

Do structural habitat modifications associated with urbanization influence locomotor performance and limb kinematics in *Anolis* lizards?

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Received 7 November 2018; revised 8 February 2019; accepted for publication 3 March 2019

Urbanization significantly alters habitats for arboreal species, increasing the frequency of very smooth substrates by substituting artificial objects, such as metal poles and painted walls, for some trees. Because they experience these novel substrates more often, urban animals may use strategies to overcome challenges from substrate smoothness that animals from natural habitats do not. We assessed locomotor performance and two-dimensional hindlimb kinematics of two species of *Anolis* lizards (*Anolis cristatellus* and *Anolis sagrei*) from both urban and natural habitats in Miami, Florida. We ran lizards on six racetracks, crossing three substrates of increasing smoothness (rough bark, concrete blocks, and smooth, unpainted wood) with two inclinations (37° and vertical). We found that on vertical tracks with smooth substrates, lizards ran slower, took shorter strides and exhibited more contracted limb postures at the end of their stance than when running on the inclined track. Urban lizards, which are likely to be exposed more often to smooth substrates, did not adjust their movement to increase performance relative to lizards from natural habitats. This result, and the similarity of kinematic strategies between the two species, suggests the locomotor responses of lizards to substrate properties are highly conserved, which may be a mitigating factor that dampens or obviates the effects of natural selection on locomotor behaviour.

ADDITIONAL KEYWORDS: *Anolis* – artificial substrates – kinematics – performance – smoothness – sprint speed.

INTRODUCTION

Substrate properties can limit the efficacy of locomotion as animals move through their habitats (Turchin, 1998; Spezzano & Jayne, 2004). For example, many animals often sprint close to, or at, maximum velocities to flee from predators or to capture prey (Irschick & Garland, 2001; Irschick & Higham, 2016), but maximum velocity is not achievable on all types of substrates (Tulli *et al.*, 2012). Arboreal animals in particular must overcome several challenges because of the properties of the substrates they use for locomotion. For example, movement up steep inclines can pose functional challenges, especially for larger animals, as increased body mass can result in increased gravitational forces that impede effective

locomotion (Huey & Hertz, 1982; Cartmill, 1985; Jayne & Irschick, 1999; Tullis & Andrus 2011). In addition, variation in substrate diameter, and its interaction with other substrate properties, has been shown to influence locomotion by affecting how an animal grips a branch (Lammers, 2004; Spezzano & Jayne, 2004; Herrel *et al.*, 2013; Hsieh, 2016).

These aspects of the arboreal habitat present trade-offs for locomotion in many animals (Losos & Sinervo, 1989; Losos *et al.*, 1993; Vanhooydonck & Van Damme, 2001). For example, one trade-off involves the speed and stability of locomotion (Losos & Sinervo, 1989; Irschick & Losos, 1999), such that specializations or behaviours for enhancing stability may also result in reduced speed, and vice versa. To prevent toppling backwards as incline increases, some animals hold their bodies closer to the substrate during locomotion and reduce stride lengths to increase contact time with the surface, resulting in decreased speed (Spezzano

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& Jayne, 2004; Lammers *et al.*, 2006; Herrel *et al.*, 2013; Birn-Jeffery & Higham, 2016). Narrow perches also pose a velocity–stability trade-off (Losos & Sinervo, 1989; Spezzano & Jayne, 2004), resulting in decreased maximal velocity and acceleration capacity for some *Anolis* lizard species (Losos & Irschick, 1996; Vanhooydonck *et al.*, 2006; Hsieh, 2016). In sum, there is a substantial body of research showing that variation in substrates associated with arboreal habitats is likely to have strong impacts on the locomotion of animal groups. However, there has been little work relating such locomotor effects to variation in habitat use in novel conditions, such as artificial structures in urban habitats.

Urbanization creates novel environments that significantly change natural habitat in numerous ways, with profound effects for many organisms (McKinney, 2006). Trees, branches and other natural substrates are reduced in density by removal of vegetation, whereas artificial structures, such as metal poles, wooden fences and painted walls, are added (Battles *et al.*, 2018). Arboreal species have an ecological and evolutionary history of moving on natural vegetation in a dense matrix of the forest, and in urban habitats they must instead manoeuvre on human-made structures (Winchell *et al.*, 2018a; Battles *et al.*, 2018). Perhaps one of the greatest contrasts between urban and natural habitats for arboreal animals is the increased frequency of vertical, smooth surfaces in urban areas, such as poles and walls (Kolbe *et al.*, 2016b). The high frequency of smooth substrates in cities may present novel locomotor challenges for animals that normally use rougher substrates, which are more common in natural habitats.

Previous research has shown that *Anolis* lizards decrease their velocity with increasing incline because of changes in both limb positioning (i.e. to hold the body closer to the locomotor surface) and stride lengths (Spezzano & Jayne, 2004). Velocity also decreases for anoles running on smooth surfaces (Kolbe *et al.*, 2016b; Winchell *et al.*, 2018b). While foot traction is generally worse on low-friction substrates (Clark & Higham, 2011), we do not know yet how gait or limb positions change for quadrupedal organisms not specialized for locomotion on smooth surfaces.

Geckos, however, which are notable for their ability to traverse smooth substrates of all orientations, provide some insight into how other quadrupeds may handle smooth surfaces. The adhesive benefits offered by highly specialized toepad morphology come with a cost of reduced maximal velocity, such that increasing incline decreases velocity more than substrate smoothness (Russell & Higham, 2009). Furthermore, their specialized toes contribute to better acceleration performance on smooth substrates compared to rough substrates (i.e. wire mesh and rough cloth) because

their toepads can adhere more completely, or create van der Waals interactions more often on the smooth surface (Vanhooydonck *et al.*, 2005; King *et al.*, 2014). Finally, geckos possess unique and complex climbing behaviours that probably allow them to move on smoother surfaces with relative ease (Autumn, 2006). Other taxa that possess similar adhesive toepad structures, such as *Anolis* lizards, are able to cling to smooth substrates, but there has been less research on the ability to move on smooth surfaces for this group and other arboreal quadrupeds.

We investigated the effects of substrate inclination and smoothness on locomotor performance and two-dimensional hindlimb kinematics for populations of two species of *Anolis* lizards from natural forest habitats and urban sites, the latter population being exposed to artificial substrates. Anoles are an excellent system to explore novel substrate use because of extensive previous research on locomotor performance and kinematics (e.g. Losos & Sinervo, 1989; Losos, 1990a, b; Irschick & Losos, 1999; Foster & Higham, 2014), and several *Anolis* species are found in both urban and natural areas (Marnocha *et al.*, 2011; Winchell *et al.*, 2018a). Furthermore, Caribbean anoles in general have a fairly conserved toepad shape (Losos, 1990a, b; Elstrott & Irschick, 2004; Losos, 2009; Crandell *et al.*, 2014). Two species, *Anolis sagrei* and *Anolis cristatellus*, occupy both natural and urban habitats in their non-native range in Miami, Florida, but *A. sagrei* has been in urban areas in Miami for at least a few decades longer than *A. cristatellus* (Salzburg, 1984; Kolbe *et al.*, 2007, 2016a). These two species are similar in overall body shape and have claws (Losos, 1990b). Although these two species have about the same number of lamellae, *A. cristatellus* exhibits greater clinging performance (Losos, 1990a), but it is unclear how clinging performance affects sprint speed. On smooth substrates, claws are unlikely to be effective and do not contribute to velocity (Winchell *et al.*, 2018b). Both species specialize in similar structural habitats, commonly occupying the ground and perches up to ~2 m (Salzburg, 1984; Losos, 2009), but whether these two species respond in the same manner kinematically to variation in inclination and smoothness is an open question. Furthermore, lizards utilize broad-diameter perches in urban areas, which are frequently artificial, smooth structures (Winchell *et al.*, 2016; Battles *et al.*, 2018).

We conducted trials in which lizards ran up two inclines (37° or inclined, and 90° or vertical) and three substrates of increasing smoothness (rough bark, concrete block and smooth wood). We predicted that (1) maximal sprint speed and stride length will decrease on vertical structures, and (2) to overcome increased effects of gravity, lizards will utilize a broader stance, bringing their body closer to the track surface (Jayne &

Irschick, 1999). We also predicted that (3) velocity and stride length will decrease on smooth substrates. Finally, because lizards from urban habitats should encounter smooth, vertical substrates more often, we predicted that (4) urban lizards should employ kinematic strategies (e.g. gait characteristics and limb positioning) that are more effective on smooth, vertical substrates. Therefore, we predict that urban lizards will perform better on these substrates than lizards from natural habitats.

MATERIAL AND METHODS

STUDY SPECIES AND COLLECTION

We studied two species of *Anolis*, small insectivorous lizards found naturally in southern North America, Central and South America, and throughout the Caribbean (Losos, 2009). *Anolis sagrei* is widespread in Miami and throughout Florida, whereas the distribution of *A. cristatellus* is more restricted, radiating out from two independent points of introduction in the Miami area (Kolbe *et al.*, 2016a). We collected adult males (snout–vent length, SVL, > 40 mm) of *A. cristatellus* from natural ($N = 13$) and urban ($N = 15$) sites, and *A. sagrei* from natural ($N = 15$) and urban ($N = 15$) sites in Miami, and shipped them to the University of Rhode Island where all experiments were performed. In natural areas, both species were allopatric. *Anolis cristatellus* individuals from urban areas were collected from a site where they were sympatric with *A. sagrei*, whereas urban *A. sagrei* individuals were collected from locations without *A. cristatellus* present. The home ranges of these species are small, and individuals do not move distances sufficient to encounter both urban and natural habitats.

MORPHOLOGY

We measured mass, SVL using a ruler, hindlimb length (hip insertion to the tip of the 4th, the longest, toe) with digital calipers, and counted the number of toepad lamellae on the 4th toe of the hindfoot for each individual. We calculated relative hindlimb lengths as residuals of a non-linear, scaled-SVL index (Peig & Green, 2009). While all of these individuals attempted racetrack trials, only a subset had runs of sufficient quality that allowed further analysis. Therefore, the morphological analyses include all lizards, whereas the kinematic analyses include only a subset as outlined below.

RACETRACK PROCEDURE

Lizards ran on three substrates (bark, very rough; cinder block, somewhat smooth, hereafter called 'block';

and unfinished pine plywood, very smooth, hereafter called 'wood') at two different inclines (37°, inclined; and 90°, vertical), for a total of six tracks. We used 37° as the inclined track because this is a commonly used inclination to test sprint speed as lizards will often run bipedally at angles closer to horizontal. Each track surface had a scale bar and distance markings every 25 cm. To keep lizards from jumping off the sides, all tracks had walls along both sides. The order of tracks was randomly determined and consistent for all lizards.

To elicit maximum speed, lizards were placed at the base of each track and allowed to run upwards. We encouraged movement with gentle taps near their tails when needed. To increase our chances of capturing the best performance, each lizard ran three to five times on each track within a single trial and was given at least 2 h to recover between trials. We filmed all lizard runs at 240 frames-per-second with a digital camera (Casio Exilim Ex-zr1000) positioned dorsally to lizards on the tracks. Such filming frequencies are effective for estimating basic movements of small lizards with these running velocities (Walker, 1998). We ran all lizards at the same temperature (~27 °C), confirmed with readings from a cloacal thermometer at the beginning and end of each trial.

We took data only from runs in which the lizard maintained constant speed over at least three stride cycles. On the smooth and vertical substrates, lizards often took multiple strides within 10 cm, while continuous runs on the rougher tracks covered at least 20 cm. For each stride of these continuous runs, we isolated still frames at the beginning and end of the stance for one hindlimb. The beginning of the stance is defined as the moment the foot contacts the substrate to begin to push the body forward. The end of the stance is defined as the moment the foot leaves the substrate, or when the lizard has pushed itself as far forward as it will with that leg, lifting it to re-plant it for the next beginning of stance. We used the number of frames including and between these stance beginnings to mark the passage of time. We performed all video manipulations in ImageJ (Schneider *et al.*, 2012).

LIMB KINEMATIC MEASUREMENTS

Of the total number of lizards collected from the field ($N = 58$), only a subset of each species completed full, steady-speed runs on all six tracks: seven of each species from natural populations and six of each species from urban populations. We measured multiple variables from the longest stride, defined as the greatest distance between stance end and the subsequent stance beginning within each

continuous, constant-speed run (multiple runs per track could result in more than one stride per lizard per track; see above). These variables included instantaneous velocity, stride length (i.e. the distance between successive footfalls), stride duration (i.e. the time for that stride), step length (i.e. the distance the body advances from when the foot is planted to when it is lifted) and duty factor (i.e. the proportion of the stride in which the foot is in contact with the surface). We used the longest stride as this is typically tightly correlated with greater speeds and maximum efforts in lizards (Irschick & Jayne, 1999). At the beginning and end of stance, we measured the angle around the hip, the angle around the knee and step width (i.e. the single-axis distance between the ankle and the midline; Fig. 1). We used the ObjectJ plugin for ImageJ (Schneider *et al.*, 2012) to measure these variables from the still frames.

STATISTICAL ANALYSES

We performed all statistical analyses in R (R Core Team, 2014), and analysed each species separately. For each kinematic variable and velocity (Tables 1, 2), we performed mixed-model ANOVA with factors including incline (inclined or vertical), substrate (bark, block or wood), population source (urban or

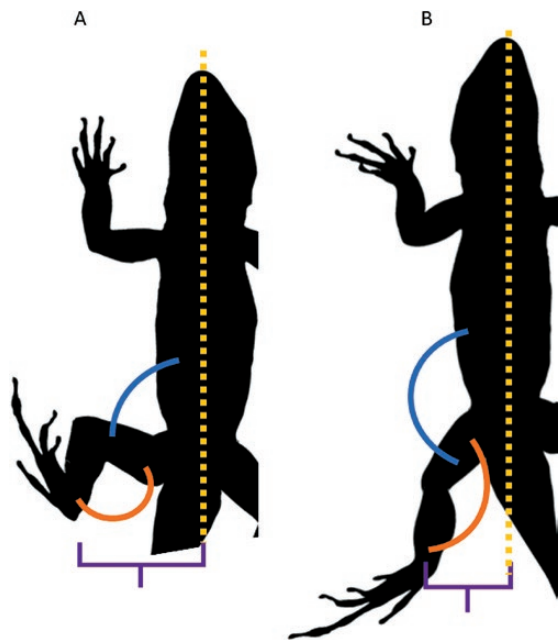


Figure 1. Diagram of measurements taken for each foot position. A, footfall, stance beginning; B, footrise, stance end. Hip angle: blue arc at hip; knee angle: orange arc at knee; step width: purple bracket between midline (in yellow) and ankle.

Table 1. *F*-values from three-way ANOVAs performed separately on each variable for *Anolis cristatellus*. Snout–vent length and relative hindlimb length were included as covariates. The full table with *F*-values of covariates, variance from lizard ID (individual) and model *R*² values is presented in Table S3

	ANOVA effects						Interactions			Model <i>R</i> ² (marginal)
	Incline	Substrate	Hab.	Incline × Substrate	Substrate × Hab.	Incline × Hab.	Three-way interaction			
Velocity	194.6**	11.4**	0.0	1.4	1.1	4.0*	0.5	0.47		
Stride length	353.4**	30.7**	0.3	1.5	4.3*	0.9	2.7	0.66		
Step length	298.3**	36.4**	0.2	4.2*	4.2*	1.5	4.4*	0.63		
Stride duration	8.3*	0.1	0.4	1.6	0.4	1.4	2.0	0.18		
Duty factor	160.4**	37.5**	0.2	5.0*	5.2*	0.0	3.5*	0.51		
Knee angle, beginning	77.3**	1.6	1.1	7.2**	0.1	0.0	1.6	0.29		
Hip angle, beginning	40.0**	6.1*	0.1	1.0	1.3	2.8	0.0	0.20		
Knee angle, end	96.3**	8.7**	0.0	2.6	0.2	0.0	0.3	0.35		
Hip angle, end	80.2**	5.4*	2.1	5.4*	0.9	0.6	0.5	0.33		
Stride width, beginning	66.5**	0.2	0.2	1.3	1.5	6.2*	2.2	0.30		
Stride width, end	59.9**	1.6	0.0	2.7	1.8	1.4	1.4	0.26		

P* < 0.05, *P* < 0.001. 'Beginning' and 'end' refer to the stance position stance beginning and stance end, respectively. 'Hab.' refers to habitat source (urban or natural).

Table 2. *F*-values from three-way ANOVAs performed separately on each variable for *Aanolis sagrei*. Snout-vent length and relative hindlimb length were included as covariates. The full table with *F*-values of covariates, variance from lizard ID (individual) and model *R*² values is presented in Table S4

	ANOVA effects			Interactions					Model <i>R</i> ² (marginal)
	Incline	Substrate	Hab.	Incline × Substrate	Substrate × Hab.	Incline × Hab.	Three-way interaction		
Velocity	368.9**	6.06*	1.4	1.9	1.3	0.0	3.4*	0.56	
Stride length	478.7**	26.9**	0.2	1.9	0.9	6.6**	0.3	0.67	
Step length	273.0**	36.6**	0.0	1.1	0.3	5.3**	0.2	0.59	
Stride duration	11.7**	2.8	0.6	2.1	1.0	1.2	2.3	0.15	
Duty factor	102.0**	34.8**	1.5	0.6	3.3*	2.8	0.5	0.45	
Knee angle, beginning	6.9*	2.4	0.6	2.6	1.3	0.2	2.7	0.12	
Hip angle, beginning	3.7	2.5	0.5	0.2	0.3	0.0	3.8	0.07	
Knee angle, end	118.3**	61.4**	0.0	5.8*	0.3	0.5	1.2	0.55	
Hip angle, end	70.2**	9.1**	0.0	6.2*	0.7	1.1	3.2*	0.32	
Stride width, beginning	59.7**	0.5	6.1*	1.3	0.1	7.0*	0.5	0.36	
Stride width, end	59.2**	0.0	0.2	0.5	0.4	0.8	1.6	0.24	

P* < 0.05, *P* < 0.001. 'Beginning' and 'end' refer to the stance position stance beginning and stance end, respectively. 'Hab.' refers to habitat source (urban or natural).

natural), and their interactions, as well as lizard ID as a random effect to account for multiple runs across all tracks by the same individual. We used SVL and relative hindlimb length as covariates, even when not significant. We calculated Pearson's correlations between the kinematic variables, SVL, relative hindlimb length and velocity (Supporting Information, Tables S1, S2). We also used *t*-tests to test for differences in morphological variables between urban and natural populations using the entire sample of lizards (*N* = 58).

RESULTS

MORPHOLOGY

Urban and natural *A. cristatellus* did not differ in SVL, mass, relative hindlimb length or the number of toepad lamellae. In contrast, urban *A. sagrei* were larger (SVL: $t_{110.7} = -4.88, P < 0.0001$; mass: $t_{108.6} = -6.90, P < 0.0001$), had relatively longer hindlimbs ($t_{105.5} = -2.84, P < 0.01$) and more lamellae ($t_{104.5} = -4.44, P < 0.001$) than those from natural sites.

PERFORMANCE

For *A. cristatellus*, velocity decreased significantly on vertical compared to inclined tracks, and on the wood tracks compared to both block and bark (Table 1; Fig. 2A). On the vertical tracks, urban and natural *A. cristatellus* ran at similar speeds, whereas on the inclined tracks, urban lizards tended to run faster (significant interaction between incline and population source; Table 1). For *A. sagrei*, velocity decreased significantly on vertical compared to inclined tracks as well as on the smoother tracks compared to bark (Table 2; Fig 2B). Population source also had a three-way interaction with incline and substrate, such that urban lizards ran slower than natural lizards on only the vertical wood (smooth) track (Table 2).

KINEMATICS

Whole-stride characteristics

For both species, stride length and step length decreased on the vertical track and on the wood substrate (Tables 1, 2; Fig. 3A, B, E, F). Similarly, step length also decreased for both species with increased incline and increased smoothness (Tables 1, 2; Fig. 3B, F). Duty factor increased on the vertical and wood substrate in both species (Tables 1, 2; Fig. 3C, 3G). Stride duration decreased significantly in both *A. cristatellus* and *A. sagrei* with an increase in incline (Tables 1, 2; Fig. 3D, H).

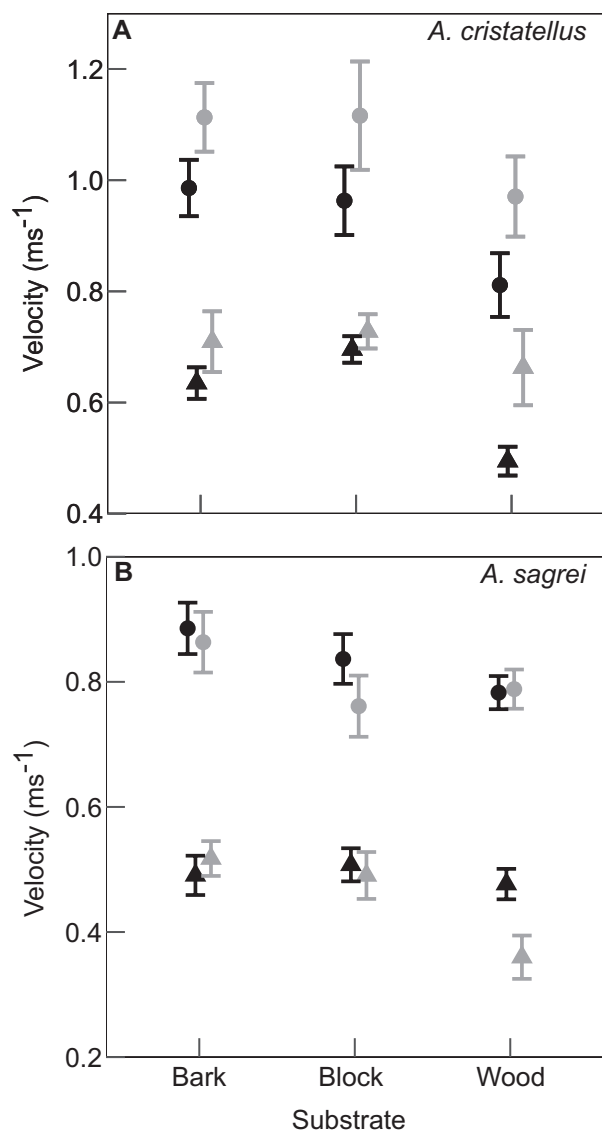


Figure 2. Velocity (mean \pm SEM) for (A) *Anolis cristatellus* and (B) *Anolis sagrei* on each substrate, with inclined tracks represented by circles, vertical tracks by triangles and colours showing the population source (black = natural, grey = urban).

Limb angles

On the vertical track compared to the inclined track, knee angles were greater at the stance beginning and smaller at the stance end for both species (Tables 1, 2; Figs 4A, B, G, 5A, B, G). At the stance beginning, *A. cristatellus* hip angles were greater on vertical tracks and smaller on wood tracks (compared to inclined and bark tracks, respectively), while this angle was not affected by any of the factors in *A. sagrei* (Tables 1, 2; Figs 4C, 5C). For both species, hip angles were smaller at stance end on vertical and wood tracks, indicating

the leg was less extended behind the body (Tables 1, 2; Figs 4D, G, 5D, G). Stride width at stance beginning increased for both species on vertical tracks, with a greater increase for urban lizards, but decreased at stance end on vertical tracks for both species (Tables 1, 2; Figs 4E, F, 5E, F). The Supporting Information provides more detailed descriptions of interactive effects.

DISCUSSION

EFFECTS OF INCLINE AND SMOOTHNESS

Our study of locomotion in two species of *Anolis* lizards from urban and natural habitats revealed several key findings. As predicted, we found that both species decreased velocity, stride and step lengths, and increased duty factor on smooth, vertical substrates compared to rough, inclined substrates (also see Kolbe *et al.*, 2016b). We found no support for our prediction that lizards from urban areas would move differently on smooth substrates compared to lizards from natural areas for either species. Despite some interactions between population source and locomotor variables, we did not find any consistent patterns for greater velocity on smooth substrates by urban lizards. However, urban *A. sagrei* ran slower than natural lizards on the smooth, vertical track, which we discuss below (Fig. 2B).

Quadrupeds often use a range of strategies to move effectively across substrates they typically encounter (Biewener, 2003). Lizards have been shown to modulate stride length and stride frequency, their primary mechanisms for modulating speed, and that these characteristics change in a regular way with incline (Huey & Hertz, 1982, 1984; Irschick & Jayne, 1998; Higham *et al.*, 2011). The decrease in velocity on inclines has been attributed to changes in stride characteristics that help lizards overcome the effects of gravity, and prevent toppling, such as holding the body and centre of mass closer to the locomotor surface (Cartmill, 1985), which we also observed in this study. Furthermore, shortened stride lengths, probably affected by more-contracted limb positions, also contributed to reduced velocity on inclines (Irschick & Jayne, 1998; Jayne & Irschick, 1999). Our results also support previous findings that as incline increases, step lengths decrease (Jayne & Irschick, 1999) and duty factor increases (Foster & Higham, 2012). In response to increasing inclines, lizards and other taxa typically cannot both run at maximal velocity and remain stable (Foster & Higham, 2012). Importantly, our results show that the strategies for overcoming the challenge of increasing incline are also effective for maintaining stability on smooth substrates. Specifically, the effects of smoothness on lizard velocity

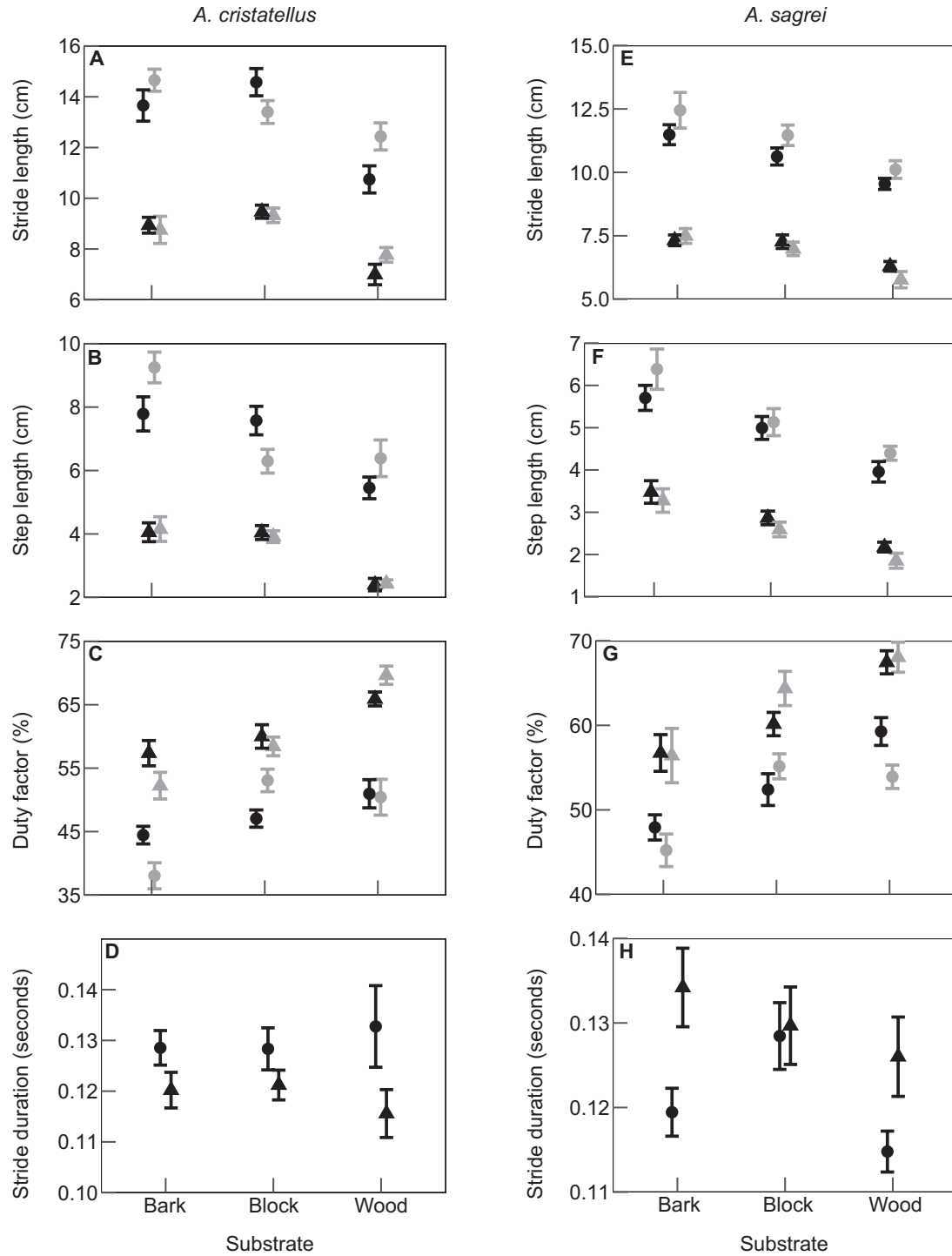


Figure 3. Gait characteristics (mean \pm SEM) by substrate for (A–D) *Anolis cristatellus* and (E–H) *Anolis sagrei*, with inclined tracks represented by circles and vertical tracks by triangles. A,E, stride length; B,F, step length; C,G, duty factor; D,H, stride duration. For gait characteristics significantly affected by population source in the model (Tables 1, 2; this figure, A–C and E–G), the natural population is in black and urban population in grey. Stride duration was not affected by population source in either species, so D shows combined populations in black.

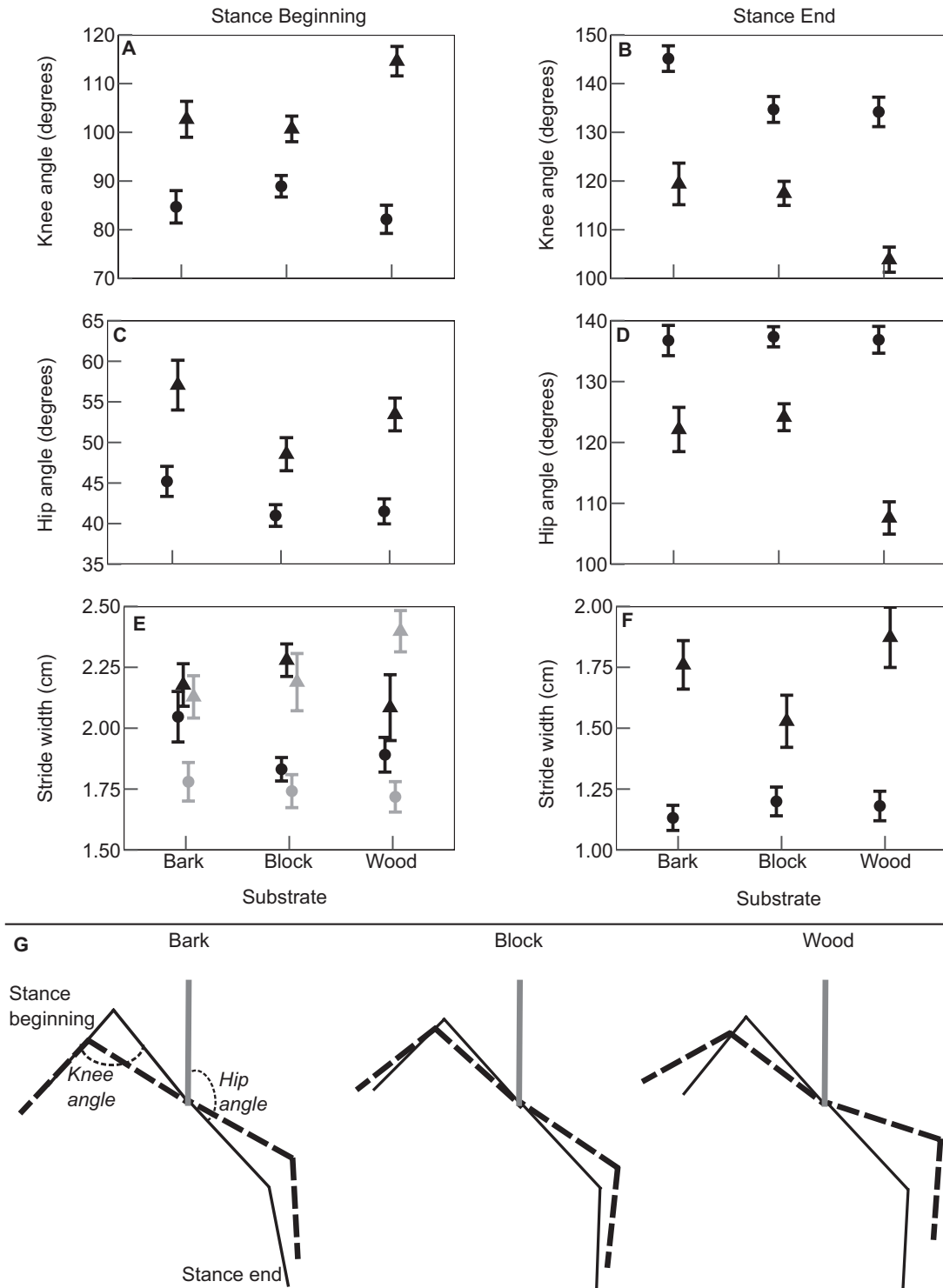


Figure 4. Mean (\pm SEM) limb angles of *Anolis cristatellus* at stance beginning: A, knee angle; C, hip angle; and at stance end: B, knee angle; D, hip angle, and mean (\pm SEM) stride widths at (E) stance beginning and (F) stance end. The inclined track is represented by circles and the vertical track by triangles. When population source significantly affected a kinematic variable (D), natural lizards are shown in black, and urban lizards in grey. G shows a schematic of mean knee and hip angles at stance beginning (left side of each cartoon) and stance end (right side of each cartoon) on each of the substrates, with solid lines showing leg positions on the inclined tracks and dashed lines showing leg positions on the vertical tracks.

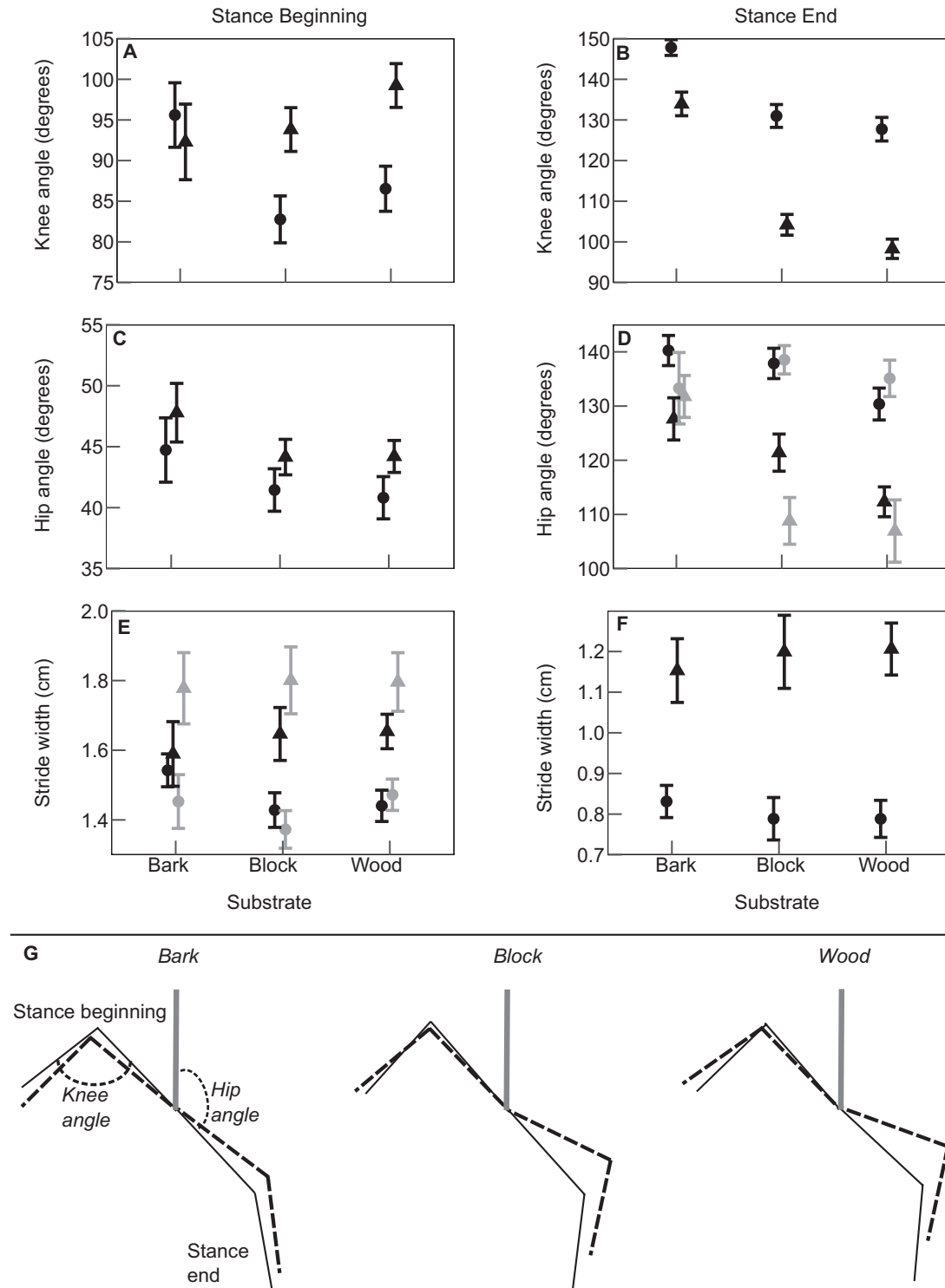


Figure 5. Mean (\pm SEM) limb angles of *Anolis sagrei* at stance beginning: A, knee angle; C, hip angle; and at stance end: B, knee angle; D, hip angle, and mean (\pm SEM) stride widths at (E) stance beginning and (F) stance end. The inclined track is represented by circles and the vertical track by triangles. When population source significantly affected a kinematic variable (D, E), natural lizards are shown in black, and urban lizards in grey. G shows a schematic of mean knee and hip angles at stance beginning (left side of each cartoon) and stance end (right side of each cartoon) on each of the substrates, with solid lines showing leg positions on the inclined tracks and dashed lines showing leg positions on the vertical tracks.

and stride characteristics are in the same direction as for inclination, but the effects are not as strong.

URBANIZATION AND LOCOMOTION

The growth of urban areas worldwide exposes countless species to this human-modified habitat, and species respond on a spectrum from avoidance of to dependence on urban habitats (Fischer *et al.*, 2015). How animals use urban habitats is increasingly studied to better understand and predict the persistence of populations in these widespread habitats (Shochat *et al.*, 2006; Sol *et al.*, 2013; Winchell *et al.*, 2018a). Urban lizards encounter very smooth artificial surfaces more often than lizards from natural habitats (Kolbe *et al.*, 2016b; Battles *et al.*, 2018), and this work shows that there is substantial flexibility in the movement patterns of anoles, a result generally consistent with the large body of work showing that climbing lizards can move on a wide range of surfaces using the same basic strategy. For instance, in a study testing acceleration, sprint speed and endurance on rough and smooth surfaces, Vanhooydonck *et al.* (2015) found that multiple species of lacertid lizards were able to attain relatively high speeds after running more than 50 cm on smooth substrates, even though the species differed in morphology.

In our study, urban lizards of both species did not alter limb angles to any advantage, and the effects of population source nearly always interacted with incline, not smoothness. However, considering that multiple, phylogenetically disparate taxa respond to increased incline with shorter stride lengths and broader limb positions (Spezzano & Jayne, 2004; Lammers *et al.*, 2006; Bergmann & Irschick, 2010; Herrel *et al.*, 2013), urbanization may not prompt novel strategies for moving on smooth substrates given the relatively recent appearance of cities on an evolutionary time scale. In Puerto Rico, the native range of *A. cristatellus*, Winchell *et al.* (2018b) found similar decreases in velocity on inclined smooth compared to flat tracks, but that urban lizards performed better on both track types. Future studies should replicate our design across the native and introduced range, which could uncover effects of the invasion process in influencing adaptation to smooth, vertical substrates.

An alternative explanation is that even with a few slips, locomotion on smooth surfaces may be 'good enough' for lizards to conduct everyday activities (e.g. capture prey, defend territories, avoid predators), and as noted previously, even submaximal locomotor effort may be sufficient in many circumstances (Irschick, 2002). Nevertheless, further research is needed to determine if the documented submaximal performance on smooth substrates has an impact on

fitness in urban areas. However, the contributions of submaximal performance to fitness may explain why urban *A. sagrei* in our study, which have been exposed to urban habitats in Miami longer than *A. cristatellus*, ran slower on the smooth, vertical track than did natural *A. sagrei*. The link between performance and fitness in urban areas may lie in morphological adaptations.

Animals often display a high degree of specialization in terms of morphological adaptations that enable them to move effectively on surfaces they commonly encounter (Biewener, 2003; Irschick & Higham, 2016). Anole species that use different structural habitats show considerable variation in hindlimb morphology, which allows shorter-limbed species to more easily move on, and occupy, narrow perches, and allows longer-limbed species to move faster on broader surfaces (Losos & Sinervo, 1989; Losos, 2009). Similarly, in urban habitats, where anoles encounter and use broader perches more often than in natural habitats (Battles *et al.*, 2018), some anole populations have evolved longer limbs (Winchell *et al.*, 2016), which should increase stability and velocity on broad substrates (Kolbe *et al.*, 2016b). However, smooth substrates may disrupt the traditional relationship between morphology and habitat use, as the broadest structures in urban habitats are often artificial (Battles *et al.*, 2018; Winchell *et al.*, 2018a), and typically much smoother than those found in natural habitats (Kolbe *et al.*, 2016b).

In our study, hindlimb length and stride length were positively correlated in *A. cristatellus*, and greater strides resulted in faster sprint speeds across all tracks. Therefore, morphological changes that improve performance on broad structures (e.g. longer hindlimbs) may also increase performance on smooth substrates. Because locomotor performance seems more likely to improve with morphological changes, we expect that smooth, artificial structures will contribute to selection for increased limb length in urban lizards. In urban populations, we did not find greater relative hindlimb lengths in *A. cristatellus*, but did in *A. sagrei*. Furthermore, anoles are characterized by the presence of specialized toepads that allow them to manoeuvre on smooth substrates where other taxa may be excluded. Indeed, Winchell *et al.* (2018a) found that *A. cristatellus* from urban areas had more toepad scales than lizards from natural areas, suggesting this morphological feature plays a role in facilitating the ability to inhabit urban environments. While we did not find this result in *A. cristatellus*, urban *A. sagrei* did have more toepad lamellae. We can think of two potential reasons why our results did not consistently follow the predicted trend. First, *A. cristatellus* was introduced more

recently than *A. sagrei*, and so may have had less time for urban and natural populations to diverge. Second, *A. cristatellus* was probably introduced to Miami from urban areas in Puerto Rico (Kolbe *et al.*, 2007), and thus may be ‘pre-adapted’ to urban habitats in Miami (Hufbauer *et al.*, 2012; Winchell *et al.*, 2018a). Future research should evaluate lizards from urban and natural sites in their native ranges, where populations have been exposed to urbanization for longer and potential founder effects associated with invasion are less relevant.

We also note that the ultimate fitness value of performance traits remains a topic of debate (Irschick *et al.*, 2008), and as noted by others (Garland & Losos, 1994; Irschick & Garland, 2001), not all ecological situations require maximal sprint capacity. Behavioural strategies are also critical for avoiding predators, and the use of such strategies often depends on the habitat occupied (e.g. availability of nearby refuges) and the predator in question (Hopper, 2001; Templeton & Shriner, 2004). Therefore, while sprint speed in lizards has been shown to decrease on smooth, vertical substrates, anoles may utilize different anti-predator behaviours when using these substrates, such as jumping instead of sprinting. When approached by a predator, they may also flee earlier when using smooth, vertical surfaces because of their reduced velocity (Avilés-Rodríguez & Kolbe 2019). Anole predators may also suffer reduced performance on smooth substrates, and future research should investigate this possibility. Furthermore, we did not test effects of substrate diameter in this study, but studies have shown strong effects of diameter on performance and kinematics (Spezzano & Jayne, 2004), and therefore interactions among diameter, incline and substrate smoothness are probably important, particularly for urban lizards. Nonetheless, urbanization presents a host of ecological challenges to non-human urban dwellers, with smooth substrates as a significant challenge for locomotion. As evolutionary ecologists gain understanding of the mechanisms behind habitat use and performance capability, we can better evaluate persistence of organisms in urban areas and other human-modified habitats.

ACKNOWLEDGMENTS

The protocols for use of vertebrate animals in this study were approved by the Institutional Animal Care and Use Committee at the University of Rhode Island (AN11-09-005). This research was funded by a grant from the National Science Foundation (DEB-1354897) and funds from the University of Rhode Island. We thank Elizabeth Shadle for help with lizard care and for help preparing and organizing video data,

and Zachary Chejanovski for collecting and shipping lizards to the University of Rhode Island. We declare no conflicts of interest for this work. We thank four anonymous reviewers for their help in improving the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. Correlations between performance, kinematic and morphological variables for *A. cristatellus* (all treatments together). Values in bold type are significant.

Table S2. Correlations between performance, kinematic and morphological variables for *A. sagrei* (all treatments together). Values in bold type are significant.

Table S3. Full ANOVA table (extension of [Table 1](#)).

Table S4. Full ANOVA table (extension of [Table 2](#)).