

RESEARCH ARTICLE

Effect of temperature on leg kinematics in sprinting tarantulas (*Aphonopelma hentzi*): high speed may limit hydraulic joint actuation

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ABSTRACT

Tarantulas extend the femur–patella (proximal) and tibia–metatarsal (distal) joints of their legs hydraulically. Because these two hydraulically actuated joints are positioned in series, hemolymph flow within each leg is expected to mechanically couple the movement of the joints. In the current study, we tested two hypotheses: (1) at lower temperatures, movement of the two in-series hydraulic joints within a leg will be less coupled because of increased hemolymph viscosity slowing hemolymph flow; and (2) at higher temperatures, movement of the two in-series hydraulic joints will be less coupled because the higher stride frequencies limit the time available for hemolymph flow. We elicited maximal running speeds at four ecologically relevant temperatures (15, 24, 31 and 40°C) in Texas Brown tarantulas (*Aphonopelma hentzi*). The spiders increased sprint speed 2.5-fold over the temperature range by changing their stride frequency but not stride length. The coefficient of determination for linear regression (R^2) of the proximal and distal joint angles was used as the measure of the degree of coupling between the two joints. This coupling coefficient between the proximal and distal joint angles, for both forelegs and hindlegs, was significantly lowest at the highest temperature at which the animals ran the fastest with the highest stride frequencies. The coordination of multiple, in-series hydraulically actuated joints may be limited by operating speed.

KEY WORDS: Arachnids, Locomotion, Running

INTRODUCTION

Instead of using muscles, spiders extend the proximal femur–patella and the distal tibia–metatarsal joints of their legs hydraulically (Ellis, 1944; Parry and Brown, 1959; Wilson, 1970; Kropf, 2013; Fig. 1). At these two joints, extension results from hydraulic pressure generated by muscles that compress the prosoma (or cephalothorax), causing hemolymph to flow out of prosoma and into lacunae within the legs (Anderson and Prestwich, 1975; Stewart and Martin, 1974). Direct muscle functions to flex these joints, during which hemolymph flows out of the legs and returns to the prosoma (Siebert et al., 2010). Hemolymph flows into the leg during joint extension and back out of the leg during joint flexion with each stride (Sensenig and Schultz, 2003).

To change walking and running speed, animals can vary stride frequency and/or stride length. The contributions of stride frequency and stride length to modulate locomotor speed differ among arthropod species, and can also change with speed within a given species. Cockroaches, for example, vary stride frequency more at lower speeds, while varying stride length at higher speeds (Full and Tu, 1991). *Grammostola* tarantulas and *Hololena* spiders increase both stride length and frequency when increasing speed (Biancardi et al., 2011; Spagna et al., 2011). However, variation in running speed is due exclusively to variation in stride frequency in *Brachypelma* tarantulas and teneriffid mites (Anderson and Prestwich, 1985; Wu et al., 2010).

Locomotor performance strongly depends on temperature in ectotherms: running speed typically increases with temperature up to an optimal temperature, above which performance declines steeply (Huey and Stevenson, 1979; Bennett, 1985; Heinrich, 1993; Huey and Kingsolver, 1989; Angilletta, 2009). Most arachnids exhibit this typical temperature-dependent pattern for locomotion (Prestwich, 1983; Shillington, 2005; Kruse et al., 2008; Carlson and Rowe, 2009; Wu et al., 2010). However, hunting ability in crab spiders remains constant across temperatures (Schmalhofer and Casey, 1999). Generally, the thermal sensitivity of locomotion reflects the thermal sensitivity of underlying physiological and biochemical processes (Swoap et al., 1993; Kingsolver, 2009).

In addition to its effects on muscle contraction and enzyme activity, temperature may also affect hemolymph viscosity. Ideally, hemolymph viscosity would remain constant across the active temperature range of spiders that use hydraulics to extend leg joints. In reality, however, hemolymph viscosity likely increases as temperature decreases, as with water and vertebrate blood (Rand et al., 1964; Dunlap, 2006; Snyder, 1974). The viscosity of water increases by 35% as water cools from 40 to 20°C (Korson et al., 1969; Kestin et al., 1978). Lower temperatures (e.g. 10 and 15°C) also cause blood to be more viscous in humans (22°C; Rand et al., 1964) and lizards (10 and 15°C; Dunlap, 2006; Snyder, 1974). In contrast, the viscosity of frog blood did not change between 5 and 25°C (Palenske and Saunders, 2003). In arachnids, the thermal effects on hemolymph viscosity (Sengers and Watson, 1986) may contribute to the thermal sensitivity of running performance (Wu et al., 2010).

In the current study, we examined the locomotion of tarantulas in the laboratory across their ecologically relevant temperature range (15–40°C; Shillington, 2002). Tarantulas are large-bodied spiders (family Theraphosidae) that locomote readily in captivity (Shillington and Peterson, 2002). Their large size facilitates video recording and analysis of their joint kinematics during running (Figs 1, 2). In addition, tarantulas in the wild can experience wide variation in environmental temperatures (Shillington, 2002), so the thermal sensitivity of locomotion is directly relevant to their field

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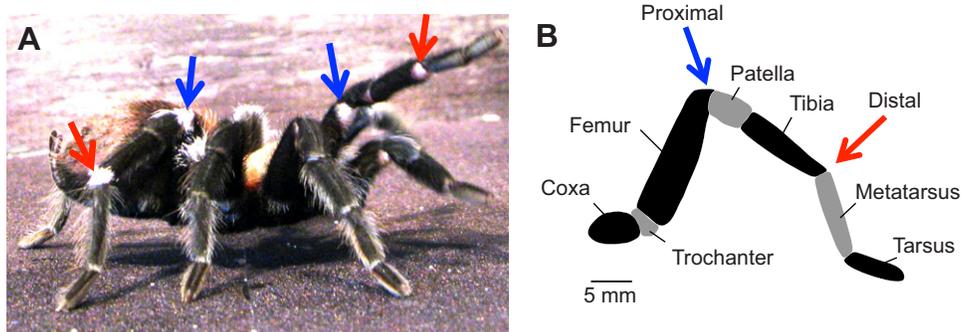


Fig. 1. Marker placement and leg anatomy of the tarantula *Aphonopelma hentzi*.

(A) Lateral view of the tarantula as it walks forward (to the right). The first (fore) and fourth (hind) leg on the animal's right side were examined. (B) A representative spider leg with the seven segments colored alternately in black and gray. Arrows point to the hydraulically extended joints examined: blue arrows indicate the femur–patella (or proximal) joint, red arrows indicate the tibia–metatarsus (or distal) joint.

ecology. During sprinting in a tarantula, joints extended by hemolymph pressure are characterized by internal volume change during movement (Blickhan and Barth, 1985). Joint volume changes to induce joint extension are measurable (8–20 μl), especially in a large spider like *Aphonopelma* (Sensenig and Shultz, 2003). Because the two hydraulically actuated joints are positioned in series within each leg, hemolymph movement into the leg and the accompanying increase in pressure would mechanically couple extension between the two joints (Fig. 1). In the current study, we tested two hypotheses: (1) at lower temperatures, movement in two in-series hydraulic joints within a leg will be less coupled possibly as a result of increased hemolymph viscosity and subsequently reduced flow of hemolymph to the distal joints of the legs; and (2) at higher temperatures, movement in two in-series hydraulic joints will be less coupled because the higher stride frequencies prevent complete movement of hemolymph, and subsequently pressure, into and out of the legs. These two hypotheses are not mutually exclusive, but rather address the possible constraints of using hydraulics for joint extension. To test our hypotheses, we quantified the joint kinematics of tarantulas sprinting, a maximal behavior (Prestwich, 1988), over the entire range of their ecologically active temperatures (Shillington, 2002).

RESULTS

Animals

The eight adult Texas Brown tarantulas, *Aphonopelma hentzi* (Girard 1852) (previously known as *Eurypelma californicum* Nentwig 2012), used for the study weighed 15.53 ± 1.91 g (mean \pm s.e.m.; Fig. 1). These animals also measured 5.52 ± 0.23 cm in total length from the anterior tip of the prosoma, near the eyes, to the posterior end of the opistosoma, near the anus. The segments of the first and fourth legs of the right side were measured separately, then summed per leg to determine total leg length. The first leg or foreleg (6.60 ± 3.08 cm) was shorter than the fourth leg or hindleg (6.98 ± 1.79 cm, $N=8$; paired t -test, $P < 0.05$; Fig. 1B).

Body temperature (T_b)

The animals were acclimated for at least 60 min in the environmental chambers at the four desired temperatures (15, 24, 31 and 40°C; Percival Scientific Inc., Perry, IA, USA). However, the measured surface T_b was used as an approximate measure of internal T_b , as small animals have very low thermal inertia (Bell, 1980; Fraser and Grigg, 1984; Stevenson, 1985). Surface T_b for the four nominal temperatures was 16.8 ± 0.6 , 24.3 ± 0.2 , 31.3 ± 0.4 and 37.9 ± 0.5 °C, respectively, determined with an infrared thermometer immediately after the running trials in the environmental chamber.

Kinematics

Spider sprint speed increased 2.5-fold as environmental temperature increased from 15 to 40°C (Fig. 3). Mean speed ranged from 3.97 ± 0.60 body lengths (BL) s^{-1} (21.0 ± 3.2 cm s^{-1}) at 16.8°C to 10.11 ± 1.04 BL s^{-1} (53.2 ± 5.3 cm s^{-1}) at 37.9°C (Fig. 3A). Running speed across the 21°C temperature range corresponded to $Q_{10}=1.56$. The change in speed was steepest between 16.8 and 24.3°C ($Q_{10}=2.75$; Fig. 3A). Stride frequency increased 2.4-fold across the overall temperature range, from 3.83 ± 0.46 Hz at 16.8°C to 9.40 ± 0.35 Hz at 37.9°C (Fig. 3B). In contrast, stride length did not vary with temperature (5.97 ± 0.26 cm or 1.13 ± 0.05 BL; $P > 0.05$; Fig. 3C). Similarly, extension fraction (or the proportion of the stride cycle during which extension is occurring) of both legs did not change with temperature ($P=0.48$). The extension fraction of the hindleg (0.50 ± 0.003) exceeded that of the foreleg (0.44 ± 0.008 ; $P < 0.001$; Fig. 3D), but did not differ between the two joints within a leg ($P=0.54$).

The range of angles spanned by each joint during sprinting did not change with temperature (Fig. 4; $P > 0.05$). Although, within an individual, the range of joint angles may have increased or decreased slightly with temperature (Fig. 4), the trends varied between individuals. Overall, any change in joint angle range with temperature was not statistically significant. In both legs, the angle range of the femur–patella (proximal) joint was approximately 2 times that of the tibia–metatarsus (distal) joint ($P < 0.001$; Fig. 4).

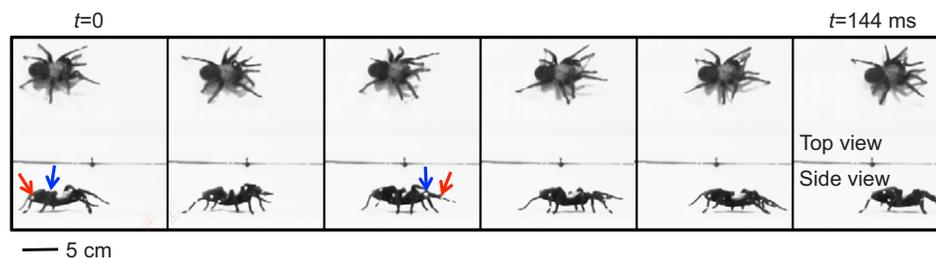


Fig. 2. Selected frames from a complete stride of an individual sprinting from left to right at 24°C. The sequential frames show the side view and the simultaneous top view of the animal provided by a mirror positioned at 45 deg to the camera. Four points on each of the first and fourth legs on the right side were digitized to provide the joint angles for the two hydraulically extended joints (blue arrow, proximal femur–patella; red arrow, distal tibia–metatarsus).

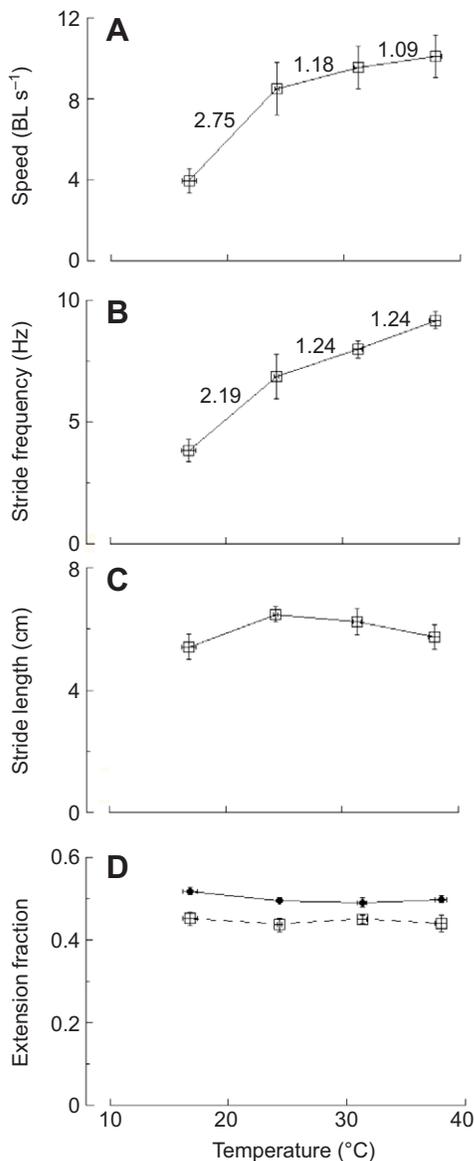


Fig. 3. Sprinting parameters change with temperature. (A) Speed increased with a Q_{10} of 1.56 across the temperature range (BL, body length). Numbers indicate the Q_{10} from 18 to 24°C, 24 to 31°C, and 31 to 38°C. (B) Stride frequency increased with temperature with a mean Q_{10} of 1.51. Numbers indicate the Q_{10} from 18 to 24°C, 24 to 31°C, and 31 to 38°C. (C) Stride length remained constant with temperature. (D) Extension fractions of the foreleg (dashed line, open squares) and hindleg (solid line, filled circles) did not change with temperature. All symbols represent means \pm s.e.m. across individuals.

In the foreleg, the proximal joint spanned 70 ± 3 deg while the distal joint spanned 36 ± 3 deg. The hind proximal joint angle range averaged 53 ± 3 deg, while the range spanned by hind distal joint was 27 ± 2 deg. Additionally, the proximal and distal foreleg joint angle ranges were greater than those of the proximal and distal hindleg joints by 32% and 36%, respectively ($P < 0.001$).

The coefficient of determination (R^2) of the relationship between the femur–patella (proximal) and the tibia–metatarsus (distal) joint angles represented the coupling coefficient between the proximal and distal joint angles of each leg. For example, if the changes in hemolymph pressure occurred simultaneously, resulting in maximally coupled hydraulic joints within a leg, then R^2 would

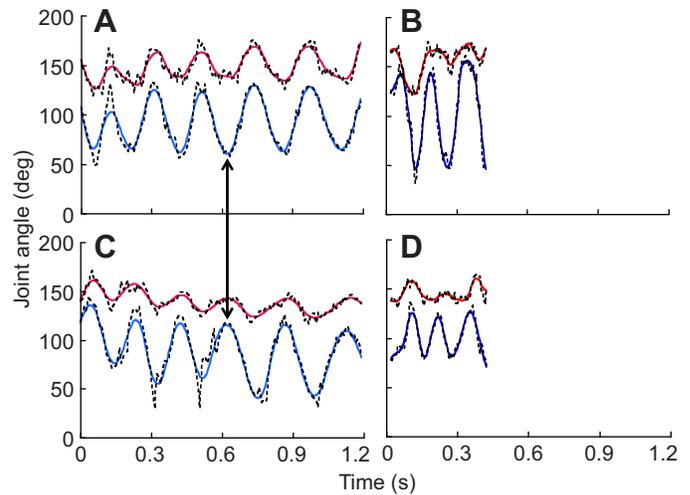


Fig. 4. Representative joint angle changes during sprinting for the proximal and distal joints at 19 and 39°C. Proximal joints are shown in blue; distal joints are shown in red. Dashed lines represent unfiltered joint angles, while solid lines represent the filtered joint angles. (A,B) Kinematics of the foreleg at 19 and 39°C, respectively. (C,D) Kinematics of the hindleg at 19 and 39°C, respectively. The vertical double-headed arrow indicates the counterphase relationship between the foreleg and hindleg.

equal 1.0. For each leg in the tarantula, the proximal–distal joint angles were more tightly coupled at lower temperatures than at higher temperatures during (Figs 5, 6). The foreleg joints were more tightly coupled than the hindleg joints at each temperature (Figs 5, 6). As temperature and running speed increased, the proximal–distal coupling coefficient decreased similarly in the forelegs and hindlegs (Fig. 6). For the foreleg, the proximal–distal coupling coefficient was highest at the lowest temperature (16.8°C ; $R^2 = 0.73 \pm 0.06$) and

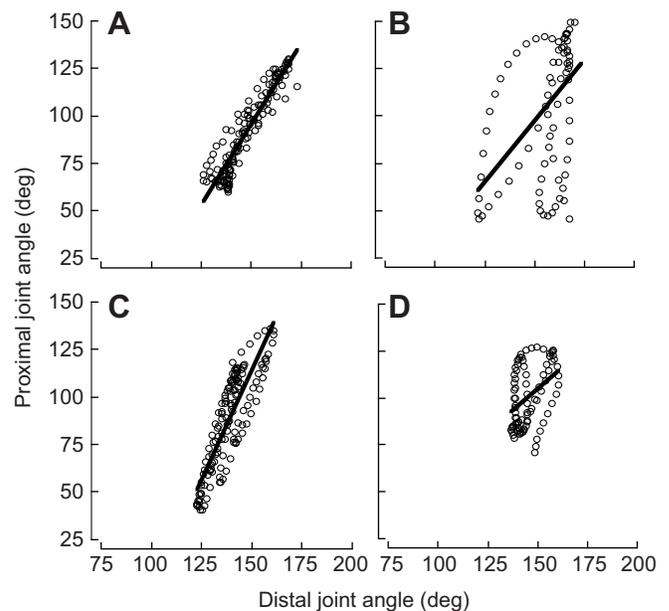


Fig. 5. Coupling of the proximal–distal joint angles at 19 and 39°C for the same trials shown in Fig. 4. Lines represent linear regressions of the proximal and distal joint angles for each trial. R^2 was used as a measure of the coupling coefficient between the proximal and distal joints within each leg. (A,B) Coupling of the forelimb joints at 19 and 39°C, respectively. (C,D) Coupling of the hindlimb joints at 19 and 39°C, respectively. Proximal–distal coupling coefficients were (A) 0.87, (B) 0.27, (C) 0.73 and (D) 0.17.

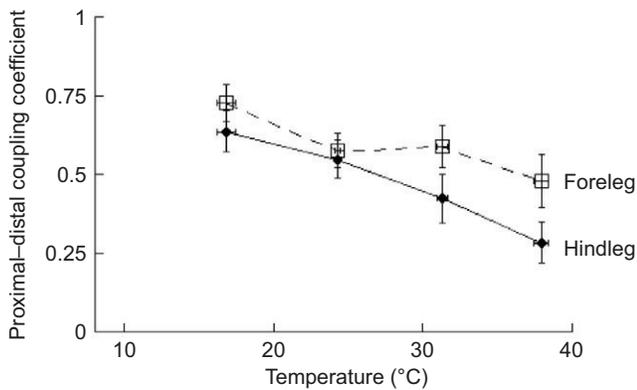


Fig. 6. Coupling coefficient decreases with temperature for both legs. The degree of proximal–distal joint coupling, represented by the coupling coefficient or R^2 , decreased for both legs as temperature increased, but less for the foreleg than for the hindleg.

was lowest at the high end of the temperature range (37.9°C; $R^2=0.48\pm0.09$). For the hindleg, the proximal–distal coupling coefficient was 0.63 ± 0.07 at 16.8°C, decreasing to 0.28 ± 0.07 at 37.9°C (Fig. 6).

DISCUSSION

At higher temperatures, the tarantulas sprinted faster (Fig. 3). As was expected for such large spiders, stride frequency and animal speed increased with temperature, whereas stride length did not vary significantly with temperature (Fig. 3; Anderson and Prestwich, 1985). For the current study, temperature was used to modulate a single behavior, maximal sprinting, in the tarantulas. Higher coupling between the two hydraulically extended joints at low temperatures rejects hypothesis 1 (Figs 5, 6). Reduced kinematic coupling of the joints at the lower temperatures would have suggested that hemolymph viscosity limited joint extension as a result of the increased resistance to flow to the distal joint of the legs. Instead, as temperature and stride frequency increased, the proximal and distal hydraulically extended, in-series joints of the forelegs and hindlegs in the tarantulas were less tightly coupled (Figs 5, 6), which supports hypothesis 2. Stride frequency may limit the time available for hemolymph to flow completely into and out of the leg.

The kinematic output of the legs and joints (stride length and extension fraction, respectively) remained constant with temperature during sprinting in the tarantulas (Fig. 3). Although some spiders increase stride length during running (Foelix, 2011; Spagna et al., 2011), only stride frequency increased as temperature increased in these large-bodied tarantulas when running maximally (Biancardi et al., 2011; Fig. 3). The constant extension fraction, despite changes in speed, indicates that the neural pattern (or central pattern generator) to control sprinting remains constant, while only the rate of pattern changes with temperature. Sprinting seems to be a single maximal effort behavior produced from a consistent neural pattern, resulting in a single sprinting gait.

During sprinting in the tarantulas, the range of angles used by the joints was always greater in the proximal femur–patella joints (50–70 deg) than in the distal tibia–metatarsus joints (26–36 deg) of the spider legs (Fig. 4). The proximal joints spanned more of their maximum range of motion than the distal joints, given the maximum ranges of 160 deg for the proximal joints and 125 deg for the distal joints in *Cupiennius* spiders (Foelix, 2011). As the muscles within the prosoma (also known as cephalothorax)

generate the pressure to move the hydraulic joints, the proximal joint experiences greater pressures than the distal joints of each leg, as hydraulic pressure will decrease with distance along a leg (Anderson and Prestwich, 1975). Subsequently, lower hydraulic pressures will be available to extend the distal joints, likely resulting in smaller changes in the distal joint angle during running (Fig. 4).

The proximal and distal hydraulic joints of a leg were most tightly coupled at the lowest temperature, suggesting that hemolymph is not too viscous to flow into and out of the entire leg when relatively cool (Fig. 5). Decreased coupling at higher temperatures suggests the hydraulic extension mechanism is limited at high temperatures and faster speeds because hemolymph movement through the length of the leg and internal volume changes may be not fast enough. If the viscosity of hemolymph changed like that of water, then the viscosity of hemolymph would be 35% more viscous at 20°C than at 40°C (Kestin et al., 1978). Even though hemolymph viscosity may be much lower at higher temperatures, at faster speeds, movement in the distal joint is less tightly controlled, although still rhythmic (Figs 4, 5; see Results).

The forelegs and hindlegs of the tarantula move oppositely, but function in stance together during running (Fig. 4). While the joints of the foreleg move counterphase to those of the hindlimb, forelegs and hindlegs are in stance phase together (vertical double-headed arrow comparing Fig. 4A,C). Joints of the foreleg extend during its swing phase; they then flex to likely pull the animal's center of mass forward (Figs 2, 4). At the same time, joints of the hindleg flex during its swing phase, then extend to push the animal's center of mass forward. Even though the foreleg joints flex and the hindleg joints extend during the stance phase, proximal–distal joint coupling decreased in both legs at faster running speeds (Fig. 6). A future experiment might test the hypothesis that both forelegs and hindlegs contribute to generate thrust forces, where the foreleg pulls while the hindleg pushes. Alternatively, the forelegs of the tarantula might decelerate its center of mass like the forelegs of insects during running (Full et al., 1991).

The current study did not consider the possible effects of desiccation and drier air at increased temperatures that could negatively affect the hydraulic mechanism by increasing the hemolymph osmolality (Punzo, 1991). Although the composition of tarantula hemolymph is known (Schartau and Leidescher, 1983; Punzo, 1989), the temperature effects on hemolymph viscosity are less well understood, including the extent to which hemolymph viscosity is affected at lower and higher temperatures. Although antifreeze proteins likely do not influence hemolymph viscosity at 15°C, ectotherms with hydraulically extended limbs may similarly use proteins to regulate hemolymph viscosity over the 25°C range of temperatures that changes water viscosity by >35% (Husby and Zachariassen, 1980; Duman et al., 2004; Kestin et al., 1978).

Coupling of the two in-series hydraulic joints, or the lack thereof, may be associated with factors other than the cycle frequency of the limbs during running. For example, changes in muscle activity patterns might vary the stiffness of one joint differentially compared with the other joint within a single leg. Although the joints extend as a result of hemolymph pressure, if the flexor muscles of the distal versus proximal joints activated differentially to modulate joint compliance, then the joints would not extend synchronously. For the current study, we assumed the muscle activity pattern for the maximal sprints across temperature would remain constant. However, future studies might test the hypothesis that the flexor muscles cause the uncoupling of the proximal–distal extension at faster running speeds.

Even without differential changes in muscle activation, mechanical forces may cause changes in joint coupling during running. For example, mechanical coupling across the animal during stance may cause irregularities of the observed limbs. Also, ground reaction forces increase with speed during terrestrial running (Biewener, 2003). However, ground reaction forces have yet to be measured on an animal that maximally sprints at different speeds as a consequence of temperature changes. Nevertheless, the higher ground reaction forces may influence distal and proximal joint moments differentially at different speeds to result in kinematic changes.

The two hydraulically extended joints were least coupled at the highest temperature when the animals ran the fastest with the highest stride frequencies. The hydraulic mechanism may hinder joint extension at very high stride frequencies, suggesting an upper limit to how fast they can extend their legs using hydraulics and perform essential cyclic activities like running or prey capture. In addition to spider ecology, the design of multi-legged walking machines has incorporated biologically inspired hydraulically extended joints (Menon and Lira, 2006; Berring et al., 2010). The advantages of minimizing space and lowering leg mass of hydraulically extended joints in a leg system may be offset by the potential disadvantage of reduced control at faster stride frequencies. The coordination of multiple, in-series hydraulically actuated joints may be limited by operating speed.

MATERIALS AND METHODS

Animals

Eight adult, female Texas Brown tarantulas (*A. hentzi*) (Fig. 1A) were purchased from Carolina Biological Supply (NC, USA). Tarantulas were housed individually in 38 l glass terraria with a sand substrate, a covered shelter and *ad libitum* access to water. Each animal was fed two adult crickets per week. The terraria were kept in a constant-temperature room (23±2°C). The room had overhead fluorescent lighting, and each terrarium had an overhead 60 W incandescent light bulb for radiant heat. The terraria offered tarantulas a thermal gradient of ~23–40°C during the photophase of a 9 h:15 h light:dark cycle.

Varying temperature to vary running speed

To examine different running speeds, we elicited maximal running performance at four nominal temperatures (15, 24, 31 and 40°C) using a temperature-controlled environmental chamber (Percival Scientific Inc., Perry, IA, USA). These temperatures included the maximum range at which the tarantulas moved in preliminary trials and also encompassed the range of body temperatures recorded in the congener *Aphonopelma anax* (Shillington, 2002).

Prior to each trial, the tarantula remained in the environmental chamber for at least 60 min to equilibrate its body temperature. We then stimulated the tarantula to begin running by directing an exhaled puff of air on to the posterior end of its opisthosoma (Parry and Brown, 1959). Tarantulas sprinted along a wooden runway (46×81 cm) with one side wall; the runway surface was covered with coarse sandpaper to ensure good traction. Each trial included 1–4 successive excursions along the runway, until we obtained a clear video record of at least three full strides in a straight direction, in which the spider did not touch the runway's side wall. Immediately after each trial, we measured the surface T_b of the animal with an infrared thermometer (Raytek Raynger, Santa Cruz, CA, USA). Surface T_b was used as an approximate measure of internal T_b , as small animals have very low thermal inertia (Bell, 1980; Fraser and Grigg, 1984; Stevenson, 1985; Shillington, 2002). Surface T_b for the four nominal temperatures was 16.8±0.6, 24.3±0.2, 31.3±0.4 and 37.9±0.5°C, respectively ($N=8$).

Joint and body kinematics

We video recorded sprinting trials with a high-speed camera (Allied Vision Technologies, Pike model, Stadroda, Germany). Recordings were made at 120 or 208 frames s⁻¹ to obtain 20–50 frames per stride. The camera viewed

the runway laterally, and a mirror was positioned at a 45 deg angle above the runway to provide a simultaneous dorsal view, allowing for three-dimensional (3D) reconstruction of the leg movements (Fig. 2).

We painted four white landmark points on the first (fore) and fourth (hind) legs of the right side of each animal: the midpoint of the femur, femur–patella (proximal joint), tibia–metatarsus (distal joint) and metatarsus–tarsus (Fig. 1). These four points on the legs allowed the determination of the femur–patella (proximal) and tibia–metatarsus (distal) joint angles during running (Fig. 1B). Movement in the patella–tibia joint was assumed to be minimal in the anterior–posterior–vertical plane and to not contribute significantly to running in the forelegs and hindlegs (Foelix, 2011). The forelegs and hindlegs were analysed because they were most clearly seen during videotaping to provide the most complete joint kinematics. Also, including both forelegs and hindlegs allowed for assessment of possible differential function of two legs positioned in opposite directions.

At the beginning of each trial or set of trials, we recorded an image of a 15-point spatial calibration frame (59.8×24.3×8.2 cm), which covered the entire area of the runway visible in the camera's field of view. 3D coordinates of each joint were determined using MATLAB™ (version R2010b, MathWorks Inc., Natick, MA, USA; dlt Data Viewer Five software, Hedrick, 2008). The 3D (XYZ) positions for the eight anatomical points on each spider were used to calculate joint angles, using a derivation of the geometric interpretation of a dot product. 3D positions were filtered with a zero-lag fourth order low-pass Butterworth filter using a cut-off frequency based on residual analysis (1.5 times the stride frequency; Winter, 2005).

Filtered joint kinematics were used to calculate several other variables using MATLAB™ programs. Joint angle ranges and stride duration were calculated from joint angle maxima and minima. Average stride frequency for each trial was determined by dividing the total number of strides by the duration. Stride length was determined by calculating the distance between the positions of the metatarsus–tarsus joint at the beginning of two successive strides. Average speed for each trial was calculated as the product of stride frequency and stride length. Extension fraction (the proportion of the stride cycle during which extension is occurring) was calculated as the duration of joint extension divided by the stride duration. Extension of the joints of the first leg did not necessarily correspond to the stance phase of the gait cycle. Therefore, extension fraction was the most direct measure of the hydraulically generated extension for both legs.

We calculated the coefficient of determination (R^2) of the relationship between the femur–patella (proximal) and the tibia–metatarsus (distal) joint angles over 2–6 strides to represent the coupling coefficient between the time-varying proximal and distal joint angles. For example, if the two joint angles extend and flex together and are maximally coupled within each leg during running, then R^2 would be close to 1.0. A high coupling coefficient between the proximal and distal joints would be expected if the changes in hemolymph pressure that extend both joints occurred simultaneously. In contrast, reduced coupling would be reflected by lower values of R^2 .

Statistics

All values are reported as means±s.e.m. We conducted statistical tests using Minitab (v. 15.1, Minitab, Inc., State College, PA, USA). We used repeated measures ANOVA (using individuals as a random-effects blocking factor) to test response variables across temperatures, between forelegs and hindlegs, and between proximal and distal joints.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

N.A.B., A.N.A. and S.C.A. conceived of and designed the experiment. N.A.B. and F.Y.S. executed the experiment and analysed the data. F.Y.S. and A.N.A. generated

the figures. A.N.A. interpreted the findings. N.A.B. and A.N.A. drafted the manuscript. A.N.A. and S.C.A. revised the manuscript.

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