



Jump takeoff in a small jumping spider

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Abstract

Jumping in animals presents an interesting locomotory strategy as it requires the generation of large forces and accurate timing. Jumping in arachnids is further complicated by their semi-hydraulic locomotion system. Among arachnids, jumping spiders (Family Salticidae) are agile and dexterous jumpers. However, less is known about jumping in small salticid species. Here we used *Habronattus conjunctus*, a small jumping spider (body length ~4.5 mm) to examine its jumping performance and compare it to that of other jumping spiders and insects. We also explored how legs are used during the takeoff phase of jumps. Jumps were staged between two raised platforms. We analyzed jumping videos with DeepLabCut to track 21 points on the cephalothorax, abdomen, and legs. By analyzing leg liftoff and extension patterns, we found evidence that *H. conjunctus* primarily uses the third legs to power jumps. We also found that *H. conjunctus* jumps achieve lower takeoff speeds and accelerations than most other jumping arthropods, including other jumping spiders. *Habronattus conjunctus* takeoff time was similar to other jumping arthropods of the same body mass. We discuss the mechanical benefits and drawbacks of a semi-hydraulic system of locomotion and consider how small spiders may extract dexterous jumps from this locomotor system.

Keywords Jumping · Salticidae · Biomechanics · Locomotion · Markerless automated pose estimation

Introduction

Animals use legs to exert forces on their physical world. Legs are used in a dizzying array of contexts, including walking, climbing, manipulating objects, predatory attacks, and communication. A particularly interesting context in which to explore the use of legs is in jumping. Jumping is a form of burst locomotion that requires large forces to be produced quickly and synchronized precisely (Biewener and Patek 2018). For a jumping specialist, optimizing the magnitude and timing of these forces can be crucial from a fitness perspective. A “good” jump allows an animal to navigate a tricky gap in terrain, capture a meal, or escape a predator. A “bad” jump could quickly lead to death (Crompton and Sellers 2007).

Jumping in animals often follows a ballistic model, in which the animal is propelled through the air by forces

generated by the legs at takeoff (Biewener and Patek 2018). The main sources of variation seen in animal jumping behavior and performance arise from variations in the force production mechanisms. These include the leg, and hence muscle length, and from variations in the body size or mass that is moved (Burrows and Sutton 2008; Sutton et al. 2016). Some animals use more complex mechanical systems, which store the energy produced by muscles and then release it far more quickly than normal muscular contraction would allow. These mechanisms of energy storage and rapid energy release are referred to as catapult mechanisms or as power amplification systems (Brackenbury and Hunt 1993; Burrows et al. 2008; Burrows 2009; Nabawy et al. 2018). Catapult mechanisms are ideally suited for jumping and are often used by arthropod jumpers (beetles, fleas, springtails, etc.) (Biewener and Patek 2018). The exception are arachnids, one of the few groups of arthropods which have never been shown to use catapult mechanisms in jumping (Nabawy et al. 2018). The only use of power amplification in arachnids has been described in the ultra-fast predatory strikes of trap-jaw spiders (Wood et al. 2016).

A lack of catapult mechanisms in jumping arachnids may be unsurprising because spiders are “something of a law unto themselves” (Vogel 2005). That is, the arachnid

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model of locomotion is quite different from other arthropods. Most animals (arthropods included) use two antagonistic sets of muscles to generate different movements: one to flex and another to extend the legs. In the canonical model of arachnid locomotion, legs only have flexors, and extension is accomplished with hydraulic pressure. To extend their legs, spiders contract muscles in the cephalothorax (head) generating a local increase in hemolymph pressure. This local increase in pressure is then transmitted via the circulatory system to compartments in the legs and joints. Within a joint, the volume increases, which rotates the joint and extends the leg (Parry and Brown 1959; Kropf 2013; Liu et al. 2019). This system is known as a semi-hydraulic system of locomotion, given that hydraulic pressure is used to extend, but not flex, the legs. This system is unique to arachnids (Spagna and Peattie 2012; Kropf 2013), and may use hydraulic principles, such as Pascal's law, for generating a mechanical advantage for jumping *in lieu* of catapult mechanisms. Much about semi-hydraulic locomotion is currently unknown, including the control of hydraulic pressure, how pressures are directed to particular legs and the extent to which leg flexors aid in or control differential leg extension (Weihmann et al. 2010; Booster et al. 2015). It remains unclear how the timing of pressure generation and transmission is modulated to generate both the slow forces for normal walking and rapid forces for jumping. However, understanding the patterns of leg use, speed and acceleration profiles during jumping may provide some insight into the force generation capabilities of a semi-hydraulic locomotion system.

Despite an unusual locomotion system and lack of catapult-assisted jumping, spiders in the family *Salticidae* are known for their jumping abilities. This large family encompasses over 6000 described species which range from about 1–25 mm in body length (Ubick et al. 2017). Surprisingly, jumping kinematics have only been studied in a few species, and mostly in large species such as *Phidippus* (body length = 15 mm) which only jump occasionally (Hill 2010; Chen et al. 2013; Nabawy et al. 2018). In this study, we explore jumping in a small jumping salticid, *Habronattus conjunctus* (Banks 1898) (body length = 4.5 mm). Spiders in the genus *Habronattus* has been studied extensively for their courtship behavior (Elias et al. 2012; Brandt et al. 2018), thermal physiology (Brandt et al. 2020), and biogeography (Maddison and McMahon 2000; Hedin and Lowder 2009; Hedin et al. 2020). *Habronattus* spiders are also known as jumping specialists, as they use jumping as their main means of locomotion and are eager and dexterous jumpers. We tracked the kinematics of spiders as they jumped between two platforms. Tracking tiny body parts in small spiders is often challenging, so we used DeepLabCut, a software package that allows for markerless pose estimation and uses deep neural networks to quickly and accurately

track different points on the animal as identified by the user (Mathis et al. 2018). This system has been used effectively to track poses in a variety of animals, but less commonly with large numbers of points, especially on small arthropods (Draft et al. 2018; Singh et al. 2019). The focus of this study was therefore twofold: first, we sought to characterize the kinematics and leg extension patterns of *H. conjunctus* jumps, especially in a comparative context with other jumping spiders and jumping arthropods generally. Second, we wanted to test the viability of using DeepLabCut to track large numbers of points on a small rapidly moving animal.

Materials and methods

Animal collection and maintenance

We collected spiders in spring 2013 from the Santa Rita Mountains in Pima County, Arizona, USA, on unceded lands of the Tohono O'odham and Hohokam peoples. No special permits were required to collect animals from these lands. We maintained animals in the lab in individual plastic containers (AMAC, Petaluma, CA, USA) with window screen provided for enrichment (Carducci and Jakob 2000). We kept spiders on a 12:12 light cycle at a constant temperature of approximately 24 °C. We fed animals weekly with *Drosophila melanogaster* and provided water as needed. Animals were never fed less than 24 h prior to any experiment.

Spider jumping trials

We elicited directed jumps (*sensu* Weihmann et al. 2010), in which the animals jumped between two platforms (Fig. 1). The experimental setup had two dowels 8 mm in diameter fixed on-end to a piece of sandpaper. Cardboard pieces topped with sandpaper (3 cm²) were glued to the top of the dowels. The dowels were either placed to leave gaps of 4 cm ($n = 4$ jumps), 5 cm ($n = 5$ jumps), or 6 cm ($n = 3$ jumps) for animals to jump across. These gaps represented ~9–15 body lengths for the spiders. We captured jumps with a high-speed camera (Fastcam SA3, Photron USA, Inc., San Diego, CA, USA) and a 105 mm 1:2.8 DG macro lens (Sigma Corp. of America, Ronkonkoma, NY, USA). We used a frame rate of 1000 fps, a shutter speed of 1 frame/second and a post-trigger mode. The camera was aligned perpendicularly relative to the platform as closely as possible. This was accomplished by measuring the angle with a protractor and weighted string. Two fluorescent light bulbs illuminated the arena for each trial. In order to elicit a jump from a spider, we placed the spider on the left-hand platform and encouraged it to jump by gently brushing behind the animal with a small dry paintbrush. We filmed jumps from a total of 11 individuals. Of the trials included in the analysis, only one

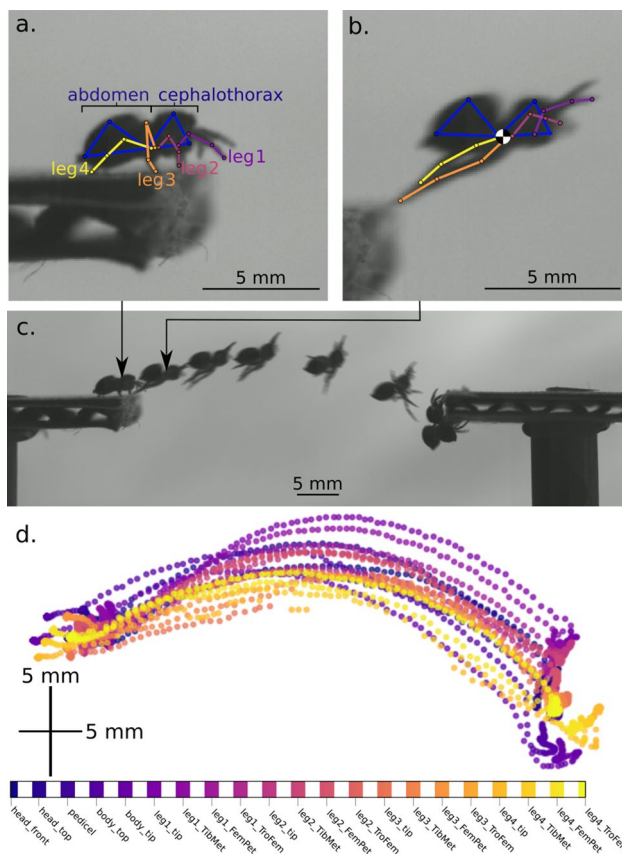


Fig. 1 Schematic of DNN based automatic tracking in the jumping specialist *Habronattus conjunctus*. **a** Illustration showing spider immediately before jump takeoff. Body parts and the 21 points used for tracking and skeleton are labeled. Only the right legs are labelled **b** Illustration of spider completing takeoff phase of jump, with tracking points and skeleton shown **c** View of an entire jump. Frames were selected that were representative of each jump stage. **d** Trajectory of each tracking point through space during a jump. Total duration for this jump was 660 ms

was unsuccessful (did not land on the opposing platform). Since we were mostly interested in the takeoff phase for this study, and because the failed jump looked similar to the others in this takeoff phase, we included the failed jump in this dataset as well.

Video labeling and tracking with DeepLabCut

We used DeepLabCut version 2.2b5 (Mathis et al. 2018) to extract kinematic data from jumping videos. DeepLabCut is an open-source software package for markerless pose estimation. The software allowed us to train deep neural networks to identify the body parts of interest using a small training set of video frames, ultimately creating fully labelled and tracked videos. We first cropped and trimmed the videos to only encompass the time from when movement started until the spider landed on the opposing platform. We

also modified the contrast and saturation to better distinguish between the various body parts of the spiders. Approximately 50 frames were then selected through k-means clustering for the training data set, as recommended by the user manual. Frames were taken from two videos (from a total of 568 frames), and manually labelled. We tracked a total of 21 points, representing the abdomen, cephalothorax, and leg joints. Specifically, we labelled and tracked the front and top of the cephalothorax (head_front, head_top), the tip and top of the abdomen (body_tip, body_top), and pedicel joint (assumed to correspond roughly to center of mass). On each of the right four legs, we tracked the tip, tibio-metatarsal, femoro-patellar, and trochantro-femoral joints. We pinned each trochantro-femoral joint to a fixed position along the axis formed from the pedicel joint to the point at the front of the cephalothorax. See Fig. 1 for schematic of tracked points. As jumps were recorded from one sideview camera, only joints on the right legs, which faced the camera, were tracked.

We used the labelled frames to create a training dataset for the deep neural network (DNN). The DNN was trained for 250,000 iterations using the pre-trained ResNet-50 network for about 6 h. We analyzed and labelled 31 jumping videos using median-filtered predictions. Because the image contrast between leg parts and body was low, the model network had trouble tracking various joints when the legs overlapped in the plane of view. For the next iteration, we continued with 15 jumps where the takeoff phase was clearly visible and remained within the narrow focal plane. We excluded 15 videos from the dataset because this phase was either blurry or occluded. The model was retrained with user-labelled frames chosen specifically with high overlap. In addition to the 50 initial frames, we now manually selected and added labels to ~100 more frames during the takeoff and aerial phases from eight additional videos (from a total of 2586 frames). As our analysis focused primarily on the takeoff phase of the jump, we selected frames to optimize tracking of this phase. The neural network was then retrained for 300,000 iterations from default weights using the ResNet-50 network for about 7 h. The retrained network was now more generalizable. We analyzed and labelled 15 jumping videos using median-filtered predictions. We present data from twelve jumps (three videos were removed due to inaccurate tracking). See SI2 for an example video of a labeled jumping trial.

Analysis of kinematics

Trajectory data were filtered and analyzed using Matlab version 2019a (Mathworks Inc., Natick, MA, USA). Statistical analyses were performed in R (R Core Team 2020) and figures generated using ggplot2 in R (Wickham 2016). All data are shown with time normalized such that $t=0$ ms

corresponds to peak acceleration of each jump. $N = 12$ jumps for all analyses. We first linearly interpolated the data to more accurately pinpoint the precise liftoff times of each of the legs. Specifically, we used the griddedInterpolant function in Matlab to insert ten equally spaced time-points between each existing time-point. Next, we calculated speed and acceleration for the data series associated with the pedicel as the approximate center of mass for the animal (Chen et al. 1997) (Fig. 1). These speed traces were smoothed with a second order elliptical filter in Matlab to account for noise in the data introduced by small inaccuracies in tracking. The filter had a passband between 5.0×10^{-6} Hz and 55 Hz, 0.1 dB of ripple in the passband, and 60 dB of attenuation in the stopband. The filtered data were phase shifted by the filter, i.e. offset from the original speed trace by about 20 time-points. We compensated for this lag by using a cross-correlation function to find the appropriate lag for each trace and shifting it appropriately. Each filtered speed trace was compared visually to the unfiltered traces and found to align well. We then calculated acceleration as a differential of the filtered speed. We calculated other kinematic variables including kinetic energy ($1/2 \times \text{mass} \times \text{speed}^2$), jumping power (kinetic energy/takeoff time), mass-specific power (power/mass), takeoff angle, leg plane angle, and body angle for all jumps. Finally, we calculated drag coefficients and drag force according to Vogel (1996), assuming the spider as a sphere of diameter equal to body length.

Analysis of leg extension

We first determined liftoff time for each leg. We did this by drawing a polygon around the takeoff platform. The time-point at which each leg tip was no longer inside the platform polygon was noted. To determine the extent to which each leg was extended throughout the jump, we also calculated effective leg length (ELL) (Fig. 2a, b). Effective leg length was defined as the distance between the measured proximal and distal points for each leg and divided by the total leg length (Fig. 2a). To determine how closely leg extension patterns corresponded to acceleration, we measured when each leg was extended relative to the acceleration experienced by the spider during take-off. To remove transient noise in the data, we first filtered the leg extension traces with a low-pass filter that filtered any frequencies below 80 dB. This was a second-order low-pass elliptical filter, with 0.1 dB of passband ripple, and 60 dB of stop band attenuation. As before, we adjusted for any temporal shift in the filtered data. We then calculated the first derivative of the filtered leg extension traces to determine the rate of extension over time. Since the jump is powered by an unfolding leg delivering an upward force, we expect that the extension rate of the propelling leg will peak at about the same time as the force, and therefore the acceleration. To determine

which leg fits this best, we performed a cross-correlation analysis between a time-series depicting the leg extension rate of all four legs and the spider's normalized acceleration over the same period. This yielded a series of correlation coefficients (how closely the two time-series "match up"), for different lags (different temporal delays between the two time-series). Next, we measured the lag at which the two traces showed the highest correlation coefficient for each leg. We expected that legs that contribute more to takeoff would have a higher correlation coefficient, and a smaller more consistent lag. Since the extension of the leg was mostly driven by the unbending of the leg at a proximal joint, we also calculated the leg joint angle at this joint for each leg as the jump proceeded. Although spider legs consist of seven segments, we could only delineate three sections in the videos (Fig. 2c). We define leg joint angle as the angle between the first (proximal) and second section of the leg.

We note that leg lengths were measured in a single plane from one camera and are thus a 2D projection of the actual length. Any leg extension outside of this plane would therefore not be captured by these measurements. However, given that the jumps occurred in-plane (remained in focus throughout the video), we find it unlikely that significant leg extension would occur outside of this plane, and that these measurements are therefore a reasonable approximation of the actual 3D ELL.

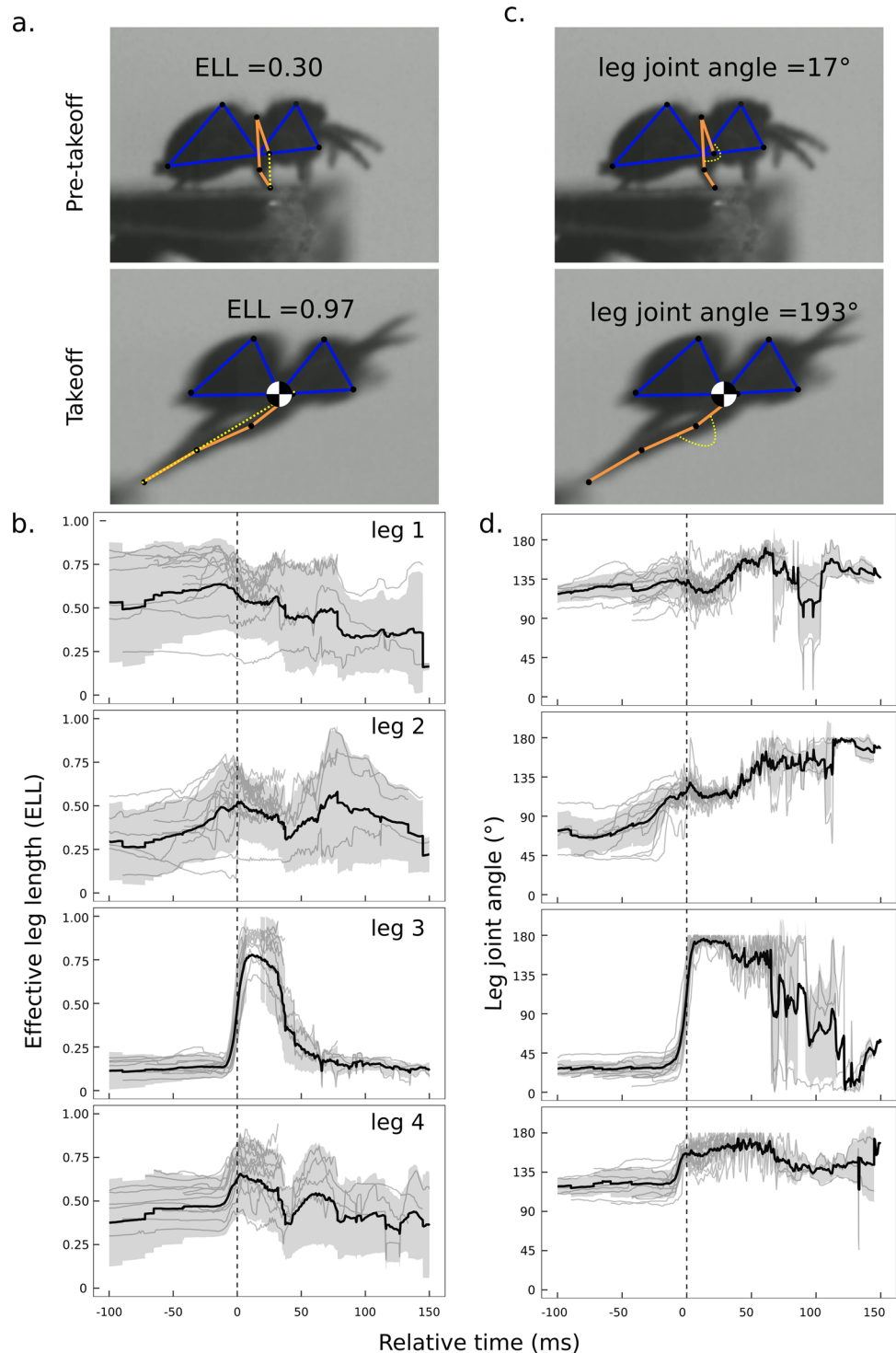
Statistical considerations

As the leg liftoff data deviated significantly from normality, we performed a paired Wilcoxon test to compare takeoff times of legs three and four. To test whether individual or jump distance had any bearing on key characteristics of the jumps, we ran a series of linear models to test these factors' effect on peak acceleration. Neither factor had any effect on peak acceleration, so we considered all the jumps together. The dataset also contained a mix of mature male and mature female individuals. We had no a priori expectations that sex would significantly affect jump performance, so we grouped the sexes together in the analysis. *H. conjunctus* are dimorphic with respect to body mass, (overall mass: 14.9 ± 5.2 mg; female: 18.1 ± 5.1 mg; male: 11.6 ± 2.8 mg), but there is no dimorphism seen in body length or leg length. For a visual representation of the variation in jump variables due to distance and body angle, leg plane angle, and takeoff angle see SI1-1.

Metanalysis on jumping performance

To compare *H. conjunctus* jumping performance with that of other jumping spiders and other jumping arthropods more generally, we used data from Nabawy et al. (2018) to perform a metanalysis. For the purposes of this analysis, we

Fig. 2 Effective leg lengths (ELL) and leg angles of *H. conjunctus* during jumps. ELL was calculated as a ratio between the distance between the proximal and distal leg joints, and the entire leg length. Leg angles were calculated between proximal and middle sections of the leg. **a** Example of a low ELL in a spider pre-takeoff, with a leg mostly flexed, and example of high ELL in a spider post-takeoff with leg mostly extended. **b** ELL over the course of the jump for each leg. Traces are aligned so that $t=0$ s corresponds to peak acceleration of the center of mass, indicated by vertical dashed line. For each time-point, an ELL value of 1 indicates that a leg is fully extended. Traces for every individual jump are shown in grey, a mean trace in black, and the lighter grey polygon indicates \pm standard deviation. **c** Example of a low leg joint angle in a spider pre-takeoff, and a large leg joint angle post-takeoff. **d** Leg joint angle over the course of the jump for each leg. Traces are aligned so that $t=0$ s corresponds to peak acceleration of the center of mass, indicated by vertical dashed line



calculated speed and acceleration following Nabawy et al. (2018). Briefly, we calculated speed as the speed at time of takeoff (when the last leg left the platform). We also measured takeoff time, which is the duration from when

acceleration first increased above zero to time of takeoff. We calculated acceleration as takeoff speed/takeoff time. We calculated g-force as acceleration/ 9.81 m/s^2 . For the sake of

this analysis, we did not include any arthropods that use a catapult system to aid in jumping.

Results

Take-off kinematics

On average, spiders reached a peak speed of 0.62 ± 0.16 m/s ($N=12$ for all analyses) (Fig. 3a). The lowest peak speed of any jump was 0.41 m/s, whereas the highest peak speed achieved in any jump was 0.88 m/s. Although this value varied between jumps, the progression of the spider's speed *during* jumps was remarkably similar between jumps, suggesting a high degree of stereotypy (Fig. 3a). In terms of their

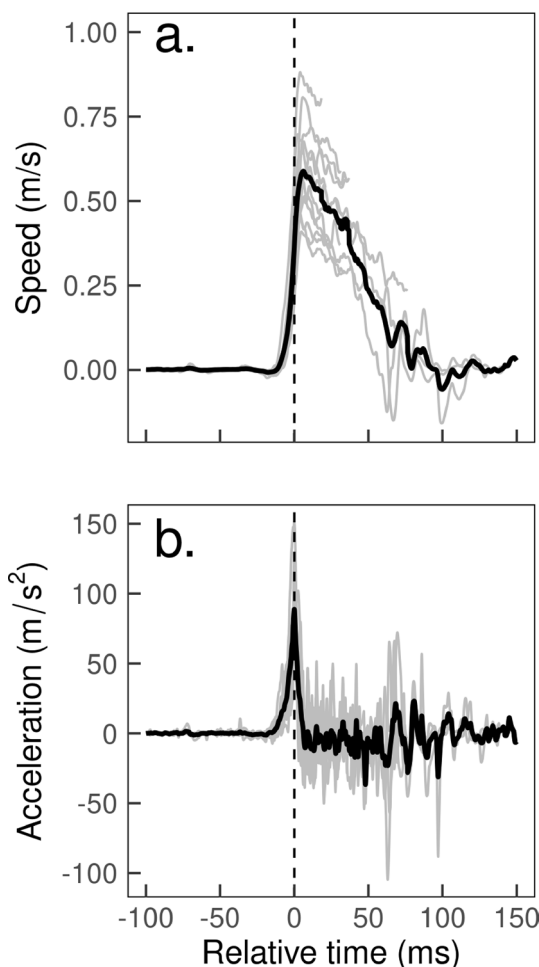


Fig. 3 Speed and acceleration of *H. conjunctus* center of mass. **a** Speed traces for every jump (grey lines) along with mean trace for all jumps (black line). Speed data were filtered using a bandpass filter (see text for details). **b** Acceleration traces for every jump (grey lines) along with mean trace for all jumps (black line). Traces are aligned so that $t=0$ s corresponds to peak acceleration, indicated by vertical dashed line

body length, given their small size, the spiders were able to achieve takeoff speeds of 120.65 ± 28.0 body lengths/s. On average, spiders reached a peak acceleration of 92 ± 28.23 m/s² (Fig. 3b). The lowest peak acceleration of all jumps was 66 m/s², whereas the highest peak acceleration achieved in any jump was 151 m/s². The shape of acceleration curves was less conserved than speed, but all jumps achieved a peak about halfway through takeoff time. Spiders achieved kinetic energies of 2.27 ± 1.23 μ J, jumping powers of 0.13 ± 0.07 mW, and mass-specific jumping powers of 9.11 ± 4.75 W/kg. Finally, with respect to aerodynamics, we found that drag forces on spiders were $2.60 \times 10^{-3} \pm 2.33 \times 10^{-3}$ mN, with drag force equaling $2.59 \pm 0.96\%$ of body weight on average.

Leg use during jumping

All *H. conjunctus* jumps showed the same pattern of leg liftoff during jumps (Fig. 4). In every jump, the pattern of leg liftoff was second, then fourth, and then third. The first legs were always raised off the platform before the jump began. Third leg liftoff was always closely associated with the time at which peak acceleration was reached (mean third leg liftoff time = 2.55 ms after peak acceleration). Fourth leg liftoff also occurred near peak acceleration (mean fourth leg

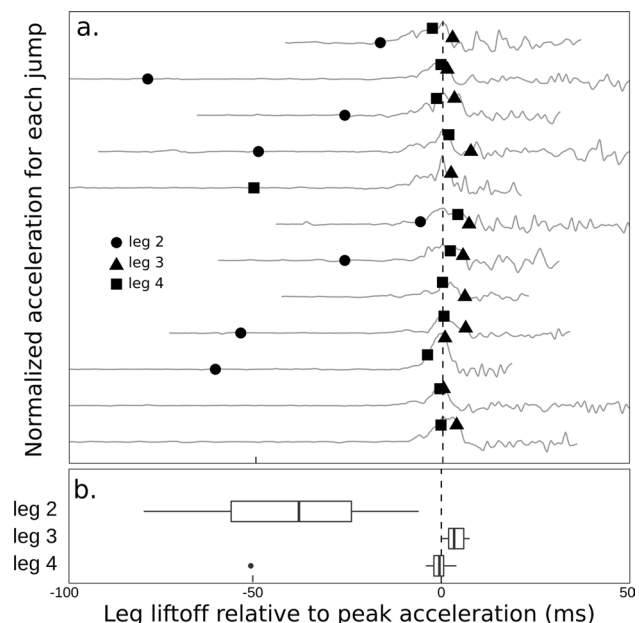


Fig. 4 Timing of leg liftoff as it relates to peak acceleration. **a** Each row shows normalized acceleration trace for an individual jump with the peak acceleration aligned vertically. Points indicate the time-point at which legs 2, 3, and 4 left the platform. Leg 1 was never on the platform for any jump, and leg 2 was not on the platform for two of the jumps. **b** Boxplot indicating overall time of leg liftoff across all jumps relative to peak acceleration. In both panels, data are aligned such that $t=0$ s corresponds to peak acceleration of the center of mass, indicated by vertical dashed line

liftoff time = 5.67 ms before peak acceleration). Third and fourth legs both showed low levels of variation in timing, but third and fourth leg liftoffs were always distinct from one another (Wilcoxon paired test, $V=78$, $P<0.001$) (Fig. 4b). In 10/12 of the jumps, the third leg left the platform shortly after peak acceleration. In 8/12 jumps, the fourth leg left the platform immediately before peak acceleration, i.e. the spider continued accelerating despite the fourth leg no longer being able to exert a force on it. There was a great deal more variation in timing for the second leg liftoff, but it typically occurred well before acceleration exceeded zero (mean second leg liftoff = 41.1 ms before peak acceleration). When we compared the rate of change in ELL with acceleration using a cross-correlation analysis, we found that leg three had the highest correlation coefficient, at 0.94 ± 0.03 , followed by leg four at 0.92 ± 0.04 . Legs one and two had coefficients of 0.84 ± 0.08 and 0.86 ± 0.06 , respectively. The change in leg three's ELL peaked 1.36 ms before peak acceleration and had a low standard deviation of 0.68 ms. The equivalent lags for leg 1, 2, and 4 were -0.878 ± 3.05 ms, 1.85 ± 5.24 ms, and 0.375 ± 2.04 ms, respectively. See SI1-1 for plots of all coefficients and lags.

To quantify the degree to which legs extended during takeoff, we compared ELL and leg joint angle at 40 ms before peak acceleration and 14 ms after acceleration. This timeframe corresponded to the largest observed change in ELL and leg joint angle. Leg three exhibited rapid and highly stereotyped extension during the acceleration phase of the jump. It extended from 0.14 ± 0.05 ELL at -40 ms to a maximum of 0.83 ± 0.08 ELL at 14 ms. Leg four showed a similar, but less dramatic pattern, starting at 0.51 ± 0.10 and extending to 0.66 ± 0.15 . In contrast, leg one flexed slightly during this interval, from 0.65 ± 0.18 to 0.57 ± 0.14 . Leg two extended very slightly, from 0.39 ± 0.14 to 0.50 ± 0.14 . The patterns of leg angle change the first over time were largely similar and explained much of the change in ELL (Fig. 2d). The joint on leg 3 unfolded in a dramatic pattern and underwent a change of almost 150° over about 20 ms, whereas the same joint on leg 4 changed only about 45° over the same interval. Therefore, leg three not only left the platform last and extended more than any other leg, but it also extended quickly, corresponding closely with the time of peak acceleration.

Comparison between the jumps of *Habronattus conjunctus* and other arthropods

On average, *H. conjunctus* had low takeoff speeds (Fig. 5a) and g-forces (Fig. 5c) compared to other jumping spiders and other arthropods whose jumps have been measured. *H. conjunctus* had a mean takeoff speed of 0.540 ± 0.12 m/s. Their g-force on average was 3.615 ± 1.268 , and take-off time was 16.7 ± 3.11 ms. For the data reported by Nabawy

et al. (2018), the jumping spider *Phidippus regius* had a higher takeoff speed than *H. conjunctus*, with a mean speed of 0.95 m/s, and a mean g-force of 4.7. A similar pattern can be seen for takeoff time. *H. conjunctus* had a takeoff time of 16.70 ± 3.11 ms. *P. regius* had a takeoff time of 33.31 ± 3.65 ms. This difference is likely due to the difference in weight (*P. regius* weighs about $10\times$ more than *H. conjunctus*). However, when looking at data points for individual jumps, *H. conjunctus* jumps have velocities that are both above and below those found in *P. regius*. Of the other two species measured, *P. princeps* had jump speed and acceleration similar to *P. regius*, whereas *Sitticus pubescens* had measurements within the range of *H. conjunctus*, likely because of similar body weight. The mean takeoff angle for *H. conjunctus* was 22.28° with a large standard deviation of 11.65° . This is very similar to takeoff angles achieved by *P. regius* when jumping between two platforms of equal height ($19.6 \pm 6.17^\circ$). Compared to insect jumpers, *H. conjunctus* are more similar in g-force to mantises and moths, which are not specialist jumpers, than to other jumping spiders (Fig. 5c). With respect to takeoff time, body size seemed to be the largest determinant across all animals. However, *H. conjunctus* seems to be on the low end of takeoff time for their body mass.

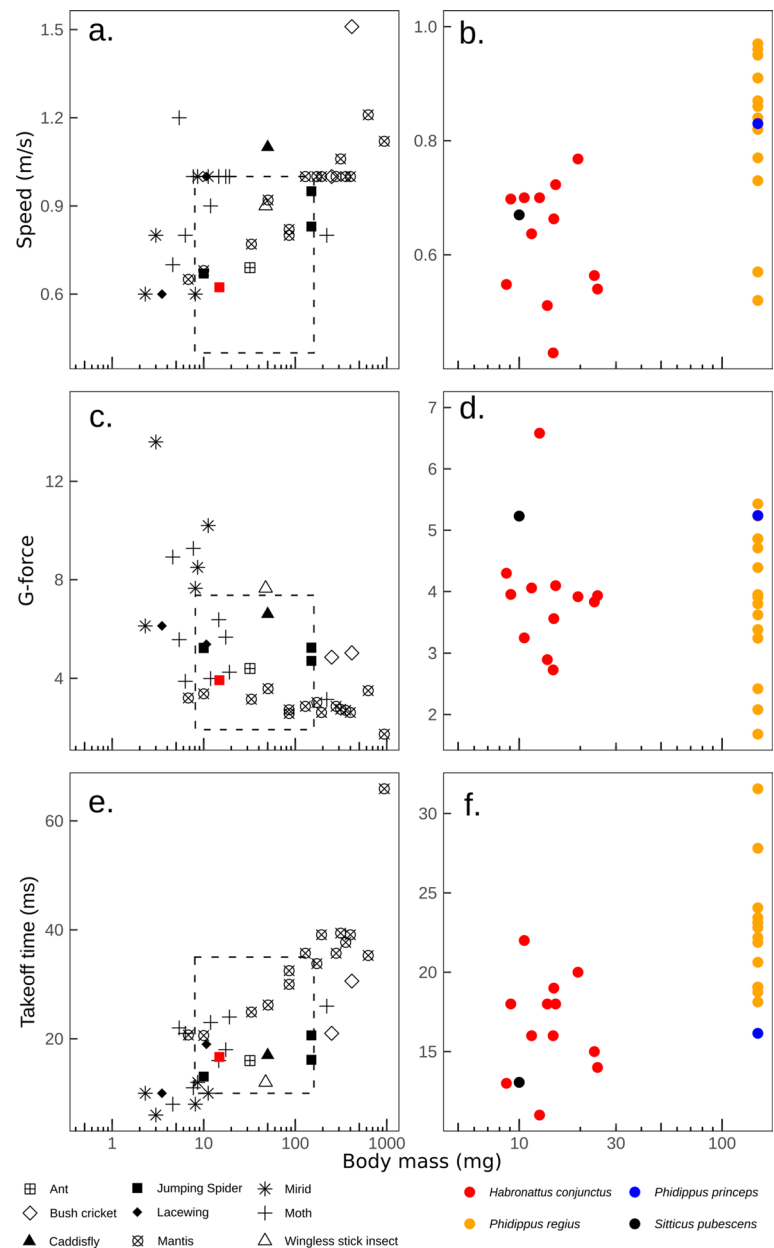
Discussion

Third legs are likely primarily responsible for powering jumps

In every jump, the third leg left the platform last (Fig. 4a). In other similar studies, it has been argued that the last leg to take off is the one that produces force (Hill 2010, 2018). However, the order of leg liftoff is not necessarily indicative of that leg exerting any ground reaction forces. Legs could be used for functions other than force production, such as aerial stabilization or righting (Zeng et al. 2017; Ribak 2020). Therefore, we also examined the extent and pattern of leg and joint extension to infer which leg is most likely to generate the ground reaction force that drives the jump. In every case, the third leg exhibited a fast, large-magnitude extension, with a very large and rapid change in joint angle during take-off that occurred just before the peak acceleration of the jump (Fig. 4c, SI1-1). Additionally, the spider body often (8/12 times) continued accelerating after the fourth legs had left the platform and could no longer generate forces. Therefore, we conclude that the third legs are primarily responsible for producing forces in jumps in *H. conjunctus*.

Third legs are known to be elongated compared to the fourth legs in *Habronattus* species (Griswold 1987; Blackburn and Maddison 2015). We found this to be the

Fig. 5 Comparison of jumping performance between *H. conjunctus* and other non-cata-pult-using jumping arthropods. All data points other than *H. conjunctus* were taken from Nabaway et al. 2018. **a** Speed as a function of body mass in an assortment of jumping arthropods. Red square indicates average value for spiders in the present study. Dashed box indicates region shown in panel (b). **b** Speed as a function of body mass for jumping spiders. Here we show all data points from the present study, as well as *Phidippus regius*, *Phidippus princeps*, and *Sitticus pubescens* data compiled by from Nabawy et al (2018), **c** g-force as a function of body mass in an assortment of jumping arthropods. Dashed box indicates region shown in panel (d). **d** g-force as a function of body mass for jumping spiders. **e** takeoff time as a function of body mass in jumping arthropods. Dashed box indicates region shown in panel (e). **f** takeoff time as a function of body mass for jumping spiders



case in *H. conjunctus* as well. Leg three was the longest, at 6.68 ± 0.80 mm. The next-longest leg was leg four, at 6.01 ± 0.978 mm. Legs one and two were shortest and had lengths of 4.32 ± 0.456 mm and 3.63 ± 0.355 mm, respectively. All else being equal, jumping ability scales with leg length (Biewener and Patek 2018). It therefore follows that animals should use their longest legs to perform a jump. It has been suggested that elongated third legs are used for jump propulsion in a number of long-legged salticid species, including several *Habronattus* species. The larger, squatter *Phidippus* species tend to favor the fourth legs, which are longer than the third legs in these species (Hill 2018). Why salticids vary in the relative length of different

legs is less clear, although some clues may be seen in their sexual behavior.

In many *Habronattus* species, males use elaborate courtship displays that incorporate substrate-borne vibrations and visual “dances” that involve waving the legs about in order to attract the attention of females. In certain species, the third legs take on a key role in these sexual displays. Many *Habronattus* species have patches of coloration and swellings on the tibio-patellar joint of the third leg (Griswold 1983, 1987) that they wave during courtship (Elias et al. 2012, Rivera et al. 2021). *H. conjunctus* and its closely related sister species do not have any secondary sexual modifications to their third legs (Griswold 1987). Phylogenetic

evidence suggests that elaborate courtship displays, including “third leg waving” are derived, rather than basal, characters in the group (Leduc-Robert and Maddison 2018). Importantly, third leg waving species also use their third legs to perform jumps (Hill 2018). This suggests the hypothesis that long third legs evolved first, either to give some locomotory advantage, or simply due to phylogenetic inertia. Some species of *Habronattus* (but not *H. conjunctus* or its close relatives) later coopted these lengthened legs as real estate on which to display sexual characteristics. The sister genus to *Habronattus*, *Pellenes* (Maddison and Hedin 2003, Leduc-Robert and Maddison 2018) does not have any secondary sexual characters on their third legs supporting this hypothesis. Other jumping spider genera (*Maratus*, *Saitus*) also use elongated third legs in sexual displays. It would be interesting to examine jumping in the broader context of the family Salticidae to test these hypotheses (Girard et al. 2011).

Jumping as an “off-label” use of the arachnid semi-hydraulic system

Jumping spiders in general, and *Habronattus* more specifically, are thought to be “jumping specialists”. Casual observation shows this to be likely: *Habronattus* species jump readily and deftly as their primary means of locomotion (Brandt et al. 2020). Yet, *H. conjunctus* are not exceptional jumpers when it comes to speed, acceleration or takeoff time (Fig. 5). Even among other arthropods that do not use catapult mechanisms, *H. conjunctus* do not stand out in these jumping metrics. In fact, *H. conjunctus* are less similar to other jumping spiders in terms of speed and g-force than they are to mantises and moths. This is especially surprising as moths sometimes use wings to assist in takeoff and presumably generate lift, whereas spiders have no such lift-generating structures (Burrows and Dorosenko 2015). One reason for this disconnect could lie in the importance of body weight in determining jumping performance, where light animals are expected to have a significant advantage (Scholz et al. 2006). This seems especially to be the case in terms of takeoff time. There is a clear trend in this metric for shorter takeoff times in lighter animals, regardless of taxonomic affiliation (Fig. 5e, f). Alternatively, the difference could lie in the function of *H. conjunctus* jumps compared to other species. Many of the more “impressive” jumpers use undirected means of escaping. There are known neural pathways in locusts that differentiate between directed and escape jumps (Simmons et al. 2010). Crucially, escape jumps often have a high degree of randomness in order to evade predators (Card 2012). One possible reason why *H. conjunctus* do not have high velocities and g-forces is because they may not have evolved jumping as a randomized escape mechanism, but rather as a more prosaic (but precise)

means of moving from point A to point B. Recent work has investigated the ways that jumping spiders see and therefore target their jumps, sometimes with surprising accuracy (Nagata et al. 2012). Perhaps the best way to truly appreciate the jumping abilities of *Habronattus* will be to understand tradeoffs between speed, force, and accuracy in their jumping. Further, understanding the ecological context of jumps will be essential to clarify the context in which these behaviors evolve and therefore the tradeoffs involved.

An additional reason why *H. conjunctus* jumps may appear to be unexceptional in comparison to other arthropod jumpers may lie in constraints imposed by the unique arachnid semi-hydraulic locomotion system. This system was unlikely to have originally evolved for effective jumping. In fact, most arachnids either do not jump or do so reluctantly. Perhaps the real strength of the semi-hydraulic system of locomotion is that, while likely evolved for slow walking motions, it *could* be co-opted for burst locomotion of various types, including jumping in small animals. Salticids themselves have a truly diverse locomotor repertoire, encompassing running, jumping, climbing, and even elaborate courtship dances (Elias et al. 2006; Girard et al. 2011). Below, we present some hypotheses about ways that the semi-hydraulic system provides advantages to jumping arachnids that would not necessarily translate to metrics commonly used to assess jumping.

The arachnid semi-hydraulic system is not in itself a force production mechanism. Rather, the forces are thought to be produced by the cephalothorax and/or abdominal muscles (Kropf 2013). Since the hydraulic system only transmits this pressure to the limbs to generate movement, it should instead be thought of as a force transmission system. This transmission system has its drawbacks, including slower speed, and energetic losses inherent in a hydraulic system, such as damping and fluid viscosity (Sensenig and Shultz 2003; Weihmann et al. 2010). However, the semi-hydraulic system could provide key advantages as well. First, a transmission system allows muscles to be located at some distance away from the contact point with the ground and the legs themselves. In the case of spiders, muscles that generate locomotor force are putatively located in the cephalothorax (Kropf 2013; Liu et al. 2019). Muscular force is generally considered to be proportional to its cross-sectional area (Alexander 1985; Rospars and Meyer-Vernet 2016). As the cephalothorax contains more space for muscle than the legs, more force should be available for locomotion than if the extensors were located in the legs. Insect jumpers such as orthopterans often have bulky extensor muscles located on the jumping legs (Chapman and Joern 1990). By offloading this muscular bulk to the cephalothorax, spiders are free to evolve extremely slender legs (Anderson and Prestwich 1975; Rovner 1980). Advantages to this could include fitting into small crevices and ease with the molting process.

The semi-hydraulic system also has a centralizing effect on force production. This centralization could offer a degree of flexibility unavailable to animals with extensor muscles distributed across legs. In spiders, a small amount of hemolymph pressure, and therefore force, distributed to many legs in rapid succession allows for walking or running. Some spiders are known to use this to great advantage in rapid running (Spagna et al. 2011) and turning (Zeng and Crews 2018), for example. For jumping, a large amount of force could be delivered to only the legs needed in producing ground reaction forces. This could explain why the first and second legs decrease in ELL through the takeoff phase and remain relatively “tucked” during the first phases of the jump (Fig. 2a, b). The tucking could give an aerodynamic advantage, but it may also serve to increase the volume of hemolymph available to push into the legs powering the jump. The role of leg four in *Habronattus* jumps is particularly intriguing in this context. Little is known about how hemolymph pressure is controlled within the internal hydraulic compartments inside spiders, especially small spiders. One potential explanation for this pattern is that instead of intentionally extending the fourth legs to exert force on the platform, leg 4 may receive “overflow” pressure from leg 3, and the extension could therefore be a byproduct of this overflow rather than force production that powers the jump. Future work could test this specific hypothesis using detailed imaging and force measurements.

Conclusions

We set out to measure a behavior that represents “maximal performance” in an animal. Although we determined how legs were used during the jump and observed a striking level of stereotypy in the jumps, we did not find *H. conjunctus* to be an exceptional jumper compared to other jumping spiders or other arthropod jumpers. However, we propose that it is important to understand not only how an animal jumps, but the context in which it jumps as well. We suggest that *H. conjunctus* may not be built for high speeds and g-forces but perhaps for jump accuracy and other measures of jump ability.

We found *DeepLabCut* to be an extremely useful method to track jumps. One strength of the method is the enormous time savings in data processing. This allowed us to track more points than would be feasible if we did the tracking by hand. Creating a skeleton of points with predefined physical constraints further increased the level of accuracy in tracking and output. A combination of the skeleton and the neural network-based approach also allowed the system to make reasonable predictions about the location of points even when the image was difficult to interpret by eye. Our dataset was somewhat limited by capturing animals in a single

plane. Other studies have noted that spiders can use complex aerial behaviors, including somersaulting and rotating about the axis of the dragline (Weihmann et al. 2010; Chen et al. 2013). Future work will perform similar experiments with multiple cameras, allowing us to characterize the aerial and landing phases of jumps.

Despite the acrobatic abilities of jumping spiders, little is known about their hydraulically activated joints or underlying control systems. The semi-hydraulic system of spiders is relatively unique across animal life yet is represented in over 98,000 known species. While a great deal can be measured using video systems, without a solid understanding of the underlying force production, force transmission and control mechanisms, these studies will always be incomplete. In the future, we will focus on these aspects of jumping spider locomotion, and will attempt to isolate, understand and model the force production and control elements that allow these small animals to support a broad and agile locomotor repertoire.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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