Overcoming an evolutionary conflict: Removal of a reproductive organ greatly increases locomotor performance

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One potential consequence of sexual size dimorphism is conflict among characters. For example, a structure evolved for reproduction can impair performance during other activities (e.g., locomotion). Here we provide quantitative evidence for an animal overcoming an evolutionary conflict generated by differential scaling and sexual size dimorphism by obligatorily removing an undamaged reproductive organ, and thus dramatically enhancing its locomotor performance. The spider genus Tidarren (Araneae, Theridiidae) is interesting because, within several species presenting extreme sexual size dimorphism (males representing ~1% of the total mass of the female), males voluntarily remove one of their two disproportionately large pedipalps (modified copulatory organs; a single one represents ~10% of the body mass in an adult) before achieving sexual maturity. Whether the left or right pedipalp is removed appears to be random. Previous researchers have hypothesized that pedipalp removal might enhance locomotor performance, a prediction that has remained untested. We found that, for male Tidarren sisyphoides, maximum speed increased (44%) significantly and endurance increased (63%) significantly after pedipalp removal. Furthermore, spiders with one pedipalp moved ~300% greater distances before exhaustion and had a higher survival after exertion than those with two pedipalps. Removal of the pedipalp may have evolved in male Tidarren because of enhanced abilities to search for females (higher endurance and survival after exertion) and to out-compete rival males on the female’s web (higher maximum speed). Our data also highlight how the evolution of conflicts can result in the evolution of a novel behavior.

A central tenet of optimality theory is that natural selection will optimize structure and performance resulting in an overall highly fit organism (1, 2). Many studies, however, have shown that the evolution of high performance in one task can lead to decreased performance during another task (e.g., refs. 3–5). In extreme cases of such apparent conflicts, a structure evolved for one activity can substantially impair performance during another activity. For example, Darwin (6) described how sexual selection for sex differences might lead to such functional conflicts, particularly in males. He depicted how the relatively elaborate feathers in some male birds result in enhanced reproductive success via female mate choice, yet also reduces or constrains flight capacities, thus potentially making the animal more susceptible to predators (7). A relatively unexplored area is how organisms cope with these constraints imposed by factors such as sexual selection, natural selection, or allometry. One view of constraints is that they limit or hinder morphological or behavioral change, but another possibility is that constraints can result in a novel phenotype or behavior (8, 9). For example, as plethodontid salamanders undergo evolutionary miniaturization (become smaller), they shift from a terrestrial to an arboreal lifestyle (10). This transition occurs because of developmental constraints on limb growth, resulting in increased toe webbing, and hence enhanced climbing ability in small salamanders.

Here, we examine a functional conflict imposed by differential scaling and extreme sexual size dimorphism in a spider, and describe how a novel behavior overcomes this conflict. We demonstrate that male spiders gain a substantial locomotor performance advantage through the removal of a structure that is normally essential for reproduction (a pedipalp). Biologists have shown that the removal of a structure occurs in a variety of taxa [lizards and tail loss (11, 12); harvestmen and leg loss (13); damselfly larvae and loss of lamellae (14)]. However, these examples are cases where animals remove structures facultatively in relatively specific situations. Here, we document a case in which all males of the species remove a reproductive organ in an obligatory manner, thus representing a case of dramatic evolutionary change in behavior.

Although sexual size dimorphism is common in spiders (15–17), it is extreme in the cobweb spider, Tidarren (Araneae, Theridiidae) (18–20). In this genus, sexual size dimorphism results from a decrease in male size (19, 21, 22) and an increase in female size (20). The male pedipalps (copulatory organs) are considered large in relation to male body size (refs. 21, 23, and 24, but see ref. 25) (Fig. 1). Vollrath (21) has suggested that the pedipalp is large to enable mechanical coupling with the reproductive organ of the relatively large female. In other words, as males decreased in body size, their pedipalps did not decrease in size at the same rate (unpublished data). In most spiders, males use as copulatory organs a pair of modified appendages disconnected from the gonads. After loading or inducting them with sperm, males search for females. During copulation, males generally use both pedipalps in an alternating fashion to inseminate the paired spermaphorae in the female. In Tidarren, however, one pedipalp is removed (either left or right pedipalp, seemingly at random, see ref. 26) before sexual maturation (20, 22, 25, 26), which has also been described for another spider of similar size and closely related to Tidarren (Echinotheridion, ref. 27). Just after molting to the penultimate instar, the male secures one of its pedipalps to a silk scaffold and then twists it off by turning in circles and pushing the bulb with the third and fourth pairs of legs (26).

Previous authors have suggested that having two extremely large pedipalps presents a functional constraint (23), and that the removal of one pedipalp would enhance locomotion. Because adult male spiders actively move about in search of females (28–31) and participate in often intense intermale competition (29, 32), any decrement in locomotor ability would have important negative consequences for their reproductive fitness. This proposed explanation for the origin of the pedipalp removal behavior has remained untested; no studies have examined the potential functional consequences of locomotion with these large pedipalps.

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In this study, we had several objectives. We first attempted to measure the size of Tidarren pedipalps to estimate the loss of mass after pedipalp removal. Second, we tested the hypotheses that removal of the pedipalp by male Tidarren sisyphoides would result in an increase in maximum speed (potentially linked to intermale competition) and endurance (potentially linked to enhanced mate searching abilities and intermale competition). Third, we examined the effects of pedipalp removal on survivorship after the above taxing endurance trials. We hypothesized that individual male spiders that have removed their pedipalp will be significantly less likely to die after endurance trials compared to males that have not yet removed their pedipalp. If the presence of the two palps causes high mortality in males, then pedipalp removal may thus provide a fitness advantage, and could provide some insight into why this genus has evolved the presence of the two palps.

Materials and Methods

Subjects. Male T. sisyphoides emerge from the egg sac in the second instar and attain adulthood in three subsequent molts (M.R., personal observation). Thirteen adult individuals and 10 egg sacs were collected at the F. Edward Hebert Center of Tulane University in Belle Chase, LA, and maintained in the laboratory at room temperature (all locomotion trials also took place at 22°C). The 956 resulting spiderlings were housed individually in 20-ml glass scintillation vials with several nylon strands for web support. They were fed small mealworms (~5 mm long), wingless Drosophilina flies, and newborn crickets, and were spritzed with water twice a week. A total of 31 males in the fourth instar (penultimate) and 38 males in the fifth instar (adult) were used in this study.

Quantification of Pedipalp Mass. The male spiders’ mean weights were estimated by weighing 15 adult specimens simultaneously. The weight of the pedipalp was estimated by calculating the volume of the different body segments of three adult male spiders by approximating them to the closest geometric figure. The bodies were divided into cephalothorax (cylinder), abdomen (sphere), legs (cone), and pedipalp (ellipse), and then each segment was photographed from two different angles and digitized.

Maximum Speed. Sixteen penultimate instar spiders were observed at least once before and after pedipalp removal. Pedipalp removal usually occurs within a few hours of the molt to this instar. Individual spiders were filmed while moving on a strand of tightly stretched silk (from a virgin adult female) the observer had placed between two 15-cm-long sticks placed 5 cm apart. A small ruler (7 cm long) was glued to a second set of sticks parallel to the first. A Redlake high-speed motionscope PCI camera (60 frames per second) filmed all locomotion and a PC digitally recorded all trials. The spiders were removed from the vial by using a brush, and were placed on one of the sticks. The trial began once the animal moved onto the silk, and the trial ended when it reached the second stick. Maximum speed was defined as the maximum instantaneous velocity achieved (quantified by using PEAK PERFORMANCE software) by an animal during the trial while it was chased with a microcapillary tube. We analyzed only locomotion in which the spider moved continuously to ensure that we examined steady-state locomotion.

Potential Confounding Variables: Hardening of the Exoskeleton, Experience, and Instar Differences. After emerging from their old exoskeleton, spiders are soft and relatively immobile (30). We therefore filmed eight adult males to examine the effects of hardening of the exoskeleton on maximum speed. Each spider was run 20, 60, 120, 180, 240, and 300 min after molting. To control for a practice effect in a manner such that there were no interactions with the effects of a recent molt, we collected 13 adults in the field from female webs and filmed them twice (1-h rest between trials) following the previously outlined procedure. These males were filmed at least 5 days after molting, because the process of sperm induction takes several days after the last molt. Finally, to demonstrate that speed did not differ according to instar, we compared maximum speed in penultimate instar (n = 16) and adult (n = 8) males bearing only one pedipalp.

Endurance. Time to exhaustion was measured in 15 penultimate instar subjects bearing two pedipalps (different from the subjects used to measure maximum speed) and 17 adult subjects with one pedipalp. The same spiders could not be examined under both one- and two-pedipalp conditions because the endurance trials were extremely taxing for the animals. Arthropods experience a high rate of water loss for a period after the molt (33). All...
animals therefore were tested 4 h after molting to minimize a potential confounding effect of water loss. Subjects were chased with a small paintbrush on a sheet of paper until exhaustion, that is, when the spider did not respond to 10 consecutive touches. During the second minute of each test, the animal’s path was traced by following the spider with a pencil to estimate the distance moved during that minute, and to provide an estimate of the average running speed (distance/time) during that minute. We quantified only the second minute because handling and placement of the spider on the test arena precluded an accurate trace of the path during the first minute of each test and because tracing the entire path would have resulted in too complex a record (375 to 1,930 s in range). The track was measured by laying a piece of string over the path and measuring its total length with a ruler. Mean speed per path was calculated by dividing this total distance by 60 s. SYSTAT (SPSS Institute, Chicago) was used for statistical analysis, and SIGMAPLOT was used for creating graphs (SPSS Institute).

Results

Quantification of Pedipalp Mass. We estimate the adult male pedipalp to account on average for 10% of body mass (Fig. 1). *T. sisyphoides* males have an average body length of 1.4 mm and weight of $7.1 \times 10^{-4}$ g, whereas females are on average 6 mm in length and weigh $5.8 \times 10^{-2}$ g (Fig. 2).

Maximum Speed. Pedipalp removal significantly increased maximum speed (Fig. 3) from an average of $2.7 \pm 0.2$ (SE) cm/s to $3.8 \pm 0.3$ cm/s (paired t test, $t = 4.41$, df = 15, $P < 0.001$). Thus, spiders ran 44% faster after they had removed one pedipalp than when they still possessed both pedipalps. We used analysis of covariance (ANCOVA) to test whether the potentially confounding process of exoskeleton hardening (time after molt) affected maximum speed. Individual spiders did not differ in their slope between maximum speed (dependent variable) and time since molting (independent variable) (homogeneity of slopes test: $F_{110} = 0.65$, $P > 0.50$), and time since molting also did not affect maximum speed ($F_{1,38} = 0.26$, $P > 0.50$). A linear least-squares regression of the first (independent variable) and second (dependent variable) runs also did not reveal a significant relationship between them ($F_{1,1} = 1.2$, $r = 0.31$, df = 13, $P > 0.25$, slope = 0.38). Thus, how spiders performed on their first run did not predict how they would perform on their second run. In addition, we did not find a significant difference in maximum speed between penultimate instar and adult males with a single pedipalp (two sample t test, $t = 0.26$, df = 22, $P > 0.1$).

Endurance. For the endurance trials, spiders with two pedipalps were able to move for an average of 17 min 30 s ± 55 s, whereas spiders with one pedipalp were able to move for an average of 28 min 30 s ± 45 s (Fig. 4; two-sample t test, $t = 9.3$, df = 30, $P < 0.001$). Thus, spiders with one pedipalp had endurance capacities that were ~63% greater than spiders with two pedipalps. In addition, spiders with one pedipalp moved significantly faster ($1.4 \pm 0.04$ cm/s) than those with two pedipalps ($0.8 \pm 0.03$ cm/s) ($t = 11.5$, df = 24, $P < 0.001$). Assuming constant speeds throughout the duration of the tests, we estimated that...
animals with one pedipalp moved an average of $2,435 \pm 118$ cm or $17,393$ body lengths (based on a mean body length of 1.4 mm taken from ref. 23) before exhaustion, whereas those with two pedipalps moved and average of $822 \pm 61$ cm or $5,870$ body lengths. In other words, spiders with one pedipalp moved on average 296% greater distances than those with two pedipalps. Of 11 spiders that died immediately after an endurance trial, significantly more spiders bore two pedipalps (eight individuals, or 53% of the total number of spiders measured bearing two pedipalps) than one pedipalp (two individuals, or 12% of the total number of spiders measured bearing one pedipalp) (Fishers’ exact test, $P = 0.021$). A multiple regression using survival (1 = spider died, 2 = spider lived) as the dependent variable and endurance and average speed during the second minute as the independent variables was statistically significant ($F_{2,23} = 7.09, P < 0.01$), but endurance was the only statistically significant (and positive) predictor of survival ($P = 0.028$, standardized coefficient = 0.77). Thus, spiders with high endurance capacities tended to more often survive after endurance trials. In addition, those individuals with two pedipalps that survived the endurance trial ($n = 4$) took up to 5 days to remove the pedipalp (mean = $3.0 \pm 0.9$ days), although this process typically takes place within a few hours after the molt.

**Discussion**

One potential consequence of extreme sexual size dimorphism is conflict among characters (e.g., refs. 6 and 7). A central issue in evolutionary biology concerns the evolution of how organisms cope with such conflicts. In *T. sisyphoides*, male body size has been reduced (19–22), whereas the reproductive organs might not have changed evolutionarily in an equal manner (21). The apparent cost of such conflict resulting from this differential scaling is a relatively large pair of pedipalps (Fig. 1) that substantially impair both maximum speed and endurance. Remarkably, rather than evolving one pedipalp to overcome this functional conflict, male *Tidarren* have evolved the behavior of pedipalp removal. In this regard, our data provide evidence for an organism overcoming an evolutionary conflict by evolving a novel behavior.

Which proximate factors could explain why *Tidarren* have greater maximum speed and endurance with one compared to two pedipalps? One explanation is that the observed performance difference is not caused by removal of the pedipalp, but rather to gross anatomical, physiological, or behavioral changes in male spiders as they molt from the fourth (penultimate) to the fifth (adult) instar. We consider this possibility unlikely. First, male size and overall morphology change very little between the fourth and fifth instars in *Tidarren* (C. Linn, personal communication), and we found no difference in maximum speed between spiders bearing one pedipalp in these two instars. In fact, relative pedipalp size increases dramatically (~370%) from the third to the fourth instar, whereas it increases only slightly from the fourth to the fifth instar (~2%) (C. Linn, personal communication). Therefore, the two pedipalps become a hindrance only after the molt into the fourth instar, and this might explain why the pedipalp is not removed earlier. In terms of physiological changes, spiders produce hormones (e.g., ecdysone) that play a vital role in the molting process (29). However, we are not aware of any hormones that would restructure the physiological system to provide the dramatic changes in performance that we observed after pedipalp removal. Moreover, the oxygen transport system of spiders is not known to undergo dramatic changes between instars (29). Finally, it is unlikely that any differences in general natural history and behavior between individuals in the fourth and fifth instars could explain the observed performance increase after pedipalp removal, because our subjects were in a period of relative quiescence, between the molting and pedipalp removal (fourth instar) or sperm induction (fifth instar) events.

A more likely possibility to explain the large difference in locomotor performance between spiders bearing one or two pedipalps is that the presence of both pedipalps might mechanically interfere with locomotion, such as impairing limb movement or by dragging on the locomotor surface (Fig. 1). Locomotion would become more energetically expensive for spiders moving long distances, and maximum speed would be reduced. The structure of male spider pedipalps is complex, and dragging when moving on flat surfaces could damage pedipalpal components needed for coupling with female genitalia. Another non-inclusive possibility is that the loss of mass caused by removal of the pedipalp enables *Tidarren* to move both faster and longer relative to their body size. *Tidarren* males are very small, so the effects of gravity on both horizontal and vertical locomotion are minor compared to larger animals (34, 35). Loss of mass will likely have a lesser impact on the locomotion of a smaller animal compared to the effect of a proportional loss in mass on a larger animal. Ideally, this latter hypothesis would most profitably be tested by adding or subtracting weights equaling the mass of a pedipalp, but adding such a small mass would be extremely difficult. However, further experiments that examine the position of the pedipalps during locomotion with one and two pedipalps might differentiate among these hypotheses. In addition, pedipalp removal does not seem to have significant proximal costs for the spider. For instance, the animals twist in circles to remove the pedipalp, thus effectively sealing the wound and apparently preventing excessive fluid loss and infection.

The endurance capacities of male *Tidarren* are remarkably high for a spider (36–42) and are comparable to many mammal species (43, 44). This high endurance is especially intriguing in the light of the lack of a sophisticated circulatory system in spiders that would enable high rates of oxygen exchange, such as found in birds and mammals (45). More remarkably, *Tidarren* males ran for very long distances at relatively fast speeds, which should lead to high levels of lactate in their tissues caused by anaerobic processes (39, 40). Indeed, in cases where the spider died after the endurance trials, the most likely cause was the build-up of lactate (39, 40). The fact that males with two pedipalps died significantly more often than males with one indicates that long movements with two pedipalps are especially exhausting.

The negative effect of pedipalp size on both aerobic and anaerobic locomotion could have profound implications for the reproductive ecology of *Tidarren*. Several lines of evidence suggest that *Tidarren* males move long distances (relative to their size). First, a large body of literature shows that male spiders, on reaching adulthood, shift from being sedentary to actively searching for females. Male spiders frequently travel long distances, often vertically, to find a mate, because females can be dispersed widely (31, 46). Second, some simple considerations show that *Tidarren* males may be forced to move long distances because of their very small size. For example, if a *Tidarren* male moves only 2 meters, that distance is equivalent to ~1,400 body lengths, which is similar to a 3-m tetrapod (e.g., an alligator) moving ~4.3 km. Added to this is intense intermale competition that might result in males often moving from female to female as is the case in other species of spiders. If *Tidarren* males do indeed move long distances, then the significantly lower mortality of one-pedipalp males compared to two-pedipalp males after endurance trials may provide an immediate fitness advantage for palp-removal. However, to test this idea rigorously, one would need detailed field data on both movement distances and speeds of *Tidarren* males during mate searching.

Another context in which locomotion might be important for *Tidarren* is during scramble competition for a female (T.E.C., C. Linn, J. DuFatta, M.R., J. Feldman, and T. Bukowski,
unpublished data). For example, once a male has located a female, maximum speed may be especially important for outcompeting rival males (47–50) on the female’s web. Up to 25 male T. sisyphoides have been observed at one time on a single, unrestrained penultimate instar female’s web (T.E.C., C. Linn, J. DuFatta, M.R., J. Feldman, and T. Bukowski, unpublished data). Furthermore, in staged encounters with a newly molted female, the first male to reach the female’s abdomen usually copulates. Endurance may also be important for the prolonged female, the first male to reach the female’s abdomen (T.E.C., C. Linn, J. DuFatta, M.R., J. Feldman, and T. Bukowski, unpublished data).

It is possible that ancestral male Tidarren spiders that first exhibited pedipalp removal were at a selective advantage relative to males that did not because of the enhanced locomotor performance that a one-pedipalp condition provides. However, one must also consider the possibility that the behavior of pedipalp removal has evolved for reasons more related to reproduction. For instance, as Tidarren and Echinotheridion males die in copula (Tidarren caneculatum, Tidarren argo, T. sisyphoides, Echinotheridion giberrosum: refs. 25, 22, 20, and 27, respectively), they can insert one pedipalp only once and inseminate only one of the female’s two spermathecae. Consequently, removal of a pedipalp might result in conservation of gametes because only one pedipalp is used in copulation. If both pedipalps were filled and only one used for copulation, the sperm in the unused palp would be wasted. Males could simply leave one palp unfilled at sperm induction. The fact that they do not suggests that there are benefits to palp removal, as noted earlier.

Finally, it is not clear why Tidarren males have not evolved a single-pedipalp condition, which would obviate the need for removing a seemingly functional organ. Pedipalps might play an important role in other functions during the early instars, such as in prey handling. In addition, the genetic traits underlying the development of two pedipalps may be more difficult to overcome than simply evolving a behavior of pedipalp removal. Thus, data that shed light on the developmental genetics of pedipalps in spiders would be especially interesting within this evolutionary context.

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