had shorter claws than *Cyrtodactylus* and *Anolis* lizards. Similarly, size-adjusted claw height also differed among genera. Claw height in *Oedura* geckos was not different to *Anolis* lizards, however, *Cyrtodactylus* geckos had taller claws than *Oedura* geckos. *Strophurus* geckos had shorter claws than both *Cyrtodactylus* geckos and *Anolis* lizards.

Function – Attachment

The binomial logistic generalised linear mixed model showed that claw removal (z = 6.55, P < 0.01), shear force (z = -4.60, P < 0.01), orientation (z = 5.29, P < 0.01) and substrate (P40 sandpaper : z = -5.15, P < 0.01; P60 sandpaper: z = 2.02, P < 0.05; P80 sandpaper: z = 2.78, P < 0.01) all had a significant effect on the probability of attachment (holding on or falling off as a binary variable). Habitat of origin did not significantly influence the probability of attachment. Most geckos successfully attached to all substrate types at an incline (45°), with and without claws (99% on P40, P60 and P80). The probability of successful attachment at 45° did not differ significantly among substrates or between geckos with and without claws (Figure 0.2). On vertical orientations (90°), the probability of attaching successfully with claws was always much higher than without claws. Attachment with claws intact was lowest on fine substrates (P80 sandpaper: 0.88 ± 0.38 SE)

intermediate on intermediate substrates (P60 sandpaper: 0.91 ± 0.40 SE) and highest on coarse substrates (P40 sandpaper: 0.96 ± 0.44 SE), but was always lower than on inclines (P40 sandpaper:0.95; P60 sandpaper: 0.90; P80 sandpaper: 0.87: Figure 0.2.A). The rate at which attachment declined with surface type was greater for without claws (setae only) than for claws. Setae alone were 28% less effective than setae with claws on the roughest vertical surfaces, 41% less effective on the intermediate vertical surfaces, and 45% less on the finest-grained vertical surface we tested (Figure 4.2B).

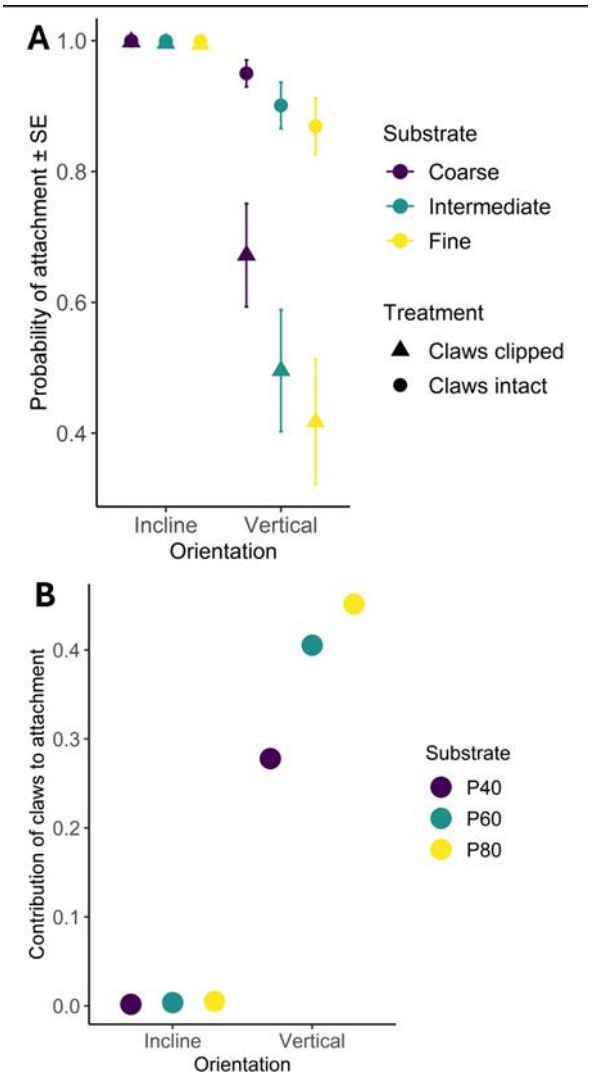


Figure 0.2 A) Role of claws in attachment on inclines and vertical surfaces. On inclines, attachment did not vary significantly with and without claws (Circles, attachment without claws; and triangles, attachment with claws intact). On vertical surfaces, overall attachment decreased, specifically, attachment without claws was significantly lower. Probability of attachment was highest on coarse vertical surfaces (P40 sandpaper: purple points ± SE) followed by intermediate vertical (P60 sandpaper: blue points ± SE) and fine vertical (P80 sandpaper: yellow points ± SE) surfaces, both with and without claws. B) Relative contribution of claws to attachment in diplodactylid geckos.

Without claws, the pattern of declining ability to attach successfully with decreasing roughness on vertical surfaces was comparable to that seen with intact claws, but was more pronounced. Without claws, the probability of attachment was lower on all vertical substrates (P40 sandpaper: $P < 0.01$, P60 sandpaper: $P < 0.01$ and P80 sandpaper: $P < 0.01$: Figure 0.2.A).

Function – Shear force

There was a significant interaction between substrate type and claw presence on shear force (on inclines at 45°) ($\chi^2 = 11.01$, $P < 0.01$). Thus, the difference in force exerted on substrates with and without claws varied among substrates. When claws were removed, there was a significant decline in shear force on intermediate (P60 sandpaper), and fine-grained (P80 sandpaper) ($P < 0.01$, 0.24, P60; 0.21, P80, estimated marginal least square means), but not on coarse-grained surfaces ($P > 0.05$) (Figure 0.3). Thus, as with attachment, setae alone were most effective on coarse surfaces, and their effectiveness declined with surface roughness.

Furthermore, the fixed effect 'microhabitat of origin' was also significant ($\chi^2 = 46.23$, $P < 0.01$). Thus, species exploiting different microhabitats showed differences in absolute shear force exerted. Generalist species in our study exerted greater shear force with and without claws compared to shrub-dwelling geckos on all substrates (P40, P60 and P80 sandpaper), with claws present ($P < 0.01$) and claws removed ($P < 0.01$). Arboreal and rock-dwelling species did not vary in ability to exert shear force with claws present ($P > 0.05$) and claws removed ($P > 0.05$) on all substrates (Figure 0.3).

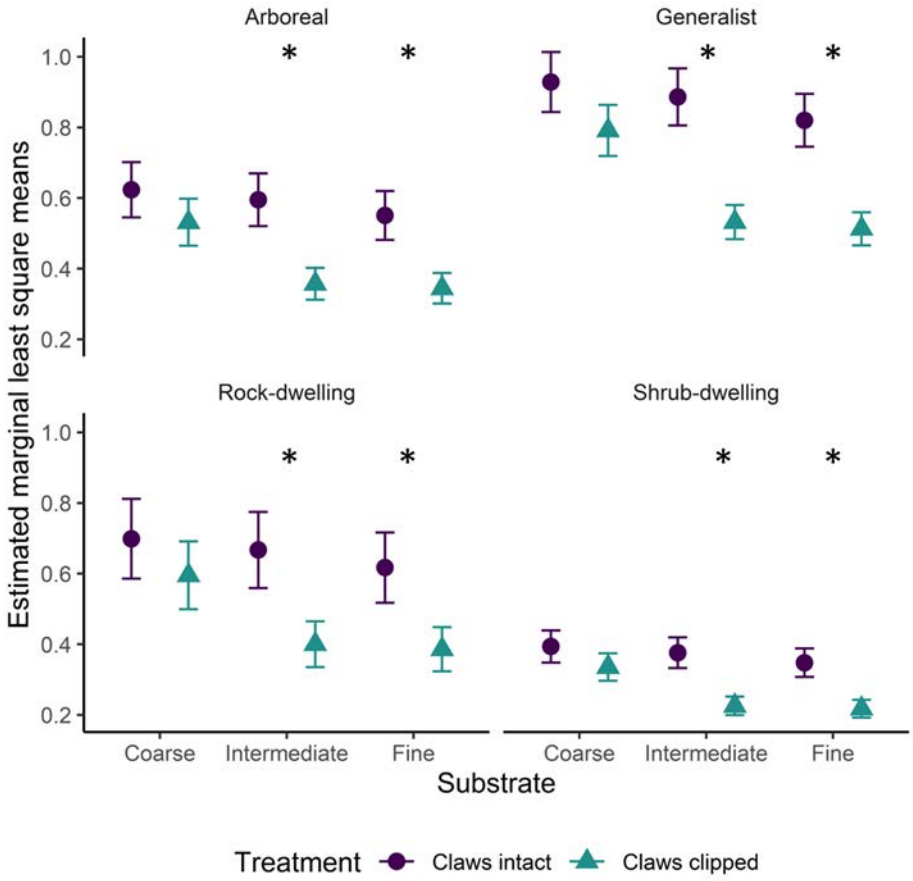


Figure 0.3. Role of claws in shear force on inclines. Shear force generated without claws (blue triangles ± SE) was lower than that with claws (purple circles ± SE). Shear force generated with and without claws differed based on microhabitats geckos used in nature. * indicates significant differences between shear force with and without claws on the same substrate.

Discussion

We found that the role of claws was critical for attachment on vertical surfaces. Claws were best for attachment on rough surfaces and less efficient on intermediate- or fine-grained surfaces. Furthermore, the ability to attach declined more rapidly with surface roughness when claws were removed, suggesting that presence of setae alone did not mitigate the failure of claws on these surfaces. Thus, we found no evidence of a trade-off between the roles of setae and claws in attachment to vertical or inclined surfaces. Instead, the role of claws and setae in attachment (holding on) was redundant on inclined surfaces; the presence and absence of claws made no difference to attachment on inclined surfaces. On the other hand, the contribution of setae and claws to attachment was additive on vertical surfaces. The patterns we observed for shear force on inclines suggested that the role of claws and setae in imparting shear force were also additive. The force exerted was always higher when claws were present, regardless of surface type.

The characteristics of a substrate determine how claws function (Wolff and Gorb, 2012; Song *et al.*, 2016; Naylor and Higham, 2019). On inclined surfaces, geckos did not need to work against gravity as much as on vertical surfaces, and were capable of attachment both with- and without claws. On vertical surfaces, geckos have to work harder against gravity to support their mass, and claws were important for attachment on all surfaces, probably because their claw tips interlocked with surface asperities. The probability of attachment was highest on vertical coarse substrates, likely because surface asperities on the sandpaper were larger than the diameter of the claw tip (Crandell *et al.*, 2014). On intermediate- and fine-grained substrates, the probability of attachment was significantly lower, likely because asperities were smaller than the claw tip.

Even with claws intact, vertical substrates posed a significant challenge. While coarse substrates usually allowed attachment, not all geckos were able to attach to the intermediate- and fine-grained vertical substrates. Previous studies have also found that claws contributed more to clinging performance on rough surfaces (in the phyllodactylid *Thecadactylus rapicauda*, Naylor and Higham, 2019). In our study, the relative differences in the probability of attachment with claws was also higher on intermediate- and fine-grained substrates, than after claw removal, indicating that in diplodactylid geckos claws do indeed contribute some component to attachment on intermediate and fine substrates.

Why do diplodactylid toepads cling so well to rough surfaces, while toepads of other taxa perform poorly on such surfaces (Naylor and Higham, 2019; Cobos and Higham, 2022)? We have demonstrated here that it is not solely the role of claws, but a combination of claws and setae that allow them to cling to rough surfaces. Further, the length and spacing of lamellae in diplodactylid geckos might be suited to the wavelength and amplitude of the rough surfaces used in this study (Gillies *et al.*, 2013). The probability of attachment by setae was highest on vertical coarse surfaces, where claw removal caused only a slight reduction in attachment (compared to on intermediate- and

fine-grained surfaces). Our findings suggest that in the absence of claws, toepads and associated setal fields may act as a backup mechanism of attachment on coarse substrates. This result is surprising, given the evidence provided in other studies of other clades of geckos, which implicate setae being better adapted to provide attachment on smooth surfaces (Persson, 2003; Russell and Johnson, 2014). The reduction in the probability of attachment on intermediate- and fine-grained surfaces in the absence of claws in our study probably occurred because the setal fields of diplodactylids lack purchase for contact on finer-grained surfaces (Pillai *et al.*, 2020b). On the other hand, the decrease in the probability of attachment on intermediate- and fine-grained surfaces when claws are intact probably occurs because claws are also less able to mechanically interlock with smaller asperities. On inclines, the probability of attachment was significantly higher on all substrates than it was on vertical surfaces, as mechanical interlocking was probably not as necessary to support body mass.

Shear force increased with increasing surface roughness in our study. On inclined, coarse surfaces, shear force generation was not reduced by claw removal, whereas, on intermediate- and fine-grained substrates, shear force generation was lower with claws removed. The lack of a significantly increased contribution of claws to shear force on coarse substrates was surprising. This may have occurred either because the claws were too blunt, too small, or insufficiently curved, to successfully interlock with the coarse substrates used in our study. This seems unlikely, however, as we would have then expected an increase in claw function on the other surfaces we tested, since they had smaller asperities. Alternatively, and more likely, toepads and setal fields may both be optimised to cling to rough surfaces comparable to those in nature. An increase in shear force generation on intermediate- and fine-grained substrates, when claws were present, demonstrates that claws play an important role in generating shear force on all surface types, in addition to the force generated by setae making contact with surface asperities.

Functional capabilities evolve in concert with habitat use, as morphological characteristics interact with habitat structure influence performance (Collins, Russell and Higham, 2015). We did not find this in our study. Attachment capabilities did not vary in relation to microhabitat use, indicating that claws were critical for attachment irrespective of where geckos live in nature (trees, rocks or shrubs). However, shear force generation did vary in relation to microhabitat use. Scansorial generalist species in this study exerted higher shear force on all substrates both with and without claws compared to specialist taxa, which is surprising, as generalists are expected to have lower functional capabilities while accommodating movement in a wider range of microhabitats (‘jack of all trades master of none’ concept, Huey and Hertz, 1984), although trade-offs of this nature do not always occur (Voight et. al. 2012). Differences in shear force generation in geckos using different habitat types are likely due to underlying morphological differences, therefore, future studies should investigate the morphology of the adhesive system in diplodactylid geckos.

Studies on the interaction of claws and adhesive pads in invertebrates show that claw removal more strongly affected attachment on rough surfaces, whereas adhesive pads functioned best on smooth surfaces (Federle and Endlein, 2004; Scholz *et al.*, 2010; van Casteren and Codd, 2010; James M. R. Bullock and Federle, 2011a; Voigt *et al.*, 2012; Winand, Gorb and Büscher, 2023). A similar trend has been observed in some pad-bearing gekkonid geckos suggesting that claws are useful on rough substrates (Dai, Gorb and Schwarz, 2002), while setae are more important on smooth surfaces (Persson, 2003). This is an example of one-to-one mapping, in which the functions of claws and setae are complementary (Naylor and Higham, 2019). Our work on diplodactylid geckos, however, suggests that, in a radiation with a different pad configuration and smaller claws, the roles of claws and setae may show greater overlap, and the two systems perform similar roles, with a different contribution to clinging of each attachment mechanism depending on surface roughness and inclination. Thus, the key differences between our study and those conducted previously are that the setae of diplodactylid geckos appear capable of both attachment and shear force generation on coarse substrates in the absence of claws; a finding opposite to the decline in shear force with increasing roughness observed in other geckos (Naylor and Higham, 2019; Garner *et al.*, 2021; Cobos and Higham, 2022). Attachment without claws was reduced on intermediate- and fine-grained substrates and claws contributed stronger to attachment on these surfaces compared to rough surfaces, even though we expected a reduced opportunity for interlocking. It appears that the dominant contributor to shear force on fine-grained and intermediate surfaces was claws in these geckos. And in the absence of claws on coarse surfaces, shear force generated by setal fields was high, and somewhat reduced on intermediate and fine surfaces.

A wide range of surfaces have been used in studies on attachment in various taxa (Ditsche, Wainwright and Summers, 2014; Garner, Lopez and Niewiarowski, 2017; Langowski *et al.*, 2019; Naylor and Higham, 2019; Falvey *et al.*, 2020; O'Donnell and Deban, 2020; Pillai *et al.*, 2020a; Burack, Gorb and Büscher, 2022; Cobos and Higham, 2022). The understanding of attachment on the extreme ends of the spectrum of surface roughness, i.e. extremely coarse and perfectly smooth, and the contribution of claws and setal fields on each are well understood. However, there has been a lack of attention on moderate- or fine-grained surfaces that exist across intermediate ranges. Future studies should investigate the prevalence of these roughness ranges in nature and microhabitat use in relation to these surfaces.

Our findings highlight that the relative contribution of claws on these surfaces was high compared to on rough surfaces despite the reduced potential for attachment on intermediate- and fine-grained surfaces in certain adhesive systems, contrasting findings in other taxa. This implies that, in lizards, the interactions between claws and adhesive pads might be clade-specific. Given this knowledge gap, there is potential for investigation as to why diplodactylid setae produced lower shear

forces on intermediate- and fine-grained surfaces, rather than contributing more, as reported in other gecko radiations.

Chapter 5. The relationship between morphology, ecology, and performance in diplodactylid geckos

Abstract

Habitat structure influences the evolution of morphology enabling effective movement.. In lizards broad-scale habitat structure (for example, open habitats versus leaf-litter habitats) affects the evolution of morphological features like limb length influencing performance traits like running, jumping and climbing. However, few studies have focused on the relationships between morphology and the habitat on a micro-scale at the level of toepads and associated setal fields. Australian diplodactylid geckos are ecologically diverse and have well-developed toepads that enable attachment. Therefore, we examined the morphology attachment system of 17 species of diplodactylid geckos in relation to their microhabitat use and clinging performance using the eco-morphology paradigm as a framework. We found that generalist geckos had large toepads with longer setae at high density compared to rock-dwelling, arboreal and shrub-dwelling geckos. Performance in the generalist species reflected their morphology and they exerted the greatest clinging ability compared to all other geckos in this study. Arboreal and rock-dwelling geckos did not vary in their morphology or ability to cling. Shrub-dwelling geckos had smaller pads, with short setae and at low density, with their clinging ability being the lowest. Setal density was higher in generalist diplodactylids compared to other clades that have been studied. Future studies should expand on our findings to examine these relationships in other clades that use different microhabitats.

Keywords – setae, clinging ability, shear force, microhabitat use, eco-morphology

Introduction

Effective movement influences an animal's success in capturing prey, avoiding predators, and acquiring mates (Aerts *et al.*, 2000; Higham and Russell, 2010), thereby affecting growth rates, survival and reproduction (Arnold, 1983; Garland Jr. and Losos, 1994; Irschick and Losos, 1999; Aerts *et al.*, 2000). As most animals use a range of topographical features within their environment, we expect natural selection to act on locomotor performance in relation to the specific challenges encountered (Collins, Russell and Higham, 2015). The morphological features that lead to increased locomotor performance within a given environment probably evolve in association with habitat use (Moermond, 1979; Irschick and Losos, 1999; Goodman, Miles and Schwarzkopf, 2008; Higham and Russell, 2010). Therefore, variation in habitat use is correlated with extensive morphological diversity of general body form and the locomotor apparatus in a wide range of organisms, including fish (Bourke, Magnan and Rodríguez, 1997; Colombo *et al.*, 2016), ants (Gibb *et al.*, 2015), birds (Zeffer, Johansson and Marmebro, 2003), bats (Marinello and Bernard, 2014), chameleons (Bickel and Losos, 2002) and other lizards (Vanhooydonck and Van Damme, 1999; Zaaf *et al.*, 1999; Irschick *et al.*, 2005, 2005; Calsbeek and Irschick, 2007; Collar *et al.*, 2010).

The study of morphology and performance in the context of ecology is called ecomorphology (Bock, 1994, Wainwright and Reilly, 1994). The 'ecomorphological paradigm' is a theoretical framework predicting covariation among individuals facing similar ecological challenges in performance, fitness and morphology (Arnold, 1983). It predicts that morphological trait variation is strongly related to ecologically relevant performance capacities, which are, in turn, correlated with Darwinian fitness (Higham et. al. 2016). Further, behaviour mediates this relationship, such that substrate choice allows individuals to match morphology to substrate, maximising fitness (Irschick and Garland, 2001). The ecomorphological paradigm suggests that morphological trait variation is strongly related to ecologically relevant performance capacities, which are, in turn, correlated with Darwinian fitness (Bock 1994, Wainwright and Reilly, 1994 and Higham et. al. 2016)(Figure 0.1).

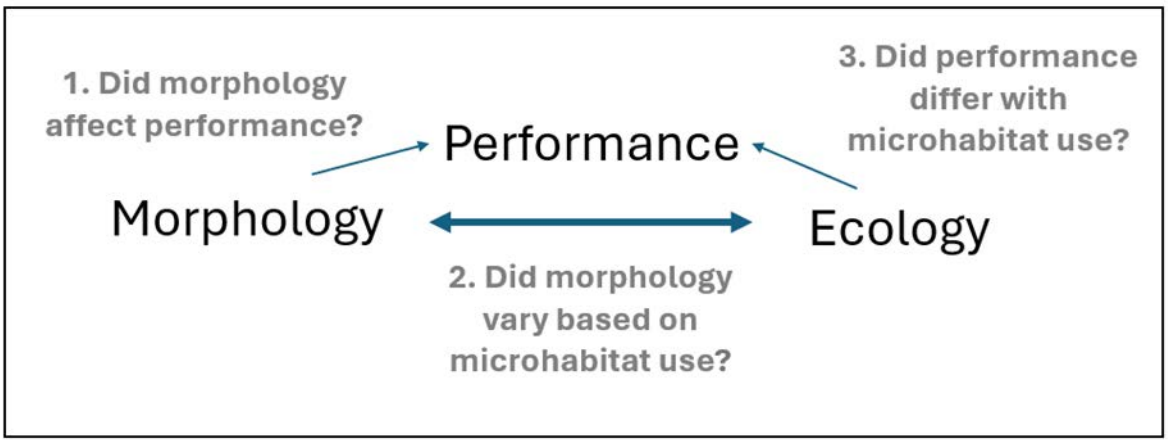


Figure 0.1. The relationship between ecology, morphology, and performance. Black text – Central aspects of the paradigm. Grey text – questions addressed in this study.

Lizards are one group in which microhabitat use is associated with morphological specialisations, such as adhesive toepads for climbing effectively on heterogeneous surfaces (Gamble *et al.*, 2012; Crandell *et al.*, 2014; Hagey *et al.*, 2014; Collins, Russell and Higham, 2015). Geckos exploit inclined and inverted surfaces on rocks, logs, and man-made structures using an adhesive system that has evolved on multiple occasions (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Glossip and Losos, 1997; Gamble *et al.*, 2012; Russell and Gamble, 2019). Adhesion occurs through finely tuned, hierarchically arranged adhesive toepads, characterised by subdigital scansors and lamellae that carry highly organised fields of microfibrillar setae (Russell and Johnson, 2007; Garner, Wilson, *et al.*, 2020; Russell and Garner, 2023). Each seta is branched multiply, and branches terminate in broadened tips called spatulae (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Huber *et al.*, 2005; Hsu *et al.*, 2012; Garner and Russell, 2021). Attachment occurs through a combination of van der Waals forces (Autumn *et al.*, 2000; Autumn, 2002), capillary interactions (Huber *et al.*, 2005; Puthoff *et al.*, 2010; Mitchell *et al.*, 2020), acid–base interactions (Singla *et al.*, 2021) and, possibly, electrostatic interactions (Izadi, Stewart and Penlidis, 2014). There is an impressive range of

morphological variation at the spatula (Garner *et al.*, 2022), seta (Hagey *et al.*, 2014; Garner, Wilson, *et al.*, 2020; Russell and Garner, 2021), scansor, lamellae, and toe levels (Peattie, 2001; Autumn and Gravish, 2008; Gamble *et al.*, 2012), which have been linked to the occupancy of different structural habitats (Higham and Russell, 2010; Collins, Russell and Higham, 2015; Riedel *et al.*, 2024).

The morphology and function of the adhesive system of lizards has been well-studied since the 1960s (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966) and this work has informed an in-depth understanding of this system, especially regarding physical properties and potential biomimetic applications (Higham *et al.*, 2019; Russell, Stark and Higham, 2019). The focus on mimicking the system led to studies occurring predominantly in laboratory settings on artificial surfaces, using smooth (acetate, glass or polyethene film) or very fine-grained rough surfaces (e.g., fibre-optic polishing film) (Russell and Johnson, 2007) although these animals utilise a variety of much rougher substrates in nature. Relatively fewer studies have focused on the function of the adhesive system on natural substrates, and their morphological associations in nature (Russell and Johnson, 2007, 2014; Higham and Russell, 2010; Collins, Russell and Higham, 2015; Garner *et al.*, 2022). Additionally, most of the studies on adhesion use only a few model organisms, such as Tokay geckos (*Gekko gecko*), and some other genera such as *Tarentola*, *Euleptes*, or *Phelsuma* (Russell and Delaugerre, 2017).

In Australia, squamate reptiles are the most species–rich vertebrate assemblage comprising over 1000 species (Brennan and Oliver, 2017). Among these vertebrates, there is exceptionally high lizard diversity, within which geckos constitute a dominant group (Riedel, Nordberg and Schwarzkopf, 2020). Australian diplodactylid geckos occupy a range of different habitats, and some species are habitat specialists and others generalists, potentially giving rise to differences in performance and morphological diversity (Norris *et al.*, 2021). Detailed ecological information for this group is available for some areas and species (Henle, 1990; Neilly *et al.*, 2018; Nordberg and Schwarzkopf, 2019b; Riedel, Nordberg and Schwarzkopf, 2020). Therefore, this group lends itself to examination of the relationship between morphology, function, and ecology.

The habitat in which an animal lives can affect the evolution of morphology, mediated by performance. For example, skinks living in open habitats have long limbs that enable them to achieve high sprint speeds, compared to skinks that live in more sheltered microhabitats, where the need for burrowing and requirement for increased manoeuvrability has led to the evolution of short limbs and slow sprint speeds (Melville and Swain, 2000; Foster *et al.*, 2018). These trends are also seen in other habitat types, for example, skinks in rocky habitats have long limbs and increased performance (clinging, jumping and climbing) capabilities compared to skinks in less rocky habitats (Goodman, 2007; Goodman, Miles and Schwarzkopf, 2008). In some geckos, performance capabilities vary depending on the fine-scale structure of microhabitats (Naylor and Higham, 2019; Garner, Pamfilie, *et*

al., 2020; Pillai *et al.*, 2020b, 2020a). Thus, it seems possible that morphology would affect performance in diplodactylid geckos that occupy different microhabitats, and therefore we should see morphological adaptations to different substrates.

Using the ecomorphological paradigm as a framework, we examined the relationship between morphology and performance in the ecologically and morphologically diverse diplodactylid geckos. We investigated three possible relationships based on the paradigm, specifically: 1) Was there a relationship between morphology and performance? 2) Was there a relationship between morphology and ecology and, lastly, 3) was there a relationship between ecology and performance?

Methods

Study species

Closely related taxa that use different habitats but are otherwise morphologically similar are ideal for investigating the relationships between performance and morphology (Pianka and Huey, 1978; Peattie, 2001; Higham and Russell, 2010; Hagey *et al.*, 2014; Hagey, Uyeda, *et al.*, 2017). We studied 17 closely related and sympatric species of gecko that use different microhabitats (arboreal, rock-dwelling, shrub-dwelling and scansorial generalists) from Queensland, Australia with leaf-like toepad configurations (Brennan and Oliver, 2017; Riedel, Nordberg and Schwarzkopf, 2020, Table 0.1). Trees and shrubs were distinguished using a combination of species, height (trees were taller than 5 meters and shrubs less than five meters) and trunk composition. Trees were defined as having a single main trunk with a distinct canopy while shrubs were defined as having multiple stems arising from the base and a bushier appearance. Geckos were detected using spotlight surveys and the microhabitat upon which geckos were sighted was recorded.. A subset of individuals were captured and returned to the nearby field station to record morphometrics and test performance.

Performance

Our findings from Chapters 3 and 4 suggested that shear force was greatest on coarse substrates (P40 grit sandpaper) and the absence of claws did not reduce shear force generation on this substrate. Pillai et al (2020a and b) showed that coarse sandpaper was also the surface most comparable to those geckos encountered in nature, therefore, in this study, we measured performance only on coarse surfaces. Further, because setal fields have evolved to aid climbing, we measured performance from vertical orientations only. Shear force was quantified using methods specified in Chapter 4 before clipping digit IV of the left pes for collection of samples for morphological investigations (Table 1). *Oedura castelnaui*, *O. monilis*, *O. coggeri*, *O. cincta*, *Strophurus krisalys* and *S. ciliaris* were tested from the long-term research collection at James Cook University collected under Scientific Purposes Permit (WA0024686 to Rishab Pillai and WA0005590 to Jendrian Riedel). These animals were euthanised and deposited at Queensland Museum in August 2023 following the requirements of the scientific purposes permit. All other species were tested in the field and released

within 24 hours of capture following JCU Ethics Permit A2691, Scientific Purposes Permit (WA0024686 to Rishab Pillai) and Nature Conservation Act Permit (P-PTUKI-100107526, P-PTC-100107528 and P-SPP-100107532 to Rishab Pillai).

Table 0.1. Sample sizes of diplodactylid geckos for performance and morphology measurements.

Microhabitat	Species	Sample size (N)
Arboreal	*Amalosia jacovae*	5
Arboreal	*Oedura castelnaui*	9
Arboreal	*Oedura lineata*	4
Rock-dwelling	*Amalosia lesueurii*	4
Rock-dwelling	*Amalosia saxicola*	3
Rock-dwelling	*Oedura argentea*	7
Rock-dwelling	*Oedura coggeri*	11
Rock-dwelling	*Oedura picta*	6
Shrub – dwelling	*Strophurus ciliaris*	7
Shrub – dwelling	*Strophurus krisalys*	6
Shrub – dwelling	*Strophurus taeniatus*	4
Shrub – dwelling	*Strophurus taenicauda*	3
Shrub – dwelling	*Strophurus williamsi*	4
Generalist	*Amalosia robusta*	5
Generalist	*Oedura monilis*	11
Generalist	*Oedura tryoni*	5
Generalist	*Oedura cincta*	10

Toepad area measurements

One hind foot and one forefoot of each individual was photographed using a digital single lens-reflex camera (Nikon D7200, Nikon Corporation, Japan) with a Tamron 100mm prime macro lens

(Tamron Corporation Limited, Japan) on a clear glass surface with a black background and a scale bar included in the image. In FIJI (Schindelin *et al.*, 2012), the 'selection brush' tool was used to choose each spatulate toepad and measure its area, based on the calibration of measurements from the scale bar. Assuming bilateral symmetry, the toepad area of one hindlimb and forelimb was doubled to calculate the total toepad area of four limbs for each animal.

Setal morphology

Digit IV of the left pes (Figure 0.2A) was removed from a subset of individuals after testing for function, and also from additional wild animals (Table 0.1) following James Cook University Ethics Protocols (permit A2690 to Rishab Pillai and Lin Schwarzkopf). Samples were stored in glutaraldehyde solution in phosphate buffer, pH 7.4. Most diplodactylid geckos exhibit paired, leaf-like toepads (Figure 0.2B), therefore, we assumed symmetry in the micromorphology on both sides. The left or right terminal pad was sectioned parasagittally, parallel to the phalanges, to yield a longitudinal transect of the setal fields for imaging via scanning electron microscopy (SEM).

Most notably, setal length increases proximodistally both within scansors and between them, and base diameter decreases proximodistally within scansors.(Garner, Wilson, *et al.*, 2020; Russell and Garner, 2021). Therefore, to standardise the region of interest we consistently imaged and measured the most representative region along the centre of each toepad (Figure 0.2C). Samples were attached to SEM stubs (12mm diameter) with double-sided carbon tape and critical point dried using a Leica EM CPD300 automated Critical Point Dryer (Leica, Wetzlar, Germany). Once dried, samples were sputter-coated in gold using a JEOL Neocoater sputter-coater (JEOL, New South Wales, Australia). The sputter-coated samples were imaged using a JEOL JSM6010LV scanning electron microscope (JEOL, New South Wales, Australia), specifically in the intermediate region of the setal array, to avoid capturing extremely long (distal end) or short (proximal end) setae. Micrographs were embedded with a scale bar to calibrate measurements.

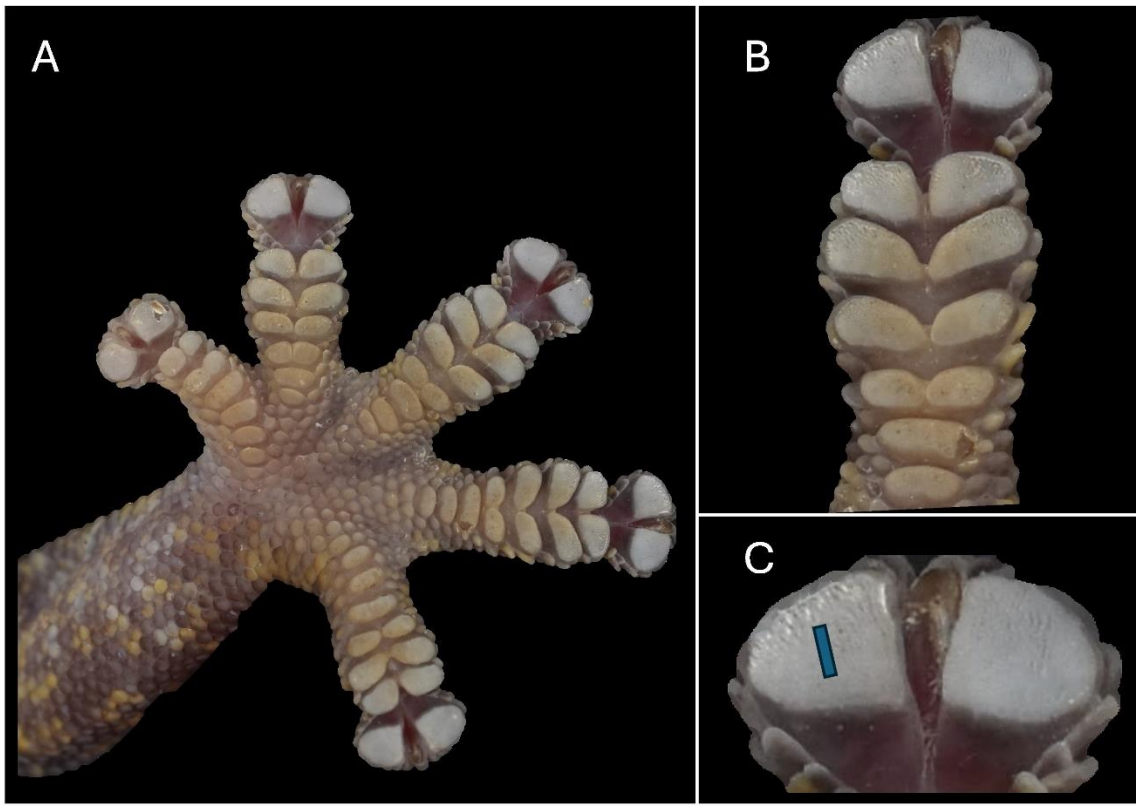


Figure 0.2. Representative images of limb and regions where morphology were measured for this study in *Oedura cincta*. A) Dorsal view of entire limb used to measure toepad area. B) Left fourth toe used to measure setal characteristics C) Terminal toepad from where setal characters were measured. Blue rectangle marks area in the middle of the pad where scanning electron micrographs were obtained.

Using FIJI (Version 2.9.0, Schindelin *et al*., 2012), we used the 'segmented line' function to measure the setal length of the entire setae. Setal length was measured from the base of the setal stalk to the tip of the highest spatula from five individual setae for each individual. Means from the five individual setae were used in all further analyses. Setal density was measured by counting the number of setae present in 10 microns, and this was then scaled to extrapolate the density based on the total toepad area of each individual.

Statistical analysis

Morphology

Geckos used in this study were of different sizes, therefore, before analysing any differences in morphology, we adjusted all morphological characters using the package 'GroupStruct' (Chan and Grismer, 2022) based on the allometric formula by Thorpe, (1976), but adjusted to differentiate within- and between-population differences in body size, with species as our grouping factor.

To visualise morphological differences, we ran a principal component analysis (PCA) (scaled and centered around 0) including size-adjusted setal length, size-adjusted setal density and size-adjusted

toepad area using the 'prcomp' function in the package 'stats'(The R Foundation for Statistical Computing, 2024). The plot was visualised using the function 'fviz_pca_biplot' function in the package 'factoextra'(Kassambara and Mundt, 2020).

Morphology and clinging ability

To analyse the relationship between morphology and performance, we tested which of the morphological variables influenced shear force generation on vertical surfaces before clipping digit IV of the left pes. We chose to analyse vertical surfaces in this study because all trials were conducted with claws intact, which led to geckos successfully attaching to a surface and exerting shear force in 383 trials out of 446. We ran a phylogenetic generalised least squares (PGLS) model (Martins and Hansen, 1997; Revell and Collar, 2009) with shear force as the response variable and svl-adjusted setal length, svl-adjusted toepad area and svl-adjusted setal density as the explanatory variables. The basic model structure was implemented using the gls() function of the 'nlme' package (Pinheiro and Bates, 2000; Pinheiro, Bates and R Core Team, 2023) and used corPagel () function in the R package 'ape' (Paradis and Schleip, 2019) to incorporate phylogeny. To account for phylogenetic non-independence, we used Pagel's $\lambda = 1$ to indicate a strong phylogenetic signal converging to a Brownian motion model of trait evolution (Freckleton, Harvey and Pagel, 2002) with a maximum likelihood (ML) estimation. The models were assessed in an analysis of variance (ANOVA) using the 'Anova()' function with type II sum of squares in the package 'car' (Fox and Weisberg, 2019). To examine if morphology varied based on microhabitat use, I ran a multivariate phylogenetic MANOVA (MVGLS) using the mvgls() function in the package 'mvMORPH' (Clavel, Escarguel and Merceron, 2015). Setal length, toepad area and setal density were combined and used as a multivariate response and 'microhabitat' was used as an explanatory variable in a phylogenetic model with Pagel's λ correlation structure. Significant terms were examined using the manova.gls() function using a Wilk's λ test statistic. Post hoc pairwise comparisons were conducted using the pairwise glh() function with Benjamini-Hochberg adjustment for multiple comparisons with 1000 permutations.

Morphology and microhabitat use

To visualise differences in morphology, we used a principal components analysis (PCA) with setal length, setal density and toepad area adjusted for body size (SVL). We scaled and centered variables of interest as they had different units of measurement.

To examine if morphology, i.e., setal length, setal density and toepad area, adjusted for size, differed based on microhabitat use, I used a multivariate generalised least squares model using the mvgls() function in the mvMORPH package (Clavel, Escarguel and Merceron, 2015). We used a matrix comprising the three morphological variables as a response, with microhabitat geckos used as an explanatory variable. I used Pagel's λ correlation as the evolutionary model with nominal leave-one-out cross-validation of the penalised log-likelihood.

Microhabitat use and performance

To examine the relationship between ecology and performance, I tested if shear force varied based on microhabitat use. I ran a PGLS model with shear force as the response variable and microhabitat use and SVL as explanatory variables with Pagel's λ correlation structure. To examine post-hoc differences in how performance varied with habitat use, we used the cld() function in the package 'multcomp'(Hothorn, Bretz and Westfall, 2008). In both models, I used a modified phylogeny based on alignments from Brennan and Oliver (2017) and recently described species to include the 17 species of climbing diplodactylid geckos used in this study. (Figure 0.3). The multilocus dataset comprised mtDNA (ND2, ND4) and nDNA (CMOS, KIF24, PDC, RAG1) markers. MAFFT (Katoh and Standley, 2013) was used to add new sequences and refined by eye. IQ-TREE2 (Minh *et al.*, 2020) was used to estimate a concatenated and locus-partitioned species tree with support calculated from 1000 ultra-fast bootstraps. Using a series of five common fossil calibrations applied to the nodes of the of the input tree, the constructed species tree was used as an input for MCMCtree to estimate divergence times. Third codon positions concatenated all nuclear loci together and provided MCMCtree with two partitions, mtDNA and nDNA were excluded to eliminate the effects of substitutional saturation. Approximate likelihoods and branch lengths were generated using 'baseml', (usedata =3), prior to running four independent MCMCtree analyses, for 200 million generations each (burnin = 20k, sampling frequency = 100, sample size = 20k). Log files were inspected in TRACER to assess convergence (ESS > 200) and runs were combined using LOGCOMBINER. A maximum clade credibility tree with median mode heights was generated using TREEANOTATOR. The R package 'ape' was used to trim the tree to 17 species of interest (Brennan, pers. comms.).

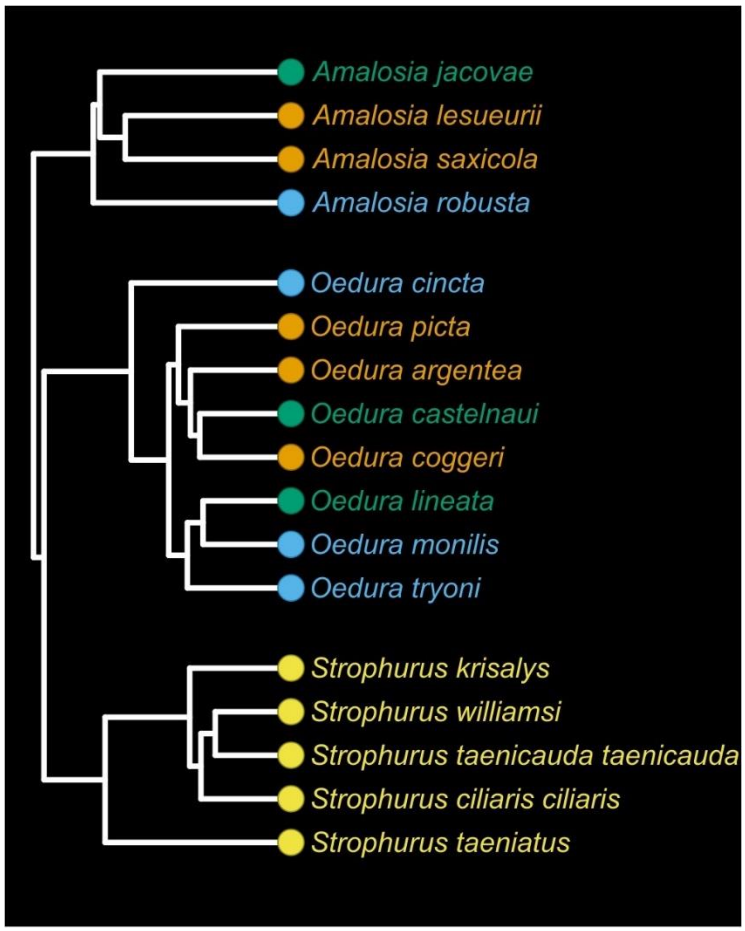


Figure 0.3. Phylogenetic relationships of the diplodactylid geckos used in this study, based upon the phylogeny of Brennan and Oliver (2017). Tip labels are coloured by microhabitat use (blue = generalists, orange = rock-dwelling, green = arboreal and yellow = shrub-dwelling).

Results

Morphology

The generalist species in this study had greater setal length, setal density, and toepad area after being adjusted for overall body size (SVL) compared to shrub-dwelling geckos. Arboreal and rock-dwelling species did not vary in their morphology (see below in *Morphology and microhabitat use*, Supplementary material 9).

Morphology and clinging ability

Phylogenetic least-squared models showed that size-adjusted toepad area ($P = 0.002$) (Figure 0.4.A) and size-adjusted setal density ($P = 0.007$) (Figure 0.4.B) had a significant effect on shear force generation in diplodactylid geckos. Size-adjusted setal length did not have a significant effect on shear force generation ($P > 0.05$).

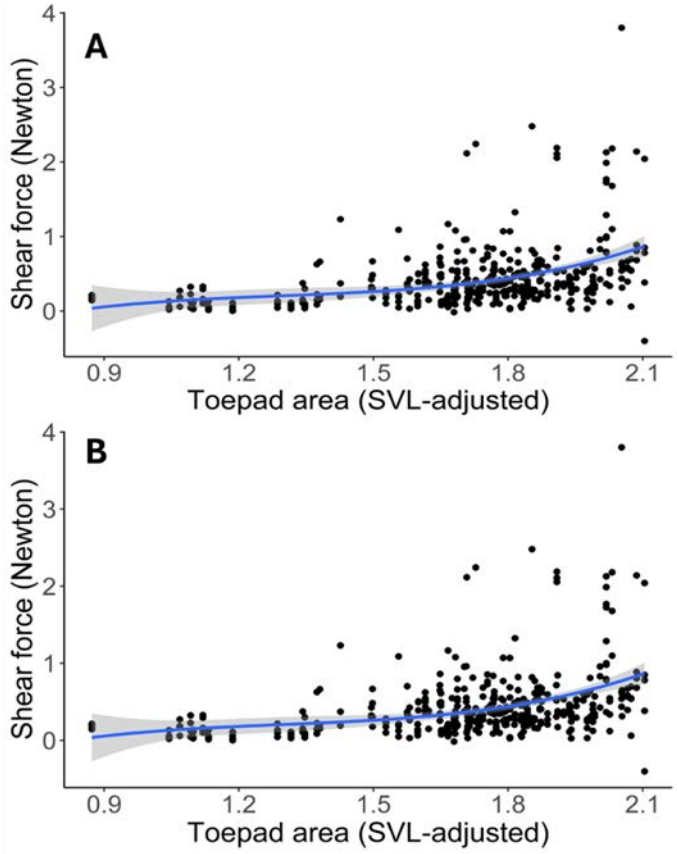


Figure 0.4. The effect of morphology on shear force in diplodactylid geckos. A) Toepad area (SVL-adjusted) had a significant positive effect on shear force generation. B). Setal density (SVL-adjusted) had a significant positive effect on shear force generation.

Morphology and microhabitat use

The first principal component explained 74.26% of the variation in morphology with an eigenvalue of 2.28 and the second principal component explained 24.79% of the variation in morphology with an eigenvalue of 0.74 (Figure 0.5).

MVGLS models showed that morphology (size-adjusted setal length, size-adjusted toepad area and size-adjusted density) varied based on habitat use ($P < 0.05$). Post-hoc comparisons showed that scansorial generalist species have different morphology compared to shrub-dwelling species ($P < 0.05$, pairwise comparisons) and shrub-dwelling species ($P < 0.05$). Morphology did not vary significantly among arboreal, rock-dwelling and shrub-dwelling species ($P > 0.05$, pairwise comparisons) (Figure 0.5).

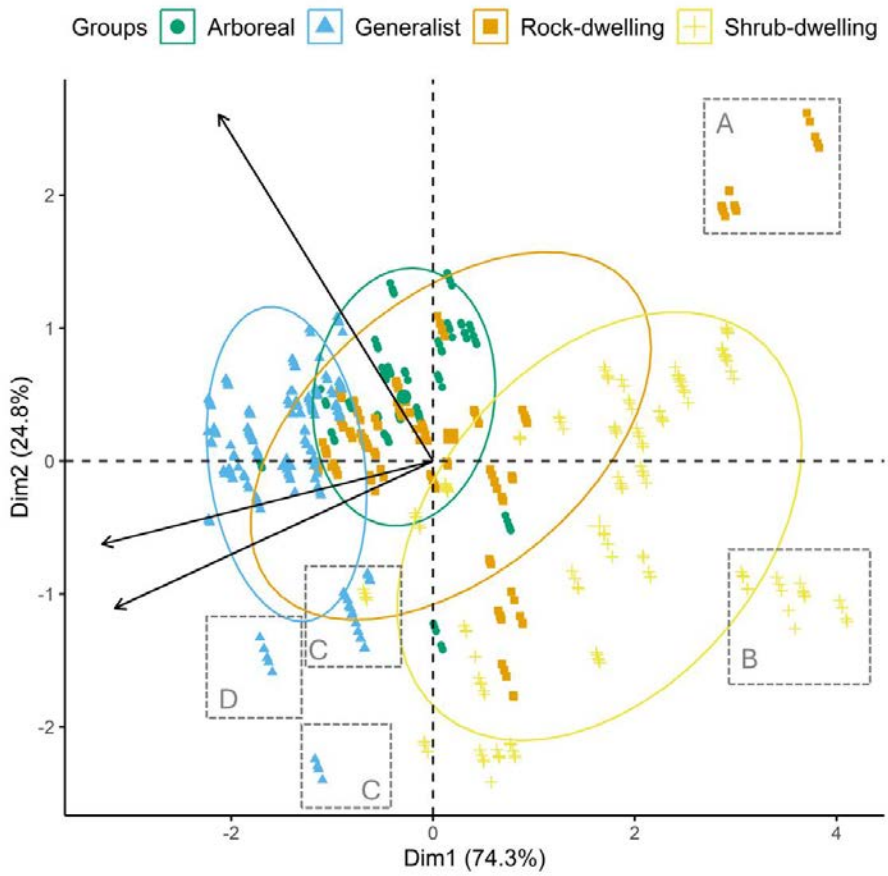


Figure 0.5. Morphological differences in individual geckos grouped by microhabitat use. Generalist geckos (blue) had significantly different morphology (significantly longer setae, larger toepad area and denser setae) compared to shrub-dwelling geckos (yellow). Arboreal (green) and rock-dwelling (orange) species were intermediate to these extremes. A) Among the saxicolous geckos, *Amalosia saxicola* (top right quadrant) had high setal density and large toepads, compared to all other rock-dwelling geckos. B) *Strophurus taeniatus* is a shrub-dwelling species that occurs in spinifex clumps and has different morphology from other shrub-dwelling species. Among generalist species, some individuals of C) *Oedura cincta* and D) *Oedura monilis* were morphologically quite different from other generalist geckos. Dots represent individual geckos and ellipses represent 50% of the data points for each group. Grey dashed boxes identify groupings within broad habitat classifications.

Habitat use and performance

Phylogenetic least square models showed that performance did vary significantly based on habitat use and svl did influence shear force ($P < 0.05$). Scansorial generalist geckos exerted greater shear force compared to arboreal geckos, rock-dwelling geckos ($P < 0.05$) and shrub-dwelling geckos ($P < 0.05$). Further arboreal and rock-dwelling geckos also exerted greater shear force compared to shrub-dwelling geckos. Performance did not vary significantly among arboreal, rock-dwelling and shrub-dwelling geckos ($P > 0.05$). (Figure 0.6).

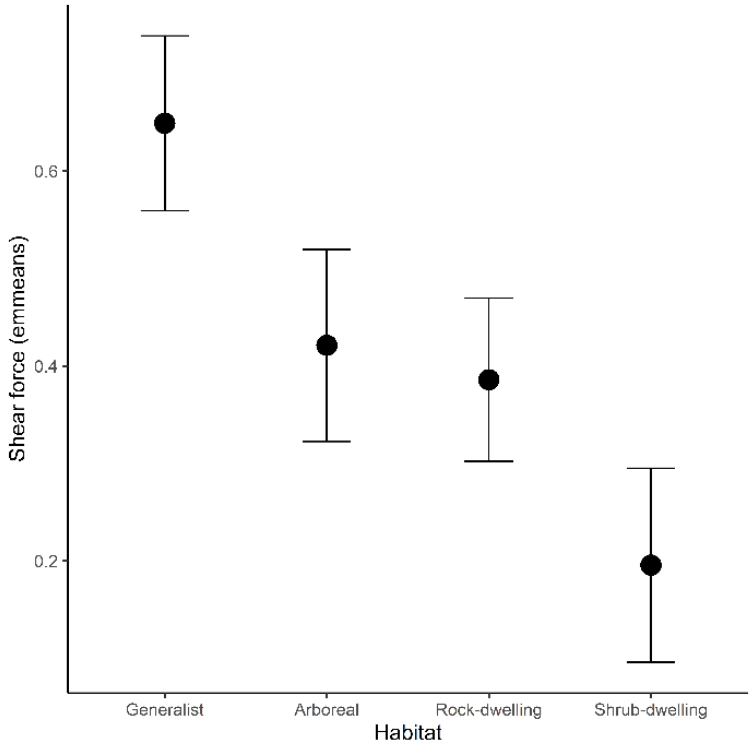


Figure 0.6. Differences in performance in relation to microhabitat use. Generalist geckos exerted the highest shear force and significantly higher forces compared to shrub-dwelling geckos. Arboreal and rock-dwelling species did not differ from each other in their capacity to exert shear force.

Discussion

We examined the relationships among morphology, performance and ecology in Diplodactylid geckos. After accounting for phylogenetic relatedness, there were clear relationships between morphology, specifically toepad area and setal density, and performance. Scansorial generalist, arboreal, saxicoline and shrub-dwelling geckos fell along a gradient in toepad area and setal density from high to low. Morphological differences among scansorial generalist and shrub-dwelling geckos were related to their ability to exert shear force, such that the species fell along a gradient with generalists exerting the highest shear force and shrub-dwelling species the lowest. Our findings provide the first comprehensive analysis of the ecomorphological paradigm in this group of geckos.

In this study, the generalist geckos had the longest setae, highest setal density and largest toepad area compared to all other groups, although only the comparison between the generalist and shrub-dwelling geckos was statistically significant once we accounted for phylogenetic relatedness. Arboreal and rock-dwelling species were similar in morphology, however, exhibited longer setae, greater setal density and larger toepad area compared to shrub-dwelling geckos. Generalist species exhibiting such extreme morphology compared to the other groups was surprising, as we expected the generalists to fall within the range of morphological traits of arboreal and rock-dwelling geckos, as generalists use both those environments. Therefore, generalist species in system might potentially be aligned with the

‘master of all’ paradigm (Barkae *et al.*, 2012) as opposed to the ‘master of none’ paradigm where morphology is intermediate to suit a wide range of habitats.

The trajectory of shear force generation in this study was similar to that observed for morphological differences, i.e., the generalists, which had the most extreme morphology also exerted the greatest magnitude of shear force. Similarly, the shrub-dwelling geckos with the shortest setae, lowest setal density and lowest toepad area exerted significantly lower shear force compared to the generalists. The arboreal and rock-dwelling geckos exhibited morphology intermediate to the generalist and shrub-dwelling species, and their clinging ability was also intermediate.

In this study, microhabitats were classified broadly, namely as trees, rocks and shrubs. Morphology did vary among these broad habitat classifications, however, distinct morphological grouping was observed in some species within these broad categories. Among the rock-dwelling species, the newly described *Amalosia saxicola* (Hoskin and Couper, 2023), exhibited lower setal density, setal length and toepad area compared to other rock-dwelling species (Figure 0.5). Despite using the same granite habitats as *Oedura monilis* and *Oedura coggeri*, *A . saxicola* has different morphology, indicating the potential for multiple solutions for the same ecological challenge, or alternatively different behaviours within the same habitat selecting for different mortality. Among the generalist geckos, some individuals of *Oedura monilis* and *Oedura cincta* also showed very different morphology from their congeners. Both these species are widely distributed and occupy a range of different habitats. The morphological differences identified in this study could inform further taxonomic investigations, perhaps revealing new species. In the shrub-dwelling category, *S. taeniatus* showed differences in morphology from other shrub-dwelling geckos. *S. taeniatus* belongs to the group of phasmid geckos that are specialised spinifex-dwellers. There are structural differences between spinifex clumps and other shrubs, and therefore highly-specialised morphology may be required to grasp the very thin perches available to move within these habitats. Future studies should investigate these differences in habitat use by, for example, establishing more fine-scale habitat classifications, and by incorporating behavioural observations to better understand the relationship between ecology, morphology, and performance.

Generalist species use a wide range of resources, therefore, they are expected to be less efficient in exploiting any given resource type. On the other hand, we expect a specialist to develop a phenotype optimised to the narrow range of resources they exploit (e.g., Barkae et. al. 2012). Based on this, we expected the generalist geckos in our study to have intermediate performance capabilities with associated, intermediate morphological traits, compared to more specialised arboreal, rock-dwelling and shrub-dwelling species. In our study, generalist geckos exhibited morphology on the higher end of the spectrum of measurements of species in this study, and their performance abilities reflected this. Surfaces in nature are rough, and generalist geckos use a larger range of surface roughnesses

compared to more specialised species (Pillai *et al.*, 2020a). Perhaps the high setal density, and length as well as large toepad area enables effective movement across these different surfaces, again, detailed behavioural observations may reveal how these geckos use their habitats.

Differences in habitat use and associated structural characteristics are thought to drive the evolution of morphological features that enable efficient movement (Berles *et al.*, 2024). In this study, the generalist geckos had different morphology (longer size-adjusted- setal length, larger toepad area and higher setal density) after accounting for body size compared to shrub-dwelling geckos. Microhabitats used in nature by scansorial generalist and shrub-dwelling geckos had similar average surface roughness (Supplementary material 8), but likely varied in other aspects, giving rise to differences in morphology. For example, generalist geckos may use higher perches, which require greater attachment abilities compared to shrub-dwelling species for safety reasons, therefore, greater setal density might increase attachment by increasing the number of spatulae that make contact with the substrate (Garner et. al. 2022). Further, shrub-dwelling geckos are likely to grasp narrow perches with their entire forefoot (pers. obs.) as opposed to contacting surfaces with flat feet which could suggest that they have evolved an attachment mechanism suitable for grasping rather than clinging and generating shear force.

Body size can influence functional traits like clinging ability (Irschick et. al. 1996; Collete et. al. 1962). Before analysing the effect of toepad area on shear force, we adjusted toepad area to account for body size (SVL) as larger geckos have larger toepads due to isometry (Cobos and Higham, 2022). In our study, toepad area was positively related to shear force generation, consistent with previous studies in other taxa (Irschick, Herrel and Vanhooydonck, 2006; Crandell *et al.*, 2014; Labonte and Federle, 2015). Larger toepads have a greater surface area of setal fields that comprise a greater number of spatulated tips to make contact, improving attachment. Therefore, relatively larger toepads were capable of exerting greater shear force.

The design of the gecko adhesive system as a hierarchical microfibrillar array permits maximised compliance between fibres and the surface (Jagota, 2002; Spolenak *et al.*, 2005). In larger animals, setae occur at greater densities to enable ‘force scaling’ in which attachment forces scale linearly with the dimensions of contact, dividing the contact zone into many microscopic subunits and increasing adhesion (Federle, 2006). This division of the adhesive area into multiple smaller contact points increases the overall adhesive force termed ‘contact splitting’ (Ruibal and Ernst, 1965; Autumn *et al.*, 2000; Autumn and Peattie, 2002; Jagota, 2002; Arzt, Gorb and Spolenak, 2003b; Kamperman *et al.*, 2010). On irregular surfaces like those encountered by geckos in this study, this interaction occurs locally, but not globally, and in this study, force generation increased with setal density. Setal fields with greater setal density are likely to include a greater number of spatulae within a small region of the field, therefore increasing the points of attachment even when the pad area does not change. On

surfaces with less potential for contact, greater setal density ensures that although the surface structure may be less than ideal, enough setae will still make contact to ensure attachment. High setal density in addition to mechanical interlocking provided by claws (Chapter 4, Dai, Gorb and Schwarz, 2002) might be the reason diplodactylid geckos can exhibit high attachment capabilities on rough surfaces that are conventionally thought to have less surface area available for setal attachment (Pillai *et al.*, 2020b, 2020a).

Variations in setal length and diameter can alter the bending stiffness of setae, and influence how much surface area can interact with the substrate (Spolenak *et al.*, 2005; Ge *et al.*, 2007; Greiner *et al.*, 2009; Greiner, Spolenak and Arzt, 2009; Johnson, Russell and Delannoy, 2009). Setal lengths in our study were between 20µm in *Strophurus taeniatus* to 128µm in *Amalosia robusta* and did not influence shear force, however, setal density did. Setal density is a function of the space present between each seta, and greater spacing reduces the likelihood of clumping while exhibiting high stiffness (Ahmadi and Menon, 2014). Shorter setae permit a higher packing density, reduce clumping (Spolenak *et al.*, 2005), and have a higher effective modulus (low elasticity or high bending stiffness, (Persson, 2003; Garner, Wilson, *et al.*, 2020). Future studies should quantify setal length and associated aspect ratio, which might affect shear force generation.

Our study was the first comprehensive examination of toepad morphology and performance in relation to ecology in Australian diplodactylid geckos. Previous studies have reported setal length and density in diplodactylids from other regions (Arzt, Gorb and Spolenak, 2003b; Russell and Garner, 2021). Unfortunately, most studies do not report the toepad regions from which toepad morphology has been measured (except Ruibal and Ernst, 1965, Russell and Garner, 2021). In our study, all characters were measured from the central region of the terminal pad of the IV toe of the left pes (Figure 0.2) where absolute setal densities (were 90,000 – 250,000 setae per mm^2) considerably greater than in other members of the diplodactylidae, in which densities ranged between 134,000 - 621,000 setae per mm^2 (Artz et. al. 2003, Russell and Garner, 2021). Further, when compared to other radiations, diplodactylid geckos in this study still exhibited greater setal density compared to gekkonids (14,400 – 110,000 setae per mm^2) and phyllodactylids (6,000 – 26,000 setae per mm^2) (Russell and Garner, 2021). This exceptionally high setal density may be the reason diplodactylid geckos are capable of generating high shear force on coarse surfaces, while shear force generation decreases with increasing surface roughness in other radiations.

This study focused on one aspect of performance, shear force, however, differences in habitat structure, shelter availability, intraspecific competition and other factors might select for other performance measures, such as jumping (Higham, Russell and Niklas, 2017) or running (Collins, Russell and Higham, 2015; Garner, Pamfilie, *et al.*, 2020). Future studies should address other

performance traits and their potential associations with differences in habitat use in diplodactylid geckos.

Chapter 6. Synthesis and future work

The attachment system of geckos has received considerable scientific attention because of its evolutionary novelty, and potential for biomimetics to create dry adhesives for human use. Following the first scientific observations in the 1930s-1940s (Dellit, 1934; Mahendra, 1941), a range of studies have described morphology (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Russell, 1975; Peattie, 2001; Hagey *et al.*, 2014), mechanisms of attachment (Autumn, 2002, 2002; Autumn and Gravish, 2008) and function of the attachment system on various surfaces (Persson and Tosatti, 2001; Campolo, Jones and Fearing, 2003; Meine *et al.*, 2004; Huber *et al.*, 2007; Stark, Ohlemacher, *et al.*, 2015; Klittich *et al.*, 2017; Pillai *et al.*, 2020b). However, since the early 2000s, few studies have addressed questions of adhesion in an ecological context (Johnson, Russell and Delannoy, 2009), although various researchers have suggested that ecologically relevant studies on surfaces comparable to natural surfaces are required in future, to better understand variations in form and function of this group (Niewiarowski, Stark and Dhinojwala, 2016; Russell, Stark and Higham, 2019). In addition, a lack of studies on the diplodactylid gecko radiation, which occurs in Australasia provided me with an opportunity to investigate relationships between ecology, morphology and performance in a relatively little-known group.

Ecdysis and clinging

Unlike most other vertebrates, squamates shed their skin in one or several large pieces, which is referred to as ecdysis. This unusual situation, coupled with the specialised clinging apparatus composed of skin structures (setae), caused me to wonder if setae accumulate damage over time, leading to reduced cling ability, and, in turn, if shedding might lead to rejuvenation of the clinging structures. The earliest studies of gecko attachment in relation to skin shedding were conducted by Dellit (1934) and Hiller (1968). They suggested that as time progressed after a shedding event, contamination or clumping of setae led to a decrease in attachment. In my second chapter, I investigated changes in clinging ability that occurred with ‘wear-and-tear’ in captivity from general use, and ‘mechanical damage’ to setae inflicted experimentally by encouraging geckos to attach to and detach from glass, which caused setae to break. We found that both general use and induced mechanical damage reduced clinging ability, however, in both instances, clinging ability was restored following ecdysis. This demonstrated the vital role of shedding in improving clinging.

In nature, geckos use a wide range of different surfaces (Higham *et al.*, 2019; Pillai *et al.*, 2020a), and may have morphological differences associated with different substrates. (Zaaf et al., 1999; Melville and Swain, 2000; Irschick et al., 2005; Collar et al., 2010) Therefore, future studies should investigate ‘wear-and-tear’ and ‘damage’ that occurs when the attachment system interacts

with natural surfaces. Furthermore, do morphological differences mitigate the decrease in clinging ability that occurs due to damage? In addition to improving the understanding of attachment systems in an ecological context, understanding the relationship between morphology and damage resistance could provide insights to improve biomimetic adhesives.

Microhabitat use and clinging ability

Performance evolves to enable effective movement in preferred habitats (Moermond, 1979; Macrini, Irschick and Losos, 2003; Elstrott and Irschick, 2004; Goodman, Miles and Schwarzkopf, 2008; Higham and Russell, 2010) and thus, conversely we would expect animals to prefer habitats where they can move effectively. Broad-scale habitat structure clearly affects performance capabilities (Melville and Swain, 2000; Goodman, Miles and Schwarzkopf, 2008), however, as the gecko attachment system interacts with habitats at very fine scales (Russell and Johnson, 2007; Johnson, Russell and Delannoy, 2009), it was important to investigate the relationship between morphology, performance and habitat selection at a small scale. In my third chapter, I used surface roughness as a measure of fine-scale habitat structure, to investigate microhabitat choice and clinging ability of geckos. Using observations and measurements of surface roughness in nature, and providing geckos with coarse- and fine-grained artificial (sandpaper) surfaces in the laboratory, I showed that geckos preferred surfaces with roughness characteristics similar to what they encountered in nature, equivalent to the coarse sandpaper. Further, I tested if they could cling better to the coarse surfaces they preferred, and clinging ability was indeed higher on coarse surfaces. By examining microhabitat choice in the laboratory on surfaces similar in roughness to those occurring in nature, and by measuring clinging ability on these surfaces, I showed empirically that behaviour and performance are clearly related. Further, I showed that the gecko attachment system is capable of performing well on a wide range of surface roughness in nature, especially in generalist and arboreal species. The variety of microhabitats used in nature, and their associated fine-scale characteristics further emphasise the need for the incorporation of ecology in studies of gecko attachment. Gekkonids are an incredibly speciose and ecologically diverse group (Uetz *et al.*, 2020) making them an suitable study system for expanding such investigations on associations between performance and habitat use that could contribute to an understanding of the way animals choose habitats and move on them.

Claws in pad-bearing geckos

Adhesive pads bearing filamentous setae have often received research attention due to their evolutionary uniqueness and biomimetic potential. Therefore, in systems where claws coexist with pads, the role of claws is often understudied. The majority of the studies addressing the function of claws in pad-bearing taxa have been in invertebrates, with a few exceptions, including some on anoles (Crandell *et al.*, 2014; Garner, Lopez and Niewiarowski, 2017), turnip-tailed geckos (Naylor and Higham, 2019) and lizards (Zani, 2000). Attachment generated from setae alone typically declines linearly with increasing surface roughness (Cole, Jones and Harris, 2005; Vanhooydonck,

Andronescu, *et al.*, 2005; Naylor and Higham, 2019; Cobos and Higham, 2022). Pillai et. al. (2020) showed that in diplodactylid geckos, when claws are present, performance on coarse surfaces was similar to that on smooth surfaces. Therefore, it was important to examine the functional role of claws in this system. Thus, in chapter four, I tested if claws played an important role at certain orientations or on different substrate roughnesses. Claws were critical to attachment ("holding on") at vertical orientations, on all substrates, as when only pads were present, geckos fell. Further when claws were present with pads, shear force generated was higher compared to that generated with pads alone. Thus, claws functioned additively to setae in generating attachment and shear force. Also, I found that, unlike turnip-tailed geckos, diplodactylid gecko pads clung best to rough surfaces, and performance declined on finer-grained (although not artificially smooth) surfaces.

Thus, I demonstrated some key differences in the trajectory of clinging ability in relation to surfaces roughness compared to other gecko species. Previous studies on other clades have suggested the shear force generation declines linearly with increasing surface roughness (Cole, Jones and Harris, 2005; Vanhooydonck, Andronescu, *et al.*, 2005; Naylor and Higham, 2019; Cobos and Higham, 2022). In diplodactylid geckos, this trajectory was opposite to what we expected. Clinging ability was greatest on coarse surfaces and declined linearly as surface roughness decreased. Furthermore, setae alone generated equal magnitudes of shear force as claws on coarse surfaces, which were traditionally considered to provide less purchase for effective attachment. These results highlight a key difference in the performance capabilities of attachment in diplodactylid geckos, in their ability to accommodate coarse surfaces. Attachment to coarse surfaces has been a challenge in the biomimetic realm (Niewiarowski et. al. 2016), therefore, more detailed investigations on diplodactylids could provide potential solutions to this hurdle in gecko-inspired biomimetics. Conversely, clinging ability in this group was lower on intermediate and fine-grained surfaces, which occur in nature (Supplementary material 8) Surface characteristics limiting the effectiveness of the attachment system should be investigated further, especially as we know surfaces in nature exhibit these levels of surface roughness. Studies should investigate microhabitat use on surfaces to which diplodactylids cannot attach well, and investigate their use and prevalence in nature.

Functional morphology of setal fields

Habitat structure on a broad-scale (for example, open versus closed habitats) influences the evolution of morphology, enabling effective movement in occupied habitats. However, the relationship between fine-scale habitat structure and associated morphological evolution is less well known. As the attachment system of geckos interacts with habitats on a fine-scale, specifically macro- (centimeters and millimeters) and micro- (μm or nanometer) level (Russell and Johnson, 2007, 2014; Higham *et al.*, 2019), there is scope to investigate the evolution of morphology of toepads and setae at this level. Further, if morphology varies, would these differences translate to different performance capabilities? In the fifth chapter, I aimed to address the links between ecology, morphology, and

performance by investigating relationships between microhabitat use, morphology (toepad area, setal length and setal density) and performance. Firstly, I established that morphology directly influenced performance, specifically, greater toepad area and setal density imparted greater shear force. I show that morphology varied based on microhabitat use, such that generalist species had greater relative toepad area, setal density, and setal length compared to all other geckos. Differences in morphology between generalist and shrub-dwelling geckos was reflected in their performance, such that generalist species exerted greater shear force compared to shrub-dwelling species. Arboreal and rock-dwelling species were intermediate to these two groups in morphology, performance and shear force generation.

Generalist species are expected to have average morphology and performance that enables use of several habitats (Barkae *et al.*, 2012), however, in my study, generalist species exerted greater shear force and exhibited larger toepads and longer setae at greater densities compared to other species. Potentially having maximised performance abilities and morphology allows these groups to accommodate surfaces with varying surface roughness. Further, setal density observed in diplodactylids was considerably higher than in other pad-bearing gekkonids (Russell and Garner, 2021). Therefore, perhaps possession of greater setal density is the underlying mechanism that allows diplodactylids to exert high clinging ability on the coarse surfaces to which other clades cannot cling effectively.

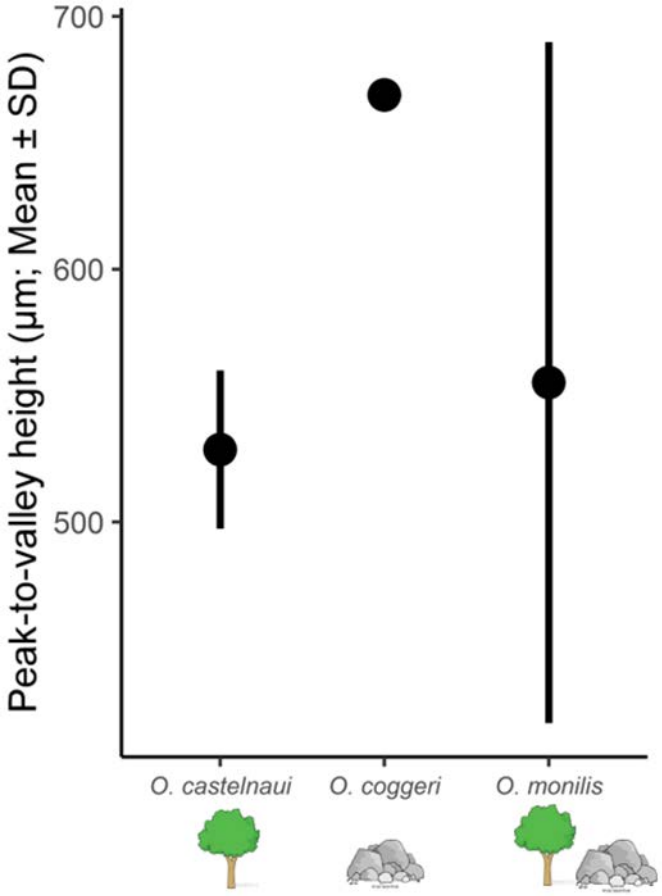
Future directions

My findings have highlighted some key differences in function and morphology in relation to ecology in diplodactylid geckos. Firstly, higher setal clinging performance on coarse surfaces contrasted with previous studies. I have described in detail the morphology of diplodactylid gecko toepads and claws, by describing setal-length and setal-density on the micro-scale, and claw height and width. Could these morphological differences be what allows this group to cling well on challenging (rough) surfaces? There is further scope to examine more morphological characters that improve the real contact area of setal fields with substrates. Firstly, calculating aspect ratios and associated bending stiffness can help in understanding the differences in elasticity that influence surface interactions. Secondly, studying morphology at the nano-scale, specifically the shape and branching of setal tips or ‘spatulae’ would improve our understanding of contact-splitting, which is a key physical characteristic that directly influences adhesion. Therefore, additional examinations of morphology in the relatively understudied diplodactylids is likely to improve our understanding of the Gekkotan attachment system.

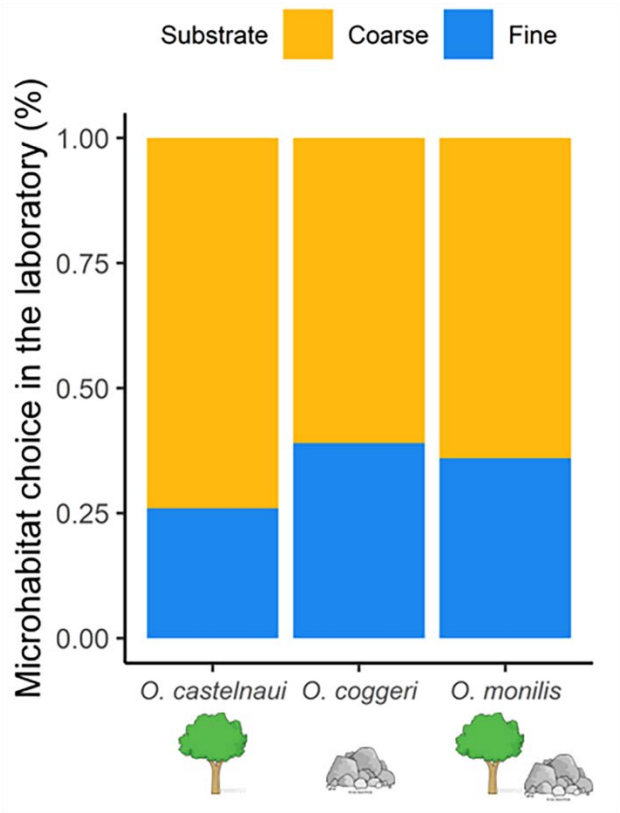
My PhD focused on clinging ability and attachment (“holding on” or “falling off”) as a measure of function. In nature, other performance traits like running, jumping or static clinging are likely to be important in relation to the tasks being performed (avoiding predators, capturing prey and acquiring mates). Little is known about diplodactylids and their feeding strategies, therefore, there is

considerable scope for investigating other performance traits in relation to behaviour in this group. Furthermore, examining these relationships in the context of broad- and fine-scale habitat structure in other pad-bearing taxa is likely to further our understanding of the relationships between ecology, performance and morphology.

Appendix A. Supplementary material for Chapter 3



Supplementary material 1**.** Peak-to-valley heights (µm; Mean +/- SD) encountered by *Oedura* geckos in nature. Mean peak-to-valley heights were plotted against number of observations recorded on each microhabitat used by each of the three species, arboreal *O. castelnaui* (28 observations on dead trees and 40 observations on *E. melanophloia*), saxicoline, *O. coggeri* (17 observations on granite) and generalist, *O. monilis* (three observations on dead trees, seven observations on *E. melnaophloia*, six observations on *E. platyphylla*, four observations on *E. similis* and 20 observations on granite).



Supplementary material 2. Microhabitat choice in *Oedura* geckos, *O. castelnaui, O. monilis* and *O. coggeri* including both vertical and horizontal observations.

Supplementary material 3**.** Candidate models including both vertical and horizontal observations. Candidate models are arranged in increasing order of ΔAIC values and top model is in bold. Abbreviation: df, degrees of freedom.

Fixed effects	ΔAIC	df	Weight	Residual Deviance
Substrate	**0.0**	**5**	**0.64**	**2612.9**
Substrate + Species	2.0	7	0.116	2610.9
Species	1298.5	4	<0.001	2344.5

Post-hoc analysis of microhabitat choice including both vertical and horizontal surfaces showed that *Oedura* geckos preffered coarse substrates compared to fine substrates (estimated marginal least square means *post-hoc* analysis, $P < 0.01$; Supplementary material 2).

Supplementary material 4**.** Clinging performance performance in *Oedura* geckos. Mean shear force (Newton; Mean ±SE) from three trials (24 individuals) and five trials (eight individuals) on coarse and fine sandpaper.

Species	Individual	Substrate	Number of trials	Shear force (Mean ± SE)
O.castelnaui	1	Coarse sandpaper	3	0.84 ± 0.06
O.castelnaui	1	Fine sandpaper	3	0.04 ± 0.02
O. castelnaui	2	Coarse sandpaper	3	0.47 ± 0.02
O. castelnaui	2	Fine sandpaper	3	0.30 ± 0.07
O. castelnaui	3	Coarse sandpaper	3	0.80 ± 0.14
O. castelnaui	3	Fine sandpaper	3	0.87 ± 0.16
O. castelnaui	4	Coarse sandpaper	3	0.46 ± 0.09
O. castelnaui	4	Fine sandpaper	3	0.38 ± 0.11
O. castelnaui	5	Coarse sandpaper	3	0.80 ± 0.22
O. castelnaui	5	Fine sandpaper	3	0.20 ± 0.04
O. castelnaui	6	Coarse sandpaper	5	0.50 ± 0.06
O. castelnaui	6	Fine sandpaper	5	0.29 ± 0.03
O. castelnaui	7	Coarse sandpaper	3	0.42 ± 0.08
O. castelnaui	7	Fine sandpaper	3	0.50 ± 0.03
O. castelnaui	8	Coarse sandpaper	3	1.18 ± 0.12
O. castelnaui	8	Fine sandpaper	3	1.27 ± 0.27
O. castelnaui	9	Coarse sandpaper	5	0.71 ± 0.09
O. castelnaui	9	Fine sandpaper	5	0.71 ± 0.05
O. castelnaui	10	Coarse sandpaper	3	0.66 ± 0.02
O. castelnaui	10	Fine sandpaper	3	0.20 ± 0.03
O. coggeri	1	Coarse sandpaper	3	0.83 ± 0.03
O. coggeri	1	Fine sandpaper	3	0.23 ± 0.02
O. coggeri	2	Coarse sandpaper	3	0.46 ± 0.04
O. coggeri	2	Fine sandpaper	3	0.32 ± 0.02
O. coggeri	3	Coarse sandpaper	5	0.51 ± 0.06
O. coggeri	3	Fine sandpaper	5	0.32 ± 0.02
O. coggeri	4	Coarse sandpaper	3	0.61 ± 0.06
O. coggeri	4	Fine sandpaper	3	0.22 ± 0.04
O. coggeri	5	Coarse sandpaper	3	0.21 ± 0.02
O. coggeri	5	Fine sandpaper	3	0.16 ± 0.04

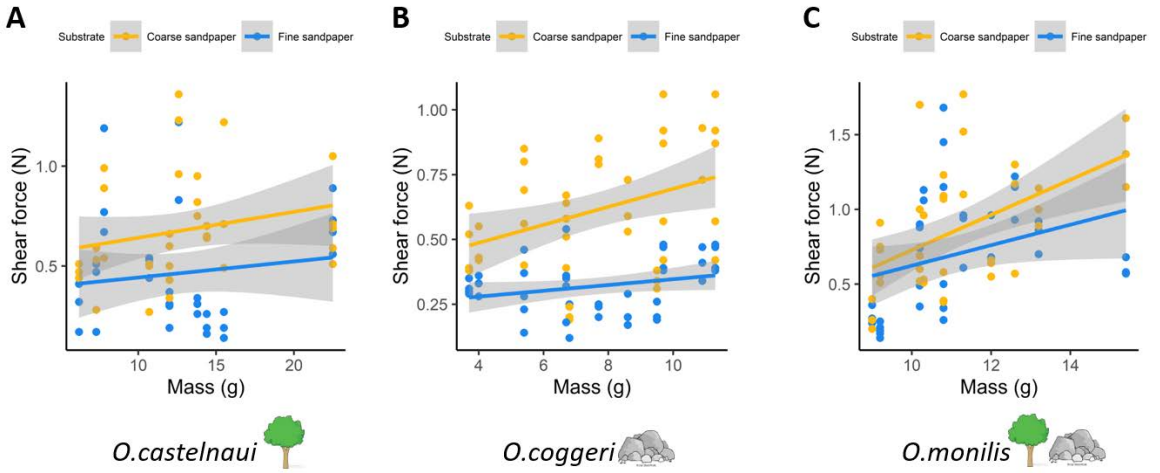
O. coggeri	6	Coarse sandpaper	3	0.86 ± 0.07
O. coggeri	6	Fine sandpaper	3	0.41 ± 0.04
O. coggeri	7	Coarse sandpaper	5	0.77 ± 0.12
O. coggeri	7	Fine sandpaper	5	0.42 ± 0.02
O. coggeri	8	Coarse sandpaper	5	0.66 ± 0.08
O. coggeri	8	Fine sandpaper	5	0.30 ± 0.05
O. coggeri	9	Coarse sandpaper	5	0.77 ± 0.12
O. coggeri	9	Fine sandpaper	5	0.42 ± 0.02
O. coggeri	10	Coarse sandpaper	5	0.56 ± 0.05
O. coggeri	10	Fine sandpaper	5	0.35 ± 0.06
O. coggeri	11	Coarse sandpaper	3	0.34 ± 0.02
O. coggeri	11	Fine sandpaper	3	0.22 ± 0.02
O. monilis	1	Coarse sandpaper	5	0.69 ± 0.07
O. monilis	1	Fine sandpaper	5	0.19 ± 0.02
O. monilis	2	Coarse sandpaper	3	1.01 ± 0.22
O. monilis	2	Fine sandpaper	3	1.10 ± 0.09
O. monilis	3	Coarse sandpaper	3	1.01 ± 0.07
O. monilis	3	Fine sandpaper	3	0.83 ± 0.07
O. monilis	4	Coarse sandpaper	3	1.38 ± 0.13
O. monilis	4	Fine sandpaper	3	0.61 ± 0.04
O. monilis	5	Coarse sandpaper	3	0.62 ± 0.03
O. monilis	5	Fine sandpaper	3	0.73 ± 0.12
O. monilis	6	Coarse sandpaper	3	0.45 ± 0.07
O. monilis	6	Fine sandpaper	3	0.37 ± 0.07
O. monilis	7	Coarse sandpaper	3	0.28 ± 0.06
O. monilis	7	Fine sandpaper	3	0.29 ± 0.04
O. monilis	8	Coarse sandpaper	3	1.46 ± 0.20
O. monilis	8	Fine sandpaper	3	0.84 ± 0.11
O. monilis	9	Coarse sandpaper	5	0.90 ± 0.22
O. monilis	9	Fine sandpaper	5	0.67 ± 0.11
O. monilis	10	Coarse sandpaper	3	1.13 ± 0.05
O. monilis	10	Fine sandpaper	3	1.43 ± 0.15
O. monilis	11	Coarse sandpaper	3	0.66 ± 0.15
O. monilis	11	Fine sandpaper	3	1.05 ± 0.05

Supplementary material 5. Observations of microhabitat use of *Oedura* geckos in nature.

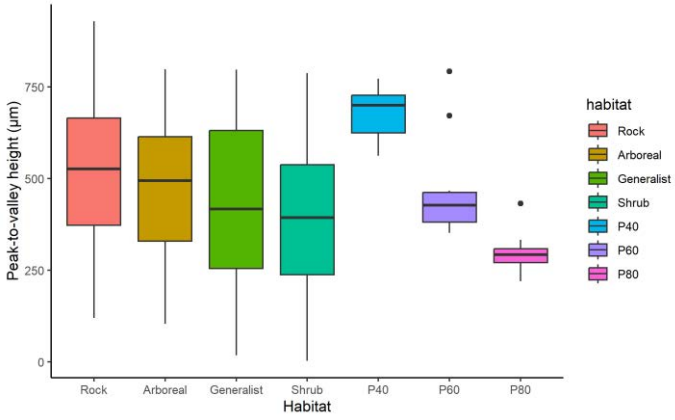
Species	Microhabitat	Locality	No. of observations	Date
O. castelnaui	Dead tree	Cape York, QLD	1	Oct 2018
O. castelnaui	Dead tree	Charters Towers Region, QLD	27	Sept 2015 – Feb 2017
O. castelnaui	*E. melanophloia*	Townsville, QLD	19	May 2016 – Dec 2017; June – July 2017
O. castelnaui	*E. melanophloia*	Charters Towers Region, QLD	21	Aug 2015 – Feb 2017
O. monilis	Dead tree	Collinsville, QLD	3	Nov 2019
O. monilis	*E. melanophloia*	Collinsville, QLD	6	Nov 2019
O. monilis	*E. melanophloia*	Hidden Valley, QLD	1	Aug 2017
O. monilis	*E. platyphylla*	Collinsville, QLD	6	May 2020
O. monilis	*E. similis*	Hidden Valley, QLD	4	Dec 2015; Aug 2017
O. monilis	*Granite*	Cape Cleveland, QLD	2	December 2015
O. monilis	*Granite*	Herveys Range, QLD	1	January 2015
O. monilis	*Granite*	Hidden Valley, QLD	10	August 2017
O. monilis	*Granite*	Magnetic Island, QLD	7	Oct – Nov 2015; Jan 2018
O. coggeri	*Granite*	Hidden Valley, QLD	12	Aug 2018
O. coggeri	*Granite*	Undara, QLD	5	Nov 2017

Supplementary material 6**.** Substrate selection testing arena. Plastic containers (70 x 32 x 12 cm; Total area – 2448 cm2) were lined on all inner surfaces, with equal areas (1224cm2 of each) of 40 grit (coarse) and 400 grit (fine) aluminum oxide sandpaper. For video recording trials, testing arenas were covered with mesh, which was wrapped in transparent plastic film (Clorox Australia Pty Ltd, New South Wales, Australia) and sprayed with canola oil (Pascoe's, Western Australia, Australia) to prevent the use of the mesh as a substrate by the geckos, while still allowing us to observe substrate choice. Surface on the left (blue) is fine sandpaper (P400) and right (grey) is coarse sandpaper (P40).

Supplementary material 7. Relationship between mass (g) and shear force (N) on coarse (orange) and fine (blue) sandpaper in *Oedura* geckos. (A) Relationship between mass and shear force on coarse and fine sandpaper in *O. castelnaui*. There was no significant influence of mass on shear force exerted by *O. castelnaui* (Linear model, df = 1, F = 2.10, P = 0.15) (B) Relationship between mass and shear force on coarse and fine sandpaper in *O. coggeri.* There was significant positive influence of mass on shear force in *O. coggeri* (Linear model, df = 1, F = 5.80, P < 0.05). (C) Relationship between mass and shear force on coarse and fine sandpaper in *O. monilis*. There was significant positive influence of mass on shear force in *O. monilis* (Linear model, df = 1, F = 14.54, P < 0.01).

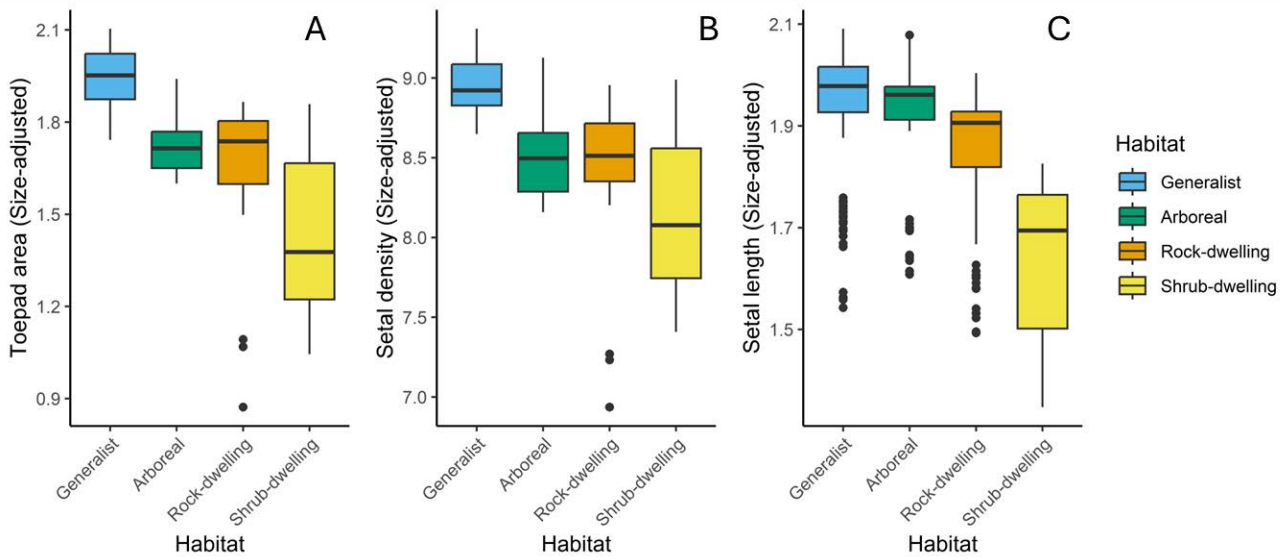


Appendix B. Supplementary material for Chapter 4



Supplementary material 8. Surface roughness (Peak-to-valley height, µm) of surfaces diplodactylid geckos used in nature and sandpapers used in this study. Coarse sandpaper (P40-grit) represented the upper end of the roughness values in geckos used in nature, while fine sandpaper (P80-grit) represented the lower end of the roughness. Intermediate sandpaper (P60-grit) represented the median roughness geckos used in nature.

Appendix C. Supplementary material for Chapter 5



Supplementary material 9. Morphological differences based on habitat use in diplodactylid geckos. A) SVL-adjusted toepad area. Generalist species had larger toepads compared to all other species. Arboreal and rock-dwelling geckos had similar toepad areas. Shrub-dwelling species had the smallest toepads compared to all other geckos. B) SVL-adjusted setal density. Generalist geckos had greater setal density compared to shrub-dwelling geckos. Arboreal and rock-dwelling geckos exhibited similar densities of setae. C) SVL-adjusted setal length. Generalist geckos had longer setae compared to shrub-dwelling geckos. Arboreal and rock-dwelling species had setae of similar length. Boxes are coloured by microhabitat use (blue = generalists, green = arboreal, orange = rock-dwelling and yellow = shrub-dwelling).

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