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RESEARCH ARTICLE

Limping following limb loss increases locomotor stability

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ABSTRACT

Although many arthropods have the ability to voluntarily lose limbs, how these animals rapidly adapt to such an extreme perturbation remains poorly understood. It is thought that moving with certain gaits can enable efficient, stable locomotion; however, switching gaits requires complex information flow between and coordination of an animal's limbs. We show here that upon losing two legs, spiders can switch to a novel, more statically stable gait, or use temporal adjustments without a gait change. The resulting gaits have higher overall static stability than the gaits that would be imposed by limb loss. By decreasing the time spent in a low-stability configuration – effectively 'limping' over less-stable phases of the stride – spiders increased the overall stability of the less statically stable gait with no observable reduction in speed, as compared with the intact condition. Our results shed light on how voluntary limb loss could have persisted evolutionarily among many animals, and provide bioinspired solutions for robots when they break or lose limbs.

KEY WORDS: Gait, Autotomy, Stability, Adaptation, Spider

INTRODUCTION

A surprisingly large number of animals have evolved the ability to voluntarily lose limbs despite their importance for locomotion. This ability, known as autotomy, is particularly widespread among arthropods and usually occurs during interactions with predators or competitors, when the loss of a limb facilitates the animal's short-term survival (Fleming et al., 2007). From a fitness perspective, the benefits of limb loss when compared with death may seem straightforward. However, the long-term consequences are less defined. From a functional perspective, autotomy poses a challenge to the individual: tasks that were once accomplished with a full set of limbs must subsequently be managed in a newly reduced state. As a result, one may predict that locomotor performance would decrease simply as a result of a reduction in power production capability (Maginnis, 2006). Yet, studies are conflicting: whereas some studies report decreased running speeds (Amaya et al., 2001; Apontes and Brown, 2005; Guffey, 1999), others report no change

in running ability (e.g. Brueseke et al., 2001), and still others document an increase in maximum sprint speed (e.g. Brown and Formanowicz, 2012). We note that determining maximum sprint speed can be problematic, owing to the difficulty of eliciting or observing maximum performance. Furthermore, field studies, though critical, may not allow for control over the number and specific legs that are lost. As a result, how animals adapt the use of their remaining appendages to cope with a new, autotomy-induced body morphology is not well understood.

The use of specific gaits has been suggested to be a feature enabling efficient and stable locomotion (Aoi et al., 2013; Full et al., 2002; Hildebrand, 1989; Hoyt and Taylor, 1981; Johnson et al., 2010; McGhee and Frank, 1968; Schmidt, 2014; Wilshin et al., 2017). Performance of an effective gait, however, is predicted to be strongly dependent on the number and spatial arrangement of legs available, making it particularly sensitive to autotomy. Likewise, the stability of a gait is at least partly determined by the location of the vertical projection of the center of mass within a base of support (McGhee and Frank, 1968; Ting et al., 1994). For example, over a broad range of intermediate speeds, six-legged insects use an alternating tripod gait in which three limbs contact the ground with each step (Ting et al., 1994), thus providing a large, triangular base of support.

The eight-legged extension of this gait is known as the alternating tetrapod, in which two sets of four legs are moved in anti-phase to one another (see Figs 1 and 2), and is a candidate for the primary gait amongst spiders (Wilson, 1967; Spagna and Peattie, 2012), including medium-sized wolf spiders similar in size to those used in the present study (Ward and Humphreys, 1981). However, spiders are also known to use a variety of locomotor patterns with a high degree of variability (Moffett and Doell, 1980; Shultz, 1987; Spagna and Peattie, 2012; Ward and Humphreys, 1981; Weihmann, 2013).

Spiders (Arachnida: Araneae) are also an excellent model system in which to study the effects of limb loss. When collected in the field, individuals of many spider species possess fewer than their usual eight legs, with estimates of individuals missing limbs ranging from 5 to 40% (Brueseke et al., 2001). Among wolf spiders (family Lycosidae), approximately 20% of individuals are missing one or more limbs (Brautigam and Persons, 2003; Brueseke et al., 2001), with those missing two or more legs as high as 9.8% (Brueseke et al., 2001). Unlike sedentary spider species that rely on chance and capture webs to ensnare their prey, wolf spiders are roving hunters that actively forage, using their powerful limbs to grapple with targets before subduing them with a venomous bite (Rovner, 1980).
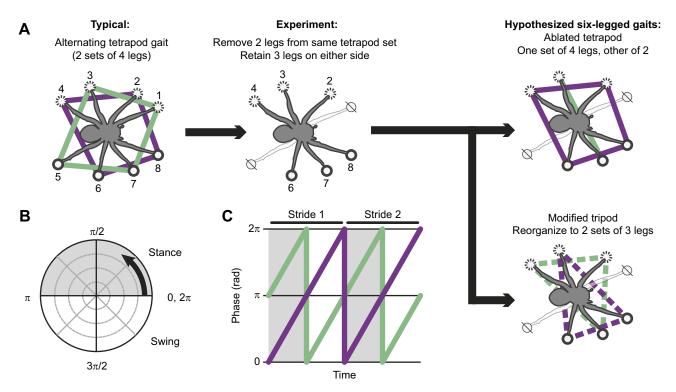
Here, we examine the consequences of limb autotomy on gait use during running, using wolf spiders as a model. Taking an approach that follows from the early work of Wilson (1967), we removed two legs from one tetrapod (Fig. 1) and asked: will the spider with six remaining legs modify its gait to move like a six-legged insect; or will it continue to use a spider gait, with two legs missing? Using high-speed video and computational gait analysis methods, we

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Fig. 1. Phase determination and expected limb coordination patterns for intact and post-autotomy spiders. (A) Diagrams of intact and ablated spiders with each color (green and purple) indicating limbs used in synchrony for each type of gait. Each set of legs (green and purple) are then moved in anti-phase to one another. Intact spiders move with an alternating tetrapod gait, for which there are two groups of four legs. Following autotomy of the limbs defined here as 1 and 5 (center), we predicted two possible post-autotomy gaits. Spiders could maintain the same limb coordination pattern, resulting in the ablated tetrapod gait (top right) in which one set of legs retains four limbs (purple) but the other only two limbs (green). Otherwise, by changing the relative phasing of their limbs, spiders could adopt a modified tripod gait (bottom right). This modified tripod would result in an equal number of legs per group (three), but would require limbs that operated in anti-phase in the intact animal gait to instead be moved in synchrony (e.g. legs 2 and 7 would move out-of-phase in the intact alternating tetrapod and ablated tetrapod but in-phase in the modified tripod gait). (B) A schematic of stride phase determination, assuming stance occupies 50% of a stride. Strides are cyclical and can therefore be characterized by a phase, ranging from 0 to 2π. (C) A graphical representation of the typical jagged pattern of two perfectly asynchronously cycling sets of limbs (green and purple) over two stride cycles, as would be for the two alternating leg groups in A. Note that 0 is equivalent to 2π.

addressed this question in a quantitative framework by exploring how gait patterns change, as well as exploring the importance of speed and static stability.

It is reasonable to suppose that quasi-static stability has the potential to be of concern for smaller spiders. Note that we refer to quasi-static stability in acknowledgement of the likely approximate and confounded role static stability is likely to play in determining limb phasing and timings. Locomotion is an inherently dynamic process, and here we look to explain this dynamic process in terms of its static process (hence quasi-static). In the Discussion, we shall consider to what extent quasi-static stability is likely to be a causal factor in our observations. It is important to note that quasi-static stability is only one amongst many factors that shape spider locomotion. Energetics, risk of injury and multi-functional constraints on limb function, for example, are all highly likely co-contributors. They are also in many cases likely to be co-variates; e.g. energetically efficient locomotion may also be stable. Disentangling these factors remains an open question, about which some recent progress has been made (Zelik and Kuo, 2012), but will not be addressed in this paper.

Support for a putative role for static stability comes from calculations concerning the physics of their locomotion. The characteristic time constant for a gravitationally induced failure mode (that is, the time it takes for a spider's body to hit the ground without support) is $\sqrt{h/g}$, where g is the acceleration due to gravity at the Earth's surface and h is the separation of the body from the

surface. For wolf spiders where body heights above the ground are less than 1 cm, this time scale is less than 33 ms, corresponding to frequencies above 30 Hz. We observed stride frequencies around 10 Hz (comparable to those of similar species; Ward and Humphreys, 1981). If an alternating tetrapod gait were maintained after ablation of two limbs on the same tetrapod, this would result in a quasi-statically unstable gait for a far longer period of time than that needed to induce a collapse without significant actuation from the two remaining limbs. That is to say, if spiders did not adjust their gait or dynamics, their bodies would drag across the substrate following the loss of two limbs from the same tetrapod set.

In addition, because of their sprawled posture, spiders require large overturning moments. Here, we estimate a characteristic torque of 2.67×10^6 mm mg^2 s^{-2} (see Materials and Methods and Fig. S1). This further suggests that quasi-static stability should be of importance to locomoting spiders.

We chose to autotomize the left foreleg and right hind leg because their loss was predicted to have a severe impact on stability that could be mediated by a change in gait (Fig. 1A). These limbs were predicted to severely hinder stability for two reasons. First, if the same pattern of ground contacts were maintained after ablation, then during the resulting double support phase the stability margin would have to be negative. Second, removing two limbs maximally separated from one another would necessarily reduce the size of the polygon of support formed by the limbs, and thus reduce stability. If spiders were to make no adjustment to their motor pattern or gait, the
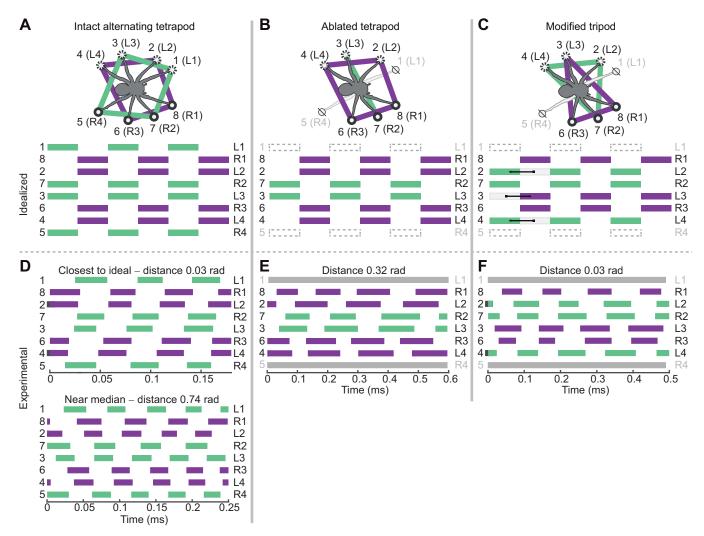
Fig. 2. Idealized and experimental octopedal gait diagrams in the intact and ablated conditions. (A–C) Upper: schematics of spiders showing leg number conventions and legs which move in phase for each gait (green and purple lines connecting leg tarsi or feet). The 1–8 leg numbering convention is used in this paper and facilitates our mathematical description, whereas the convention in parentheses (L1–L4, R1–R4) is frequently used in the literature. Lower: conventional gait diagrams indicate stance times for the tarsi, with color denoting groups of legs moving together in phase as in the spider schematics, with leg number conventions displayed at either side. Spiders are thought to use an alternating tetrapod gait in the intact condition (A), although their gait is generally more variable than in, for example, hexapedal insects. Upon ablation of legs 1 (L1) and 5 (R4), if spiders do not alter their leg coordination, they would exhibit what we term the ablated tetrapod gait (B), consisting of alternations of two legs (green) and then four legs (purple). Spiders could adapt their gait such that they exhibit what we term a modified tripod gait (C), shifting legs across grouped sets (arrows) such that they alternate between sets of three legs after ablation, with legs recruited from different original tetrapods. Ablated limbs are shown in light gray and their corresponding alternating tetrapod footfalls as gray dashed boxes. (D–F) Examples of experimentally observed gait diagrams before and after ablation. Distances to each idealized gait were calculated using our gait distance metric and are indicated above each gait diagram. (D) The trial containing strides close to the median value (0.74 rad) for all intact spider strides shows more variability than the trial closest to an ideal alternating tetrapod (0.03 rad). (E) An example of ablation trial with strides near the ablated tetrapod gait. It should be noted that a substantial portion of the time is spent with three legs in contact despite this gait being classified as an ablated tetrapod. This is because our gaits are defined in terms of phase relationships, and therefore a synchronization pattern, as opposed to contact patterns. This is manifest in Fig. 4C. (F) A trial with strides near to the modified tripod gait. Gray lines in the gait diagrams (E,F) denote missing legs.

loss of the limbs targeted by our experiment would result in spiders assuming an 'ablated tetrapod' gait, being forced to stand on only two legs for half of every complete stride. However, because the spiders still possessed three legs on either side of the body, spiders alternatively could use a gait similar to the alternating tripod gait used by insects. This 'modified tripod' gait would require novel limb pairings, however, because legs that moved out of phase in the intact animal (e.g. limbs 2 and 7 in Fig. 1A) would then need to operate in phase. We hypothesized that spiders missing two limbs would switch to the novel, modified tripod gait, at a measurable cost to running speed, reflecting the prioritization of the locomotor control system to maximize locomotor stability.

We show that although a subset of the spiders do change their limb co-ordination pattern in the expected way following this ablation (switching to an ablated tripod gait), for some strides they continue performing a gait similar to that of the intact tetrapod (Fig. 2). Although modifications to this gait are minimal in terms of the limb ordering, the duration of stance during the unstable bipod phase was shorter.

To test our hypothesis, we created a gait classifier based on projecting relative limb phases onto multidimensional gait space. This allowed us to visualize spaces occupied by observed limb phasing patterns, and to assign gait identities based on the combined distance to each gait (see Materials and Methods). We also calculated

static stability margins and temporal patterns of movements to determine how gait choice affected locomotor stability.

MATERIALS AND METHODS
Subjects
Six adult wolf spiders (Lycosidae, *Pardosa* sp. Koch 1847) were collected from a field in Hertfordshire, England. All spiders were housed individually and fed small insects two to three times weekly.

Body masses were estimated via the silhouette method (Ristroph et al., 2009). Still images of the spiders were thresholded to obtain the body shape, and the area of the silhouette was used to estimate mass through calibration and validation. The silhouette-to-mass relationship was calibrated and validated by weighing and photographing 30 wolf spiders (17 males and 13 females/juveniles). Of these 30 spiders, 27 individuals were successfully weighed and photographed. The silhouette area was determined by adjusting the threshold for the silhouette until the legs just became visible. A conversion factor from the area of the silhouette to the mass of the spiders was constructed by fitting a linear model (with forced zero offset) for body mass against this area. The relationship between silhouette area and mass is linear and the fit is generally good (R^2=0.963). The observed masses were 21.9±3.86 mg (mean±s.d.).

The body (cephalothorax plus abdomen) lengths of the spiders were obtained from the same images; these were observed to be 7.22±0.911 mm (mean±s.d.). A characteristic torque for the system is given by the typical body mass multiplied by the acceleration due to gravity at the Earth's surface multiplied by the body length (which for this species is comparable to their leg length and therefore moment arm due to spiders' sprawled posture), and is $\sim2.67\times10^6$ mm mg^2 s^{-2}. A similar number describes the overturning moment, because the leg length in these animals is the same order of magnitude as their body length. Calculation of the overturning moment is illustrated and further described in Fig. S1.

Data collection
Trials were run in a Perspex arena (1500 cm^2), lit ventrally to generate silhouettes of the spiders. Paper was placed on the arena floor and changed between individuals to prevent behavioral responses owing to accumulation of silk or chemical cues (Persons et al., 2001). Videos of each trial were recorded from the dorsal view at 1000 frames s^{-1} (X-PRI; AOS Technologies, Baden Daettwil, Switzerland), and were framed to permit five to eight complete strides within the field of view.

After 30 trials were recorded for a given spider, autotomy of the left foreleg and posterior right leg was induced by firmly grasping the leg with forceps distal to the coxa until the limb detached. We then recorded as many further trials as possible, up to a maximum of 30. These limbs were chosen because their loss was predicted to have the most severe impact on spider locomotion while still maintaining three legs on both sides of the body (Fig. 1A). Typically, intact spiders move with an alternating tetrapod gait in which two sets of four legs are moved in anti-phase to one another (Fig. 2A). In terms of static stability, if spiders were to make no adjustment to their motor pattern, the loss of the limbs targeted by our experiment would result in the greatest reduction in static stability possible while retaining three legs on either side. In terms of gait, if spiders made no adjustment, upon the loss of these legs they would be forced to stand on only two legs for half of every complete stride, a gait we have termed the 'ablated tetrapod' (Fig. 2B). However, because they still possessed three legs on either side of the body, spiders could use a gait similar to the alternating

tripod gait used by insects, though this would require that legs that moved out of phase in the intact animal (i.e. limbs 2 and 7 in Fig. 1A) would then need to operate in phase (Fig. 2C). Following autotomy, spiders were given a minimum 24-h rest before another 30 trials were recorded in the post-autotomy condition.

Spider locomotion is particularly sensitive to temperature variations (Booster et al., 2015). The room in which the experiment was conducted was heated and air conditioned. The thermal properties of the building in which the experiment was performed were characterized during a separate time period using two Dallas 18B20 one wire digital temperature sensors attached to a third-generation Raspberry Pi using a custom bash shell script to record temperatures every 2 min over 3 weeks. The external recorded temperature during the experiment never dropped below 3.9°C (according to the three closest Met Office stations: Oxford, Cambridge and Heathrow). Days during the characterization period where the temperature fell below this threshold were excluded, leaving 12 days of recordings spanning all observed external temperatures during experimentation. The observation time was restricted to between 10:00 and 17:00 h during the recording, and the temperature sensors at most disagreed by 0.8°C. Pooling the temperature recordings across both sensors, the mean observed temperature was 23.1°C with a standard deviation of 1.3°C. Thus, laboratory temperatures were similar to temperatures that spiders would have experienced in the field during the times that they are active (daytime during spring, summer and fall).

Data analysis
Only those trials that represented straight runs with at least three complete strides were selected for analysis, leaving 209 trials. From within these trials, only strides in which the phase of all leg oscillations could be estimated without artifacts (method described below) were chosen, resulting in 469 strides. Custom MATLAB code was used to isolate and track the spiders' body and limbs. Because of the oscillatory nature of limb movement during a stride, the fraction a leg is through its cycle can be quantified using a phase between 0 and 2π. Limb phase was estimated in Python using the Phaser algorithm (Revzen et al., 2013; Revzen and Guckenheimer, 2008). Different gaits can then be considered as a function of phase differences between legs. Such a representation has been useful when considering such diverse phenomena as multistability (Haynes et al., 2012), gait transitions (Haynes et al., 2009) and dynamical modeling (Revzen and Guckenheimer, 2012). For example, in bipedal systems, gaits exist along a one-dimensional circle (a single phase relationship between the two legs). By extension, quadruped gaits exist on a three-dimensional hyper-torus, hexapod gaits (as in insects or ablated spiders) on a five-dimensional hyper-torus, and eight-legged gaits (as in intact spiders) on a seven-dimensional hyper-torus. Gaits can be mapped onto these gait spaces (N−1 torii) regardless of the dimensionality of the system (i.e. regardless of the number of legs, N). Indeed, a similar analysis to that presented here was used to identify the effect of uneven terrain on dog gaits (Wilshin et al., 2017). Although it is possible to achieve sub-stride resolution of gait using these methods, here we averaged over the full stride such that each stride was represented by a single value.

We require a measure of similarity (technically inverse similarity) between gaits so that we can compare with which of our two prototypical gaits a given pattern of motion is more similar. We will quantify this in terms of the distance between gaits. Closer gaits are more similar, more distant gaits less similar. Here we will define a measure of distance d between two gaits described by limb phase

differences ϕ and φ. This distance between ϕ and φ is a measure of how much one pattern of locomotion would have to change phase in order to be identical to the other. We start by calculating a distance for limb configurations in terms of phases, and then quotient out the difference owing to global phase advance.

For each stride, we then calculated this distance in gait space between the observed gait and the two idealized gaits hypothesized to result from our experimental manipulation (the ablated tetrapod or modified tripod). To calculate a distance in this space, we assumed that distances between gaits could be quantified by putting a Euclidean metric on the six-dimensional hyper-torus of limb phases for the ablated spiders. That is, we assumed that all limbs were functionally equivalent.

It should be noted that in practice, this assumption of limb equivalence is likely not strictly accurate, because limbs often have non-locomotor functions. For example, even in wolf spiders the forelimbs seem to be especially important in capturing and subduing prey, a dual-usage strategy employed by many other legged animals. A future study may account for these potential functional differences between the limbs by adjusting the metric G_{ij} discussed below. This could, for example, be used to infer how detrimental to locomotion the loss of specific limbs will be or the degree to which their role in locomotion has been compromised.

For now we will assume all limbs are equivalent. If the tuple ϕ=($\phi_0,\phi_1,\ldots,\phi_5$) contains the six limb phases (corresponding to the remaining limbs 2, 3, 4, 6, 7 and 8 in the ablated condition; Fig. 1), then the distance between the two limb configurations ϕ and φ, $d(\phi,\varphi)$, is:

$$d(\phi,\varphi) = \sum_{i=0}^{5}\sum_{j=0}^{5}(\phi_i - \varphi_i)\mathbf{G}_{ij}(\phi_i - \varphi_j), \qquad (1)$$

where $\mathbf{G}_{ij}$ is 0 unless $i=j$, when it is 1. Note that Latin indices will always run from 0 to 5. We note that this distance d is not unique owing to the topology of the torus on which it is defined. We will address the concern shortly by finding the unique minimal distance on the phase differences. We want to compute a distance in our space of phase differences, which was done by calculating the induced metric, $\mathbf{g}_{ij}$. We defined our phase differences by the tuple θ=($\theta_1,\theta_2,\ldots,\theta_5$) with elements:

$$\theta_\mu = \phi_\mu - \phi_{\mu-1}, \quad \mu \in \{1,2,\ldots,5\}, \qquad (2)$$

and the tuple ϑ=($\vartheta_1,\vartheta_2,\ldots,\vartheta_5$) with elements:

$$\vartheta_\mu = \varphi_\mu - \varphi_{\mu-1}, \quad \mu \in \{1,2,\ldots,5\}. \qquad (3)$$

We note that Greek indices always run from 1 to 5. The vectors ϕ and φ are therefore the phase of each limb. Intuitively, these represent where in the cycle a limb is; if component 3 of the vector ϕ has a value of π/2 rad or 25% of a cycle, then the third leg is a quarter of the way through a cycle. The vectors θ and ϑ are limb phase differences. If component 3 of the vector θ has a value of π/2 rad or 25% of a cycle, then the third leg is a quarter of a cycle ahead of the second leg.

Our objective with projecting onto the torus of phase differences was to remove the contribution to the distance from the overall phase advances. This was estimated by:

$$\psi = \frac{1}{6}\sum_{i=0}^{5}\phi_i, \quad \chi = \frac{1}{6}\sum_{i=0}^{5}\varphi_i. \qquad (4)$$

Substituting Eqns 3 and 4 into Eqn 1, we find:

$$d(\theta,\psi,\vartheta,\chi) = \sum_{\mu=1}^{5}\sum_{\nu=1}^{5}(\theta_\mu - \vartheta_\mu)\mathbf{g}_{\mu\nu}(\theta_\nu - \vartheta_\nu) \\ + 6(\psi - \chi)^2, \qquad (5)$$

where:

$$\mathbf{g} = \frac{1}{6}\begin{pmatrix} 5 & 4 & 3 & 2 & 1 \\ 4 & 8 & 6 & 4 & 2 \\ 3 & 6 & 9 & 6 & 3 \\ 2 & 4 & 6 & 8 & 4 \\ 1 & 2 & 3 & 4 & 5 \end{pmatrix}. \qquad (6)$$

Because our objective is to ignore differences in overall phase advance, we drop the final term, leaving us with a distance between phase differences given by:

$$D(\theta,\vartheta) = \sum_{\mu=1}^{5}\sum_{\nu=1}^{5}(\theta_\mu - \vartheta_\mu)\mathbf{g}_{\mu\nu}(\theta_\nu - \vartheta_\nu). \qquad (7)$$

In our limb convention, the modified tripod has phase differences ϑ_{MT} given by (π,π,π,π,π), whereas the ablated tetrapod has phase differences ϑ_{AT} given by (π,π,0,π,π). To classify a gait with limb phase differences θ, we evaluate $D(\theta,\vartheta_{MT})$ for the modified tripod and $D(\theta,\vartheta_{AT})$ for the ablated tetrapod and assign that stride to the class with the smaller distance (Fig. 3). This was performed in a custom Python script.

We note that owing to the topology of the space of limb phase differences (a five-dimensional hyper-torus), the distances given by D are not unique, because any elements of the tuple which make up the arguments can be shifted by 2π and be equivalent. We therefore defined the distance to be the minimal value of D from those possible by performing such shifts.
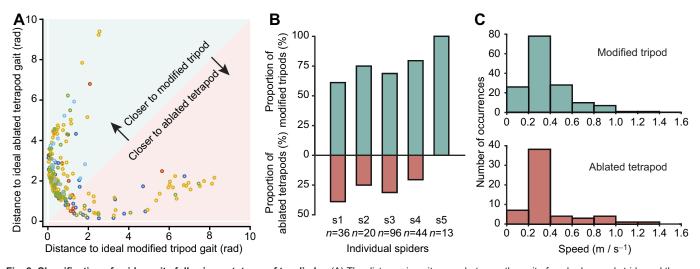
Locomotor stability was calculated as the static stability margin, or the minimum distance between the center of mass and the nearest edge of the base of support (Ting et al., 1994) (see Fig. 4A). The location of the spider's center of mass was approximated as the junction between the cephalothorax and the abdomen. The base of support in each frame was defined by a convex polygon connecting any feet engaged in the stance phase of the limb cycle.

Statistics

To determine whether speed correlated with the use of ablated tetrapod or modified tripod gaits post-ablation, we used a generalized linear model (GLM) with the binomial distribution, because behaviors were categorical. Speed and individual spider were used as independent variables. All other comparisons among the intact alternating tetrapod and ablated gaits were performed using an ANOVA with Tukey's honestly significant difference (HSD) *post hoc* comparisons.

RESULTS

We found that limb loss had no detectable effect on running speed (two-way ANOVA, $F_{2,461}=1.51$, $P=0.2217$). In total, 469 strides were included in the analysis. The tortuosity of the trajectories was estimated in the following manner. First, we identified the point of maximum perpendicular excursion of the spider from the line connecting the start and end of the running path. Second, we fit the arc of a circle through this point and the start and end of the trajectory. The tortuosity of the trajectory was estimated by taking the ratio of the length of this arc divided by the distance between the start and end

Fig. 3. Classification of spider gaits following autotomy of two limbs. (A) The distance in gait space between the gait of each observed stride and the two idealized post-autotomy gaits (modified tripod and ablated tetrapod). Points of the same color represent strides from the same individual. (B) The relative use of ablated tetrapod (red) and modified tripod (blue) gaits for each spider. Total number of strides per individual are indicated by n-values. (C) Distributions of running speeds observed for each stride, based on post-autotomy gait type. Both gaits were used over a comparable range of speeds.

points of the trajectory. The maximum observed tortuosity was 1.47, indicating that all trajectories were reasonably straight.

We characterized gaits by quantifying the phase differences between limbs. Given that limbs oscillate during locomotion, it is possible to represent leg position in cyclical terms (Revzen et al., 2013; Revzen and Guckenheimer, 2008), assigning it a phase that varies between 0 and 2π during a stride. Gaits can then be considered quantitatively as a function of phase differences between legs, permitting us to map gaits onto multi-dimensional 'gait spaces', or hyper-tori, regardless of the number of legs (see Materials and Methods). For each stride, we then calculated the distance in gait space between the observed gait and the two idealized gaits hypothesized to result from our experimental manipulation (i.e. the ablated tetrapod and the modified tripod; Fig. 3A).

We found that the modified tripod gait was used in more strides than the ablated tetrapod (modified tripod: 151; ablated tetrapod: 58), with the majority of individuals (four of five) using both post-autotomy gaits (Fig. 3B). One individual used the modified tripod gait exclusively. Although the modified tripod gait was used more frequently following limb autotomy, most spiders used both gaits across the range of observed speeds, with no significant relationship between speed and gait (GLM with binomial distribution, $P=0.215$; Fig. 3C). Examination of the change in distances to each idealized gait within and across trials showed no indication of learning. A comparison of the theoretically predicted and several experimentally observed gait diagrams are shown in Fig. 2. Here, we use the gait distance metric to find example trials in the intact condition with strides closest to the ideal gait and near the median observed gait (Fig. 2D), near the ideal ablated tetrapod (Fig. 2E), and near the modified tripod (Fig. 2F).

We expected that limb loss would negatively impact locomotor stability, which we represented using the static stability margin (Ting et al., 1994). Stability decreased in concert with the number of feet in stance when fewer than four legs were in stance, and was approximately constant for four, five and six legs (Fig. 4A). Likewise, both post-autotomy gaits were less statically stable than the intact alternating tetrapod gait (two-way ANOVA: $F_{7,409}=4.46$, $P<0.0001$; Fig. 4B). When comparing the stability of the post-autotomy gaits, however, we expected that if spiders did not modify

their gait (i.e. they ran with the ablated tetrapod gait), this would be less stable than the modified tripod gait. This is because we expected that the phase of the stride with only two supporting legs would dramatically reduce overall stride stability. Surprisingly, our results showed that spiders had similar stability margins in the ablated condition irrespective of whether they modified their gait (t-test: $t_{151}=0.36$, $P=0.721$; Fig. 4B). As a result, we hypothesized two mechanisms by which spiders could avoid the stability penalty: (1) spatially altering the placement of the feet (tarsi) relative to the center of mass to improve stability; and (2) temporally altering the fraction of the stride spent with fewer supporting legs in stance.

We analyzed the relationship between the number of legs simultaneously engaged in stance and the resulting static stability, grouped by gait, to assess the impact of spatial positioning on stride stability. Although the overall pattern was the same across gaits, with a positive correlation between the number of legs in stance and static stability, the stability margin was reduced for similar numbers of limbs in stance in the ablated condition, implying that the spatial arrangement of the ablated legs reduced stability as compared with the intact configuration (Fig. 4E, Table 1).

To address potential temporal modifications, we looked at the relative amounts of time spent with a given number of feet in stance for each gait. As expected, spiders spent most of a stride with four legs on the ground when intact, and three legs on the ground when using the modified tripod gait post-autotomy (Figs 2F and 4C). Ablated tetrapod strides were characterized as most commonly having two to four legs in stance at any moment. However, spiders most frequently alternated between the two- and four-legged configurations, spending a greater proportion of the stride with four legs on the ground ($69.2\pm0.3\%$; mean±s.d.) than with two (Fig. 4D). In other words, spiders appear to improve static stability within gaits through gait-specific changes in foot position and temporal strategies akin to limping, biasing stance durations to favor more statically stable foot configurations.

Prior work in the literature has enjoyed success treating intact octopods as two loosely coupled quadrupeds where the four fore-most limbs and the four hind-most limbs are considered independently (Biancardi et al., 2011). For the purposes of better illustrating our results, we draw inspiration from this approach and treat our ablated spiders as pairs of coupled 'quadrupeds', and the
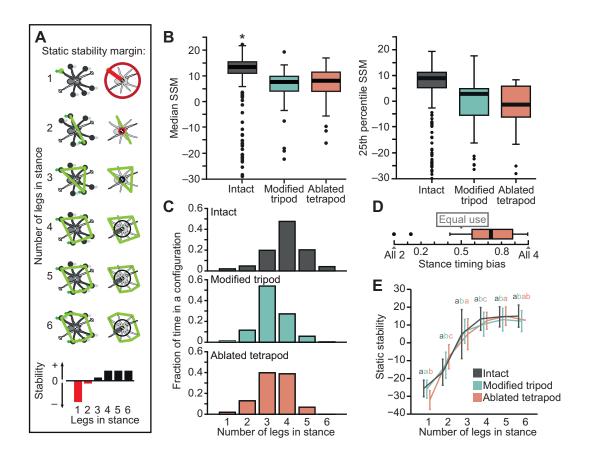
Fig. 4. The relationship between static stability and gait patterns in spiders post-autotomy. (A) A schematic demonstrating static stability. Static stability margins (SSMs) were defined as the distance between the center of mass (CoM) of the animal and the nearest edge of a convex polygon of support, formed by limbs that are in contact with the ground. Left: for six example configurations, feet in stance are marked with a green, posteriorly oriented arrow. Right: the quartered circle denotes the CoM of the animal, whereas the thin surrounding circle and radial line show the distance to the nearest edge of the support polygon. Black circles and radii show positive stability values, whereas red denote negative values where the CoM falls outside the support polygon and is statically unstable. Bottom: the relative stabilities of the limb configurations, showing a direct correlation between number of limbs in stance and stability margin value. This plot is quantitatively correct but shown here for illustrative purposes; the data plotted correspond to the gray line for 'intact' shown in E. (B) Box plots showing median (left) and 25th percentile (right) static stability of each stride, grouped by gait type. The asterisk indicates a significant difference in stability margin from the other gaits (*$P<0.0001$). Dark lines represent the medians, box edges extend to 25th and 75th percentiles, whiskers cover ±2.7 s.d. (99.3%) of the data. Data lying outside this range are shown as circles. (C) Distributions of the fraction of time within a stride spent with a given number of legs in stance for intact, modified tripod, or ablated tetrapod gaits. (D) A box plot showing the ratio of time spent with two legs in stance versus four legs in stance for each stride in which the ablated tetrapod gait was used, demonstrating a timing bias that increases the fraction of time spent in the four-legged configuration across a stride. Box plot conventions are as in B. The equal usage point, at which half of the stride would be spent on two legs and half on four, is indicated at the center. (E) The static stability observed when a given number of legs were simultaneously in stance, for each gait type. Mean values are shown with standard deviation. Different letters indicate significant differences in stability within a given leg configuration between gaits.

observed effects are reflected in the Hildebrand diagrams associated with these 'quadrupeds' as highlighted by the boxes in Fig. 5. The alternating tetrapod corresponds to a trotting gait, whereas the modified tripod corresponds to a bounding gait for each set of four limbs, when intact.

The observed changes in coordination pattern correspond to an increase in the frequency of patterns consistent with the modified tripod gait (a bound in the dual quadruped interpretation), and therefore a decrease in patterns consistent with alternating tetrapod (a trot in the dual quadruped interpretation), reflecting the shift from

Table 1. Results from an ANOVA comparing stability margins among gaits for different numbers of legs in stance (see also Fig. 4E)

Legs in stance	Intact	Ablated tripod	Modified tripod	F (within d.f.)	P
1	−25.6±0.2[a]	−32.1±0.5[b]	−26.0±0.4[a]	65.6 (778)	<0.0001
2	−16.5±0.2[c]	−7.8±0.3[a]	−12.5±0.2[b]	296 (3443)	<0.0001
3	4.9±0.1[b]	4.9±0.2[b]	6.5±0.1[a]	45.9 (14,506)	<0.0001
4	13.4±0.1[c]	12.4±0.2[b]	10.5±0.1[a]	323 (17,965)	<0.0001
5	14.6±0.1[a]	14.9±0.3[a]	13.0±0.2[b]	26.7 (6396)	<0.0001
6	15.0±0.2[b]	12.8±6.2[d]	12.2±0.8[a]	5.4 (1135)	0.0048

Stability margins are presented as means±s.e.m. Different superscript letters indicate statistically significant similarities or differences between conditions (Tukey's honest significant difference *post hoc* comparisons; the between-group degrees of freedom for the F statistic was always 2).
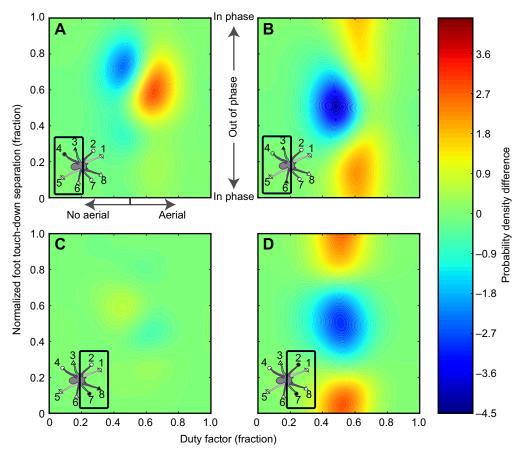
Fig. 5. Hildebrand diagrams showing shifts in the frequency of footfall timings and duty factors following limb ablation. Octopods have been thought of as loosely coupled quadrupeds such that A and B refer to the hind-most quadruped and C and D refer to changes within the fore-most quadruped, as highlighted by the black box in the lower left corner of each panel. The duty factor, presented on the *x*-axis, is calculated from the legs in the black box. The black filled symbols highlight the focal legs for which the relative footfall timings are plotted on the *y*-axis (always the timing of fore-leg touchdown within the hind-leg stride; or the left within the right leg stride). Limbs that would be paired during an alternating tetrapod gait are colored as groups of purple and green. The square and triangle symbols at each leg tip indicate limbs that would be paired during an ablated tripod gait. Hot colors (reds and oranges) indicate that a given duty factor and footfall timing was used more frequently after ablation of limbs 1 and 5 (gray); cool colors (blues and cyans) indicate the opposite. Green colors indicate no change in footfall characteristics following ablation. Upon limb loss, the hind-most quadruped shifts to longer duty factors (A,B), whereas no change is noted for the fore-most quadruped (C,D). (B) Limbs 3 and 6 and (D) limbs 2 and 7 shift from asynchronous touch-downs to synchronous touch-downs (hot colors positioned close to 0 and 1 along the *y*-axis). The probability density differences were estimated with a kernel density estimator using a von Mises kernel with the scale factor κ set to 100 (corresponding to a characteristic scale of around 0.1 as a fraction of a cycle). Normalized foot touch-down separation – an estimation of phase difference between limbs – is calculated as the difference between the focal legs divided by the difference between consecutive touch-downs of the hind-most left limb. For example, in A, the average duty factor of the hind-most four (or three for the ablated case) limbs plotted on the *x*-axis, and on the *y*-axis has the difference between consecutive touch-downs of legs 3 and 4 divided by the difference between consecutive touch-downs of leg 4.

an alternating tetrapod gait to a modified tripod gait following limb loss. For the hind-most quadruped (Fig. 5A,B), we observed an increase in duty factor and a shift from patterns consistent with trotting to those consistent with bounding (Fig. 5B). For the fore-most quadruped (Fig. 5C,D), we observed no clear change in duty cycle and a shift toward bounding (Fig. 5D).

The shift towards increased duty factor in the hind-most quadruped but not in the fore-most quadruped highlights that the timing differences observed in Fig. 4C,D are not uniformly distributed amongst the legs within tetrapods. The shift from duty factors of approximately 0.4 to 0.7 (Fig. 5A; blue to red region) and 0.4 to 0.6 (Fig. 5B) requires that a substantial fraction of a stride is spent with three legs in stance. This would be impossible if the shift in timing was uniformly distributed.

It is clear from the changes in duty factor that the timing differences observed in Fig. 4C,D are not uniformly distributed amongst legs within tetrapods. This clarifies the observation in Fig. 4C (and Fig. 2E) where a substantial fraction of a stride is

spent with three legs in stance. However, the Hildebrand diagrams (Fig. 5) make it clear that it is changes in the duty factor of the hind limbs that are responsible for the increased time spent with three legs in stance, as is also seen in the gait diagrams derived from experimental data in Fig. 2E,F.

The dynamic stability of the spiders was quantified using the variability of the body yaw within a stride. This does not in any way exhaust the possible measures of dynamic stability one might consider when examining locomoting spiders, but owing to a fixed camera position, the variability in the pitch and roll of the subjects could not be obtained. A linear mixed-effects model was fit to the log-transformed yaw variances (because of the presence of heavy tails), with the ablation conditions as independent variables and a variable error term (varying by subject) to account for heteroscedasticity. A random effect of the subject within the intercept was added. No effect on yaw was observed ($z=-0.206$, $P=0.837$). The within-stride yaw variances are plotted in Fig. 6 with accompanying box plots. These also suggest there is no
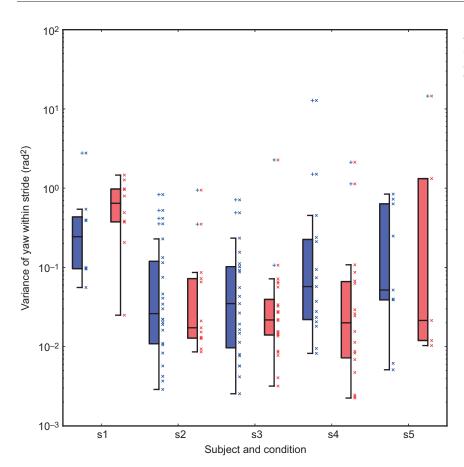
Fig. 6. Box and raster plots of the within-stride variability of the body yaw of the spiders prior to (blue) and after (red) ablation. Note that the *y*-axis is on a log scale. No change in the variance of the yaw within the stride was observed.

substantial effect of the variability of the yaw within a stride owing to ablation.

DISCUSSION

The use of both post-autotomy gaits by individual spiders suggests that limb coupling patterns are relatively flexible in these animals (Figs 2F and 3B): to achieve the modified tripod gait, some of the limbs that move in phase in the ablated tetrapod gait must operate out of phase in the tripod. Previous work has noted flexibility in limb coordination patterns in spider locomotion (Spagna and Peattie, 2012), including the early work of Wilson (1967), who observed the modified tripod gait described here during limb ablation experiments with a single tarantula. Other arthropods have also demonstrated a similar capacity for flexibility in limb timing characteristics when subjected to limb loss. For example, cockroaches will adjust their gait if the distal part of a leg is lost, and then return to a typical gait if the load-bearing capability of the leg is restored using a simple 'peg' prosthetic (Noah et al., 2004). Likewise, if the middle legs are removed in hermit crabs – crustaceans that use six legs during normal locomotion – individuals produced a quadrupedal trot (Herreid and Full, 1986), an adjustment in gait that is functionally analogous to the switch from the intact alternating tetrapod to the modified tripod gait reported here. However, the timing-based strategy reported here adds a novel dimension to how legged arthropods adaptively adjust limb coordination to achieve robust movement.

Our results are not sufficient to establish quasi-static stability as the sole causal factor in determining the observed changes in limb phasing and stance timing. A more comprehensive model is needed to incorporate and weigh the contributions of multiple determinants

of gait. Recent modeling work in an ambush predation specialist arachnid shows a promising approach (Zeng and Crews, 2018).

How spiders maintain locomotor performance despite a one-quarter reduction in leg number is still unclear. Indeed, a metric of dynamic stability – the yaw variability in the stride – showed no substantial change post-ablation. Although there are various metrics for measuring locomotor performance (e.g. maximum speed, sustained speed and acceleration capacity), our results add to the evidence that wolf spiders are remarkably resilient to the effects of autotomy on locomotor output.

While studying the fitness effects of limb loss in opilionids – another order of arachnids in which limb autotomy is common – Guffey (1998) noted a similar lack of measurable cost to running speed post-autotomy and proposed the spare-leg hypothesis. This hypothesis posits that some species possess limbs with redundant functions, such that the loss of a given limb has minimal impact on subsequent fitness. Brueseke and colleagues (2001) invoked this hypothesis to explain the persistence of overall locomotor performance in wolf spiders. Our results further support this and extend it to include measures of stability.

The gait classifier presented here also has wider applicability to both biological and mechanical systems; for example, refinement of the classifier could be used to reveal the trade-offs between the other functions of limbs and their capabilities in locomotion.

Finally, biologically inspired designs of mechanical systems have led to some rapid advancements in the field of robotics, based on research in insects, quadrupeds and bipeds (Ijspeert, 2014). In one instance, a hexapedal robot was able to detect and compensate for a leg-breakage using a gait transition (Johnson et al., 2010). The locomotor resilience displayed by wolf spiders should therefore be

of interest to engineers and roboticists, providing a simple solution for combatting destabilizing perturbations that are common during natural, untethered locomotion (Cully et al., 2015). This work may also generate hypotheses for the mechanisms that underly gait adaptation. In some animals, robustness to autotomy can emerge with only simple local control laws (Kano et al., 2012). Spiders may serve as a particularly useful taxonomic group for future study regarding failure-resistant multi-legged systems.

Data availability
All data are available in the main text and by request from the corresponding authors. Custom Python code for kinematic phase extraction and gait parameter calculation is available for download at: http://purl.org/spencelab/spidergaitablations.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.174268.supplemental

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