

Developmental asynchrony: A potential cost of extreme sexual size dimorphism in seasonal environments

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Abstract. Female-biased sexual size dimorphism reaches an extreme in the genus *Trichonephila* Dahl, 1911 (Araneae: Nephilidae). Large female size is accomplished through the addition of juvenile instars and is likely the result of fecundity selection. However, additional instars in only one sex could result in significant developmental asynchrony. To minimize asynchrony, males should grow more slowly and females more rapidly. To test this prediction, we reared spiders from six disjunct populations of *Trichonephila clavipes* (Linnaeus, 1767) on three different diets, inducing variation in growth rates. Males on all diets grew more slowly than females, but still matured significantly earlier. In the field, mature males cohabit with juvenile females, and in the laboratory, mean male age at maturity corresponded to the age at which females achieve the minimum size observed to host males. However, a significant fraction of males mature earlier than concurrently-emergent females reach that minimum size—and before any females reached sexual maturity. Thus, early-maturing males may perish before reproducing, especially in temperate environments. We propose that (1) sexual size dimorphism and early male maturation evolved in tropical environments characteristic for *T. clavipes*, where adult females are found all year round. Univoltinism is likely a secondary trait in highly seasonal environments. Seasonal populations are likely mixes of local individuals and recent tropical immigrants, which could mitigate the loss of early males and retain this apparently maladaptive trait outside the tropics. (2) Environmental variation generates asynchronous emergence at the beginning of the growing season, allowing later-emergent males to mate with early-emergent females.

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Sexual size dimorphism (SSD) due to female gigantism, male dwarfism, or a combination thereof, has evolved repeatedly in arthropods. Among terrestrial arthropods SSD is particularly pronounced in spiders (Hormiga et al. 2000; Neumann & Schneider 2015; Kuntner et al. 2019). For example, *Trichonephila* Dahl, 1911 displays extreme sexual size dimorphism (eSSD) defined as female to male body length ≥ 2 , a phenomenon that is relatively rare outside spiders (Kuntner & Coddington 2020). The common, and likely correct, evolutionary explanation for this dimorphism is selection for increased fecundity in females coupled with selection on males favoring small size relative to the female (Darwin 1871; Head 1995; Prenter et al. 1999; Foellmer & Moya-Laraño 2007; but see Kuntner & Elgar 2014). Selection on relatively small male size could be explained by a variety of factors and may be species or clade specific (Kuntner et al. 2019; Kuntner & Coddington 2020). Gravity and increased small male agility (Moya-Laraño et al. 2009; Corcobado et al. 2010; Grossi & Canals 2015; but see Quinones-Lebrón et al. 2019), scramble (Danielson-François et al. 2012; Herberstein et al. 2017) and sperm competition (Neumann & Schneider 2015), stealth mating, and other factors may all favor small males (Kuntner & Coddington 2020), though evidence for any given explanation remains ambiguous. Furthermore, access to virgin females and copulation with molting females increase male fitness and would select for rapid maturation and consequently small size in males (e.g., Uhl et al. 2015; Biaggion et al. 2016, Neumann et al. 2017). Large female size can come about by maintaining the same number of instars for both sexes while the females grow more per instar by lengthening each instar, or by the females going through additional instars before reaching maturity. In either case, the evolution of sexual size dimorphism in arthropods carries with it the cost of potential asynchrony of maturation times between the sexes (Calabrese & Fagan 2004; Teder 2014; Kuntner & Coddington 2020),

resulting in so-called incidental protandry when the smaller sex is male (Matsuura 2006).

This cost may be negligible in relatively aseasonal tropical environments where mature females may be found year-round. In such populations, males and females may have different development times (Herberstein et al. 2017; Quinones-Lebrón et al. 2021; Harvey 2022), and the larger and older females mate with smaller and younger males of later generations (Robinson & Robinson 1973; Higgins 2002; Kleinteich & Schneider 2011). In strongly seasonal environments with univoltine populations, however, extreme protandry may have a cost, at least during the earliest part of the mating season for males and likely also at the end of the season for females.

The potential negative impacts of protandry could be ameliorated by changes in timing of maturation or rate of development. In this scenario, development times must diverge along with size if male and female maturation times are to be compatible. One possible path to synchronized maturation times is for the two sexes to evolve different developmental rates, with the larger females developing faster than the smaller males (Blanckenhorn et al. 2007). This can be done either by accelerating female development, at the cost of increased metabolic needs of the larger and faster developing females (Legrand & Morse 2000; Badyaev 2002; Higgins & Goodnight 2010; Blackledge 2011), or by delayed development in the males, at the cost of delayed reproduction and potentially increased mortality in the juvenile stages. If the two sexes maintain the same developmental rate, they will mature asynchronously: the larger females maturing later than the smaller males (Thornhill & Alcock 1983; Teder 2014; Kuntner & Coddington 2020). In highly seasonal environments, this strategy has the cost that males that develop early in the season may have to wait for an extended period of time before mature females become available (Kuntner & Coddington 2020), which will not be a viable option in species with high male mortality or in species where the male depends on the female for resources.

Similarly, females that develop late in the season may not be able to find mature males with which to mate (Higgins 1989). Such asynchrony can, at least theoretically, increase the risk of local extinction (Calabrese & Fagan 2004).

The orb-weaving genus *Trichonephila* (Nephilidae) is an extreme example of female biased sexual size dimorphism in spiders (although the WSC currently includes *Trichonephila* and other nephilids in the Araneidae, here we follow Kuntner et al. (2013, 2023) and retain the Nephilidae). *Trichonephila* males are only about 10% the mass of females in most species (Kuntner & Coddington 2009, 2020; Kuntner & Cheng 2016). Thus, this genus is an excellent model system for investigating the ecological and evolutionary consequences of this phenomenon. Size dimorphism in this genus is attained by adding juvenile instars to female development (Higgins 2002; Esperk et al. 2007; Higgins & Goodnight 2011), and in some related nephilid spiders, by molting of adult females (Kuntner et al. 2012). Recent analysis of size variation within the orb-weaving genus *Trichonephila* and the family Nephilidae indicates that male and female sizes are evolving independently (Kuntner & Coddington 2009, 2020; Higgins et al. 2011; Kuntner et al. 2019). Without concurrent changes in the relative rates of development of the two sexes, additional juvenile instars in female development will lead to protandry, with the males maturing earlier than the females. Importantly, in the golden silk spider *Trichonephila clavipes* (Linnaeus, 1767), free-living males are very short-lived unless they can locate a juvenile female willing to host them as kleptoparasites (Vollrath & Parker 1992; Burnham & Anderson 2002; however, see Meraz et al. 2012).

The failure to observe extreme differences in timing of maturity in field studies of several sexually-size dimorphic arthropods led Vollrath (1998) and Blanckenhorn et al. (2007) to conclude that unless there is strong selective advantage to protandry, the two sexes will have significantly different rates of growth and development. Nevertheless, lab fed *Steatoda grossa* (C. L. Koch, 1838) maturation rates were highly food dependent, predicting that asymmetric access to resources in nature may cause a skew in timing of maturity (Harvey 2022, see also Quinones-Lebrón et al. 2021). As pointed out by Badyaev (2002), population-level studies of adult SSD cannot answer questions about the evolution of the developmental differences producing SSD. However, tracking juveniles over their entire development in the field is difficult. Moreover, wandering males are notoriously difficult to locate (Kasumovic et al. 2007) and therefore early-maturing males are unlikely to be observed 'in transit'. However, they might be located in the webs of juvenile females that they seek to monopolize (Danielson-François et al. 2012; Uhl et al. 2015; Biaggion et al. 2016; Herberstein et al. 2017).

To our knowledge, the prediction of sex-dependent differences in developmental rate has never been tested through experimental rearing of an extremely SSD species.

METHODS

Study organism.—*Trichonephila clavipes* is a widely-distributed orb-weaving spider (Kuntner et al. 2008). It is found in a wide variety of habitat types (Higgins 2000), but the majority of its distribution range is tropical (World Spider Catalog 2022). It is well known for the large size of the females (reaching 3 g in some populations) and the small size of the males (about 10% of the female mass). In natural settings, the populations are protandrous, with males maturing

2–8 weeks before the females (Vollrath 1980; Higgins 2000). Mature males are physiologically incapable of building a capture orb (they lose the ability to spin sticky silk, Foelix 1982), and must co-habit with a female, living as kleptoparasites by eating silk, feeding with the female, or stealing prey (Christenson & Goist 1979; LEH pers. obs.). In the field, males that fail to find a female's web survive on average 3 days (Vollrath & Parker 1992; Kasumovic et al. 2007; however, see Meraz et al. 2012).

Study populations.—In 1989 and 1990, Higgins conducted regular population surveys of six populations in Mexico (Higgins 2000). Surveys involved bimonthly visits to each site to determine the size distribution of the population, including counts of males resident on juvenile and mature female webs. We use records of five of these populations (discarding Arroyo Frio because of a gap in records at the time when cohabitation began) to determine the minimum size at which juvenile females will tolerate cohabitation by kleptoparasitic males. Pooling across census dates and across years Higgins (2000) showed little difference within sites in phenology between 1989 and 1990. For this analysis, we do not separate data from juvenile and mature females because, from the perspective of the early-maturing male, the ability to find and become established on any female's web is key to survival (Christenson & Goist 1979; Kasumovic et al. 2007).

In fall 2006, we collected gravid female spiders from six disjunct sites in Mexico. These sites include two lowland wet tropical forests, Nanciyaga (18° 27' N 95° 4' W, <50 m altitude) and Quihuitztlán (19° 40' N, 96° 25' W, 170 m altitude) Veracruz; two mid-altitude temperate forests, Fortín de las Flores (18° 54' N 96° 60' W, 990 m altitude) and Xalapa (19° 30' N 96° 53' W, 1000 m altitude) Veracruz; and two seasonally-dry lowland forests in the Isthmus of Tehuantepec, Sayula de Alemán, Veracruz (17° 52' N 94° 59' W 80 m altitude) and Tolosita, Oaxaca (17° 20' N 95° 5' W 50 m altitude). We collected in all sites in Mexico under a Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) permit issued to Juan Nuñez Farfán (Mexican permits allow foreign collaborators; number FLO 0014).

All of these populations are normally univoltine due to strong seasonality: the Veracruz sites are seasonally cold and the Tehuantepec Isthmus sites are seasonally cool and dry (Garcia 1973; Higgins 2000). In all of these sites, eggs hatch in the late summer and early fall, and spiderlings pass the cold or dry season as first-instar juveniles in the egg sac. In the lab, we held egg sacs under warm moist conditions until hatching and first molts had occurred then moved them to over-wintering conditions until we were ready to use them. For the rearing experiments, one haphazardly chosen egg sac represents each population, thus our analyses focus on between-sex differences within each diet treatment, with family/population as an independent random variable. A total of 193 spiderlings from Mexico started the experiment, ranging from 28 to 36 from each of the egg sacs.

Rearing and experimental diets.—We reared spiders in individual boxes, housed in a long-day growth chamber for the first 100 days (Percival incubator set at 26°C, 70% relative humidity (RH), 13:11 L:D) and thereafter in a short-day walk-in environmental chamber (11:13 L:D; average temperature 24°C and 72% RH established by AirKing 45.4 humidifier). Because of the large time investment in hand-feeding spiders, we staggered the emergence of the spiderlings from different egg sacs by keeping them at 16°C (lowland sites) or 4°C (highland sites), 10:14 L:D in boxes with damp paper towels to maintain high humidity until needed. Among the Mexican populations, we found no correlation of mean time to

Table 1.—Number of spiders in each treatment group and mean spiderling size within each population at the fourth instar, and date of molt to the third instar (initiation of experimental treatment). † The variation in the range of dates for molt to the third instar (initiation of experimental treatment) among families reflects differences in tendency of spiderlings to emerge synchronously and differences in homogeneity of growth in the first two instars (group living).

Population	Diet			TPL mean, cm (SD)	Julian date of molt to third instar, mean (SD†)
	High	Medium	Low		
Nancyaga	15	11	15	0.13 (0.19)	97 (12)
Quihuitzilán	9	8	11	0.12 (0.14)	61 (4.3)
Fortín de las Flores	10	7	11	0.13 (0.24)	102 (15)
Xalapa	13	11	12	0.14 (0.17)	49 (3.2)
Sayala	11	11	9	0.12 (0.14)	63 (21)
Tolosita	9	10	10	0.13 (0.20)	162 (5.0)

maturity with mean date of initiation (Table 1) for either males ($F_{(1,4)} = 0.27$, $P = 0.62$) or females ($F_{(1,4)} = 0.02$, $P = 0.90$); the two populations with the shortest development time were the first and the last to be brought into the experiments.

When we wished to start a new batch, we moved spiderlings within their egg sacs to the Percival chamber (long-day conditions), hanging them from 2" mesh chicken wire (which also served for web support) in clear plastic boxes (31 cm wide x 23.5 high x 11 deep cm, Pioneer plastics). Mild disturbance (pulling on silk of the egg sac with forceps) and release of protein-enhanced *Drosophila melanogaster* (Mayntz et al. 2003) encouraged emergence, generally over 1–5 days although spiderlings from Nancyaga took more than 10 days to emerge. Emerging spiderlings built communal webs and only rarely captured live prey; dispersing spiderlings built solitary orb-webs. We fed spiderlings protein-enhanced *D. melanogaster ad libitum* until they molted to the 3rd instar, as determined by leg I tibia + patella length (TPL) ≥ 0.1 cm. The experimental treatment (day 0) started when we moved 3rd instar spiderlings to small individual boxes with chicken wire mesh (11 cm wide x 11 cm high x 4 cm deep) and randomly assigned each to a feeding treatment of low, medium, or high food availability. Egg sacs were haphazardly chosen from each population. For Nancyaga and Sayala, we had one large and one small clutch hatch and for the remainder we had one egg sac. There were no significant differences between the families from Nancyaga or Sayala, and we pooled the data for each population. Our analyses focus on between-sex differences within each diet treatment, with population as an independent random variable. A total of 193 spiderlings started the experiment, ranging from 28 to 36 from each population.

Since spiders build larger webs as they grow, we moved individuals to medium-sized boxes (22 cm wide x 10 high x 10 deep) when they molted to the 6th instar (TPL ca 0.3 cm). We oriented the boxes horizontally for smaller spiders ($0.3 \text{ cm} \leq \text{TPL} < 0.5 \text{ cm}$) and vertically for larger juveniles ($\text{TPL} \geq 0.5 \text{ cm}$). Juvenile females required an additional move when they molted to TPL ≥ 0.7 cm (large box 31 cm wide x 23.5 high x 11 deep cm). All but 3 males reached sexual maturity prior to TPL = 0.6 cm. To mimic photoperiod cues in natural populations, we moved all spiders to short-day conditions in the walk-in chamber approximately 100 days after starting the experimental treatment (mean 101 days \pm 2). Most males were sexually mature at the time of the move.

We checked and fed all spiders live prey twice weekly, recording if the spider had molted and recalculating diet after each molt. Analysis of a subset of spiders showed that, on average, spiders captured 98% of all flies provided. We calculated food as a percentage of the mass of the spider (spider mass estimated from published equations based upon linear dimensions; Higgins 1992). Initial treatments were Low: 35% body mass/week; Medium: 56%; High: 84%. For spiders TPL < 0.5 cm, we based diets upon the mean mass of the first 4–7 spiders reaching those instars. After TPL ≥ 0.5 cm, we calculated diets individually for each animal. These food levels are within the range observed in the field (Higgins 2000) but avoided levels resulting in death through overeating (Higgins & Rankin 2001). We increased food levels by 50% when spiders molted to the ninth instar because prior results indicated that dietary requirements increase non-allometrically for larger juvenile females (Higgins & Goodnight 2010). Most spiders experiencing this increase in food availability were females.

Diets varied quantitatively across the different treatments and qualitatively across the development of the spiders: spiders in the same instar received the same quality diet. The qualitative shifts were necessary for logistical reasons: if we had fed only *D. melanogaster* through the entire development, the large, late-instar juvenile females would have required hundreds of fruit flies each week. We calculated the prey numbers provided based upon the mean mass of each prey type: high-protein *D. melanogaster* (mean mass 0.748 mg, SD = 0.110, $n = 11$); normal *D. virilis* (mean mass 1.60 mg, SD = 0.239, $n = 15$; *D. virilis* on protein-supplemented diets failed because of fungal overgrowth). At the tenth instar, we added commercially-reared high-protein house-flies to juvenile females' diets (*Musca domestica*, SpiderPharm Inc; mean mass 11.65 mg, SD = 2.077, $n = 10$).

Collection and analysis of developmental data.—Males and females can be distinguished only when males molt to the penultimate instar (last juvenile instar) and develop the secondary sexual characteristics of swollen pedipalps (non-walking legs used for sperm transfer characteristic of Araneae). Juveniles dying before the sixth instar without swollen pedipalps cannot be sexed. We classified as female all individuals that did not show male secondary sexual characteristics at the sixth instar. Only three males were penultimates in the seventh instar and would have been misclassified if they had died in the sixth instar. For analyses of developmental trajectories, we discarded all data from unsexed individuals that died prior to the sixth instar without displaying secondary sexual characters.

Spiders grow between instars by molting, and during instars by gaining mass stored in the abdomen. We measured size as leg I tibia + patella length, (TPL, cm; using Helios[®] needle-point calipers). The shed exoskeleton after each molt serves as a physical record of the actual TPL of the prior instar. We used measurements of TPL from the exoskeletons to verify and, on occasion, correct errors in measurements of live animals. To test for effects of population, diet, and sex on juvenile development, we compared the time individual spiders took to pass through each instar. Since intermolt duration increases with spider size (Higgins 1993; Higgins & Goodnight 2011), we included instar number as a covariate in this analysis. We compared timing of maturation as days from initiation of experimental treatment until maturity of males and females. For completeness, we also report the mean and variation in the instar of maturation in males and females of each population.

Spider size and instar duration were natural log-transformed to normalize the distributions prior to statistical analyses. Total development time was normally distributed, so we did not transform those

data. We used JMP version Pro 11 for all statistical analyses. For the analyses of developmental data, we chose to do ANOVA, using Akaike information criterion (AIC) to select an appropriate model (Burnham & Anderson 2002). Technically this data set violates the assumption of independence of measurements for ANOVA, since measurements across instars for each individual are interdependent. However, common alternatives to standard ANOVA are also not acceptable. If instar is treated as a nested factor, the interactions between instar and diet or sex will be undetectable because each individual has only a single sex, and experiences only one diet regime. Alternatively, a repeated measures ANOVA is not applicable since developmental data are necessarily serially correlated and violate the assumption of sphericity (equal correlations between all pairs of observations).

To test whether it was valid to remove the nesting factor and treat the instar number as an independent rather than nested factor, we did a one way ANOVA with individual, instar, and the individual by instar interaction as factors. Individual and instar were significant ($P = 0.0002$, $P < 0.0001$ respectively), and the individual by instar interaction was not significant ($P = 0.1146$). In calculating the ANOVA, the sums of squares due to instar nested within individual can be decomposed into the effect of instar and the instar by individual interaction (Goodnight 1988). By ignoring the nesting factor we are, in effect, pooling the instar by individual interaction with the error term. Because this interaction was not significant, we decided that this simplification was justified.

To test for differences in survivorship during development on all three diets across all populations, we used data from all spiderlings that established webs in individual boxes, analyzing survivorship (survival to molt again, or death) in each instar. The data are combined for male and female juveniles because we cannot sex younger individuals; juveniles after the sixth instar are all females. We “censored” maturation of males and females – mortality after maturation is not included. In this analysis, we excluded the data from Xalapa and Quihuitztlán females because of exaggerated mortality due to a week-long failure of the humidifier in the walk-in chamber (all mortality occurred within the week of, and week following, the failure of the humidifier). We include data from these populations predating this accident in the analyses of development described above; because start-dates for populations were staggered, the accident affected only two populations (Higgins & Goodnight 2011). The analyses of survivorship used a regression model with a Weibull distribution.

RESULTS

Experimental growth and development.—Of the 193 spiders that started the experiment, 148 lived until they could be sexed. The survivors were distributed fairly evenly across all populations (Fortín: 15, Xalapa: 32, Nanciyaga: 30, Quihuitztlán: 21, Sayula: 28, Tolosita: 22). Size following the first experimental molt (to instar 4) did not vary across diets (ANOVA: $F_{(2, 140)} = 0.962$, $P = 0.383$) and males were slightly but non-significantly larger than females ($F_{(1, 140)} = 3.75$, $P = 0.0546$). Spiderlings from different populations did differ significantly in size at this stage (Table 1; $F_{(5, 140)} = 5.148$, $P = 0.0002$); spiders from Xalapa and Nanciyaga were larger than those from Sayula and Quihuitztlán; those of Tolosita and Fortín were intermediate (*post hoc* Tukey HSD).

We compared male and female juvenile development by truncating the data at the sixth instar, the stage at which half of the males

Table 2.—ANOVAs of individual juvenile growth parameters across all families with instar number as covariate, truncated at the 6th instar and excluding molts to male maturity and molts resulting in death.

A. Instar duration (days, log-transformed)				
Source	DF	Sum of squares	F	P
diet	2	14.1	50.1	<0.0001
sex	1	2.67	18.9	<0.0001
population	5	2.55	3.61	0.0031
instar	1	55.9	396	<0.0001
population x instar	5	2.12	3.01	0.0108
diet x instar	2	1.69	5.97	0.0027
population x diet	10	2.76	1.96	0.0353
error	745	105		
B. Change in size (log (postmolt TPL - premolt TPL)).				
Source	DF	Sum of squares	F	P
diet	2	5.57	17.2	<0.0001
sex	1	3.95	24.4	<0.0001
instar	1	23.4	145	<0.0001
sex x instar	1	1.40	8.68	0.0033
error	683	111		

were mature and all but three of the remainder were in the penultimate stage. A fully-factorial ANOVA of log-transformed instar duration with instar number as the covariate (Table 2a) revealed that male intermolt periods were significantly longer than female intermolt periods (Least-squares mean intermolt duration, days (back-transformed) \pm SE: Males: 21.4 ± 1.03 ; Females: 18.6 ± 1.02). Instar duration varied among populations and increased with increasing size (instar) and decreasing food levels as observed for females earlier (Higgins & Goodnight 2010). The increased intermolt was not associated with increased growth at ecdysis (ln postmolt TPL minus ln premolt TPL; Table 2b): males grew slightly less at each molt than females (least-squares mean change in size, cm, (back-transformed) \pm SE: Males: 0.080 ± 1.03 ; Females: 0.094 ± 1.02). Spiders on the high diet and at larger instars grew more at each molt, and the effect of sex increased at larger instars.

As found in prior field observations (Higgins 1993), males molting to sexual maturity grew less than juveniles molting to a juvenile instar in instars 3–6 (Table 3; Least-squares mean of growth/molts, cm (back-transformed) \pm SE: juvenile molt: 0.101 ± 1.02 ; molt to maturity: 0.071 ± 1.06). The difference between maturation and non-maturing molts was less in spiders on the medium diet, resulting in a significant diet effect and interaction between molt and diet. The difference increased slightly at later instars (significant instar effect).

Sixty-two males reached sexual maturity, distributed across all populations but not across all diets (recall that the design could not be balanced by sex within diet because dispersing spiderlings cannot be sexed). Males matured between the third and seventh instar. The different populations varied in the mean number of juvenile instars prior to male maturity and this was unaffected by diet (ANOVA: $n = 56$; population: $F_{(5,51)} = 12.8$, $P < 0.0001$; diet: $F_{(2,51)} = 0.156$, $P = 0.856$). *A posteriori* comparisons revealed that males from the Quihuitztlán and Xalapa populations matured on average one instar earlier than males from the other four populations. Note that the failure of the humidifier does not explain these differences, as males matured before being moved to the walk-in chamber where that accident occurred. Reflecting the

Table 3.—ANOVA of change in size (log (postmolt TPL-premolt TPL, mm)) in individuals molting to a juvenile instar versus males molting to maturity at that instar.

Source	DF	Sum of squares	F	P
population	5	0.936	1.17	0.322
diet	2	1.35	4.23	0.0150
instar	1	14.8	92.6	<0.0001
mature v juvenile	1	5.04	31.5	<0.001
diet x (mat v juv)	2	1.34	4.19	0.0156
error	528	84.3		

strong dependency of size at maturity on number of juvenile instars, ANOVA of male size at maturity showed the same pattern, with significant effects of population and no significant effects of diet (ANOVA: $n = 56$, population: $F_{(5, 47)} = 10.2, P < 0.0001$; diet: $F_{(2,47)} = 1.44, P = 0.248$). Spiders that added extra instars matured at larger sizes (across all populations, ANOVA $F_{(1, 53)} = 99.5, MS = 2.31, P < 0.0001$). We could not test for population x diet effects in either analysis because there were too few males in the high diet in several populations.

Sex affects survival to and age at maturity.—Females and males matured after a different number of juvenile instars: females in the eleventh or twelfth instar and males at a median instar of six. The delay in female maturation for several instars was accompanied by increased resource requirements beyond that predicted by their size (Higgins & Goodnight 2010). We thus wanted to test for differences in juvenile survivorship to maturity in males and females under dietary stress; survival past maturity is not included in this survival analysis. Before examining survivorship, we removed the spiders from Xalapa and Quihuiztlan from the analysis because of exaggerated female mortality due to humidifier failure in the walk-in chamber (see above). Of the spiders in the remaining four populations, 69 died prior to maturation. Across the entire developmental period, population had no effect on survival (population: Likelihood ratio chi-square = 5.06, $df = 3, P = 0.17$; population x diet: Likelihood ratio chi-square = 1.03, $df = 6, P = 0.98$). Survival was strongly influenced by diet (Fig. 1; Likelihood ratio chi-square = 27.5, $df = 2, P < 0.0001$).

Since the average male matures in the sixth instar, we can use juvenile survival to the sixth instar as a conservative estimate of juvenile male survivorship on the experimental diets (vertical line in Fig. 1; only one penultimate male died). We assume that smaller juveniles are a mixture of males and females, and we have no evidence of sex-specific mortality of juveniles. About 90% of the spiders fed medium and high diets survived to the sixth instar, and on the low diet over 70% of juveniles reached the sixth instar (Fig. 1). This indicates that males are likely to survive to maturity even with low rates of prey capture.

In contrast, female spiders were less likely to reach sexual maturity on any diet (Fig. 1: area to right of vertical line; Higgins & Goodnight 2010). Survivorship was highest for individuals in the “high” diet, where 17 out of 29 known females from all families except Xalapa and Quihuiztlan reached sexual maturity. Details of female developmental response to diet are described elsewhere (Higgins & Goodnight 2011) and here we focus on the total time to development.

Our final analysis of the developmental data compares male and female total development time (days to maturity) as a function of sex, population, and diet for four families (we did not attempt to test for differences among families in response to diet with these small

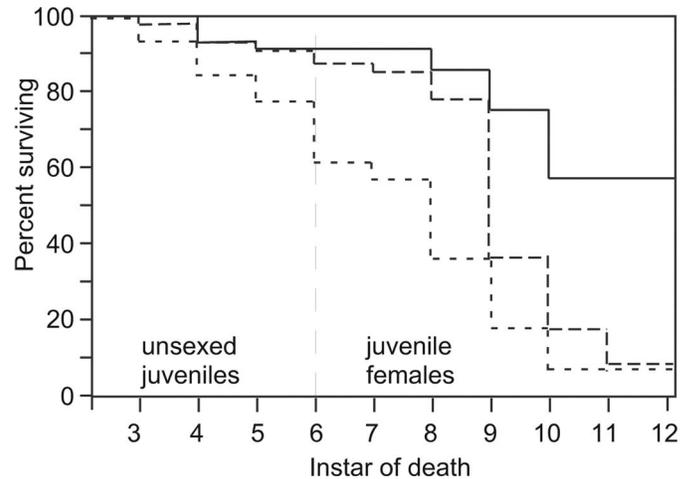


Figure 1.—Survival on each diet pooled across four populations (excluding Xalapa and Quihuiztlan; see text), censored by maturity and excluding individuals that died without molting. The vertical line at instar 7 indicates the latest instar at which males reach maturity; male survival to maturity is thus survival to instars 6 or fewer. All individuals in instar 7 and higher are juvenile females, which reach maturity at instars 11 or 12. Short dash: low diet; Long dash: medium diet; Solid line: high diet.

samples). Total development time was normally distributed and, unlike the prior analyses, we proceeded with untransformed data.

Among these four families, females matured on average 77 days later than males (females: mean 194 days \pm 8.32 SE; males: mean 117 days \pm 4.01 SE; Fig. 2). Across families, spiders reached maturity at the same time (no population effect) and responded similarly to diet (no population x diet effect; Table 4). Only males on the lowest diet (slowest development) overlapped temporally with females on the highest diet (fastest development). The significant interaction of sex with diet reflects the fact that female development changed much more strongly in response to dietary restrictions compared to their male siblings (Higgins & Goodnight 2011). The data were insufficient to test for a three-way interaction of sex, population, and diet.

To visualize the developmental trajectories of males and females, we plotted age at each molt (as days since treatment started) against spider size (TPL, cm) for all spiders (Fig. 3; excluding individuals

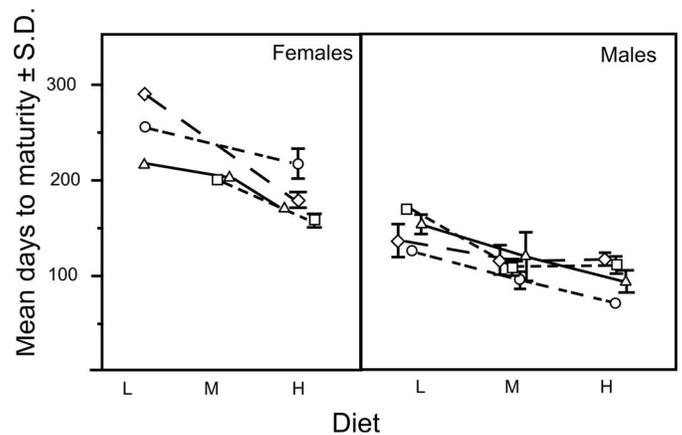


Figure 2.—Norm of reaction time to maturity for females and males from each population on each diet (X axis), excluding Xalapa and Quihuiztlan (see text). Diamonds: Nanciyaga, Circles: Fortín de las Flores, Squares: Sayala; Triangles: Tolosita.

Table 4.—ANOVA of days to maturity in males and females from four Mexican populations (excluding Xalapa and Quihuiztlan; see text).

Source	DF	Sum of Squares	F	P
population	3	1116	0.903	0.448
diet	2	18,173	22.1	<0.0001
population x diet	6	1396	0.565	0.7558
sex	1	61,845	150	<0.0001
population x sex	3	4792	3.88	0.016
diet x sex	2	2765	3.36	0.044
error	42	17,295		

that died before being sexed). Females grew more rapidly than males, supporting our hypothesis that there are sex-dependent differences in development. However, as indicated in the analysis of age at maturity, these differences are insufficient to ensure synchronous maturation.

Female size distribution and male residence.—In the field, only juvenile females larger than 0.75 cm TPL regularly hosted kleptoparasitic males (Fig. 4). Juveniles smaller than 0.5 cm TPL cannot be sexed and were never observed with males ($n = 848$ spiders across all populations). Similarly, only 6% of juveniles of TPL between 0.5 and 0.75 cm (which also cannot be reliably sexed)

hosted males ($n = 93$ spiders), despite the strongly male-biased operational sex ratio early in the growing season (Higgins 2000). Why males are absent from webs of small juvenile females is unknown. To estimate the likelihood of an early-emergent mature male finding a host female, we compared the distribution of age at maturity for all laboratory-reared males to age at 0.75 cm TPL for all laboratory-reared females. We found that average male age at maturity corresponded to average female age at the size most likely to tolerate male kleptoparasitism, but many males mature earlier (Fig. 5). Roughly 40% of males reach maturity prior to 100 days of age (Fig. 5a). In contrast, fewer than 10% of females reach 0.75 cm TPL before 100 days of age (Fig. 5b). Early-emergent males maturing younger than 100 days of age are unlikely to find a host female and thus are likely to die without reproducing.

DISCUSSION

Extreme female gigantism seen in arthropods generally appears to evolve via the addition of juvenile instars in female development (Esperk et al. 2007). *Trichonephila* spiders follow this pattern, with females going through four or more additional instars beyond the six instars seen in male development (Hormiga et al. 2000; Kuntner & Coddington 2009, 2020; however, see Vollrath & Parker 1992). The maintenance over evolutionary time of small male size while

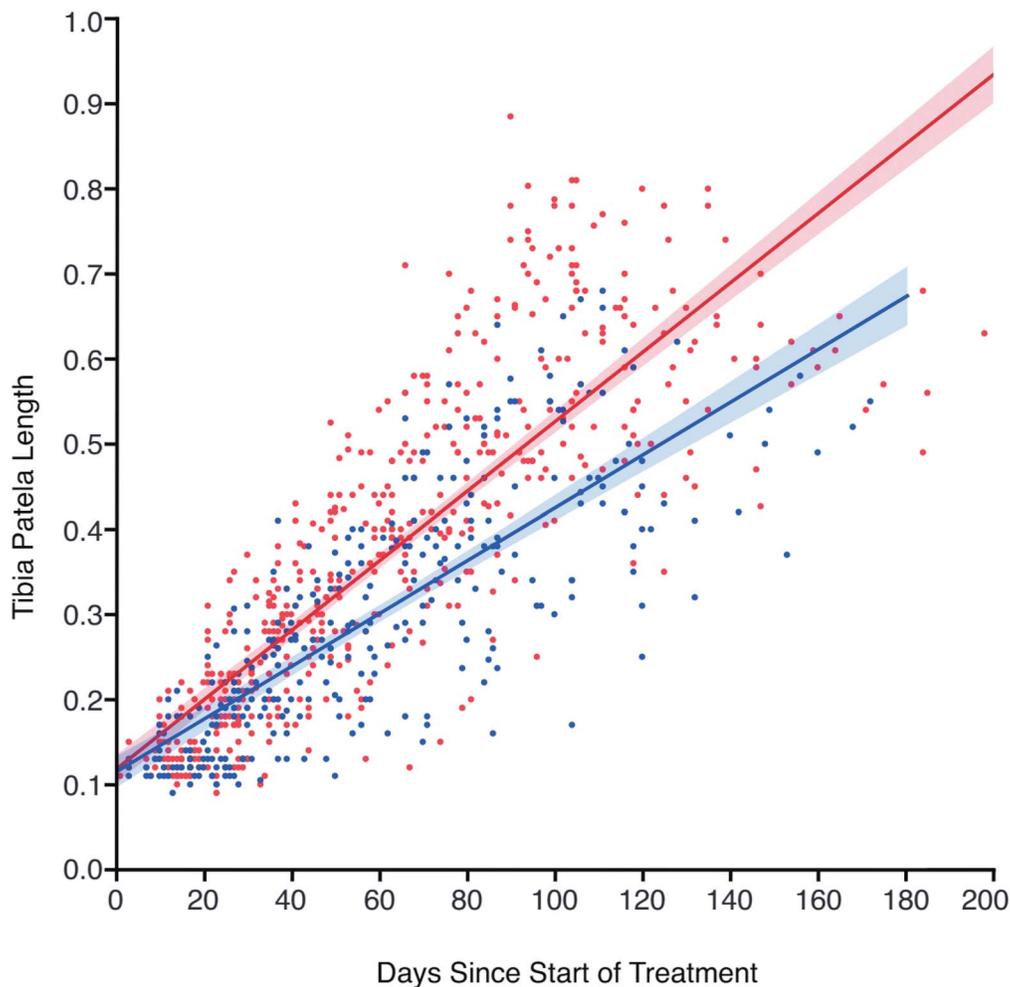


Figure 3.—Size of male (blue) and female (red) spiders in cm at each molt date following initiation of treatment (X axis).

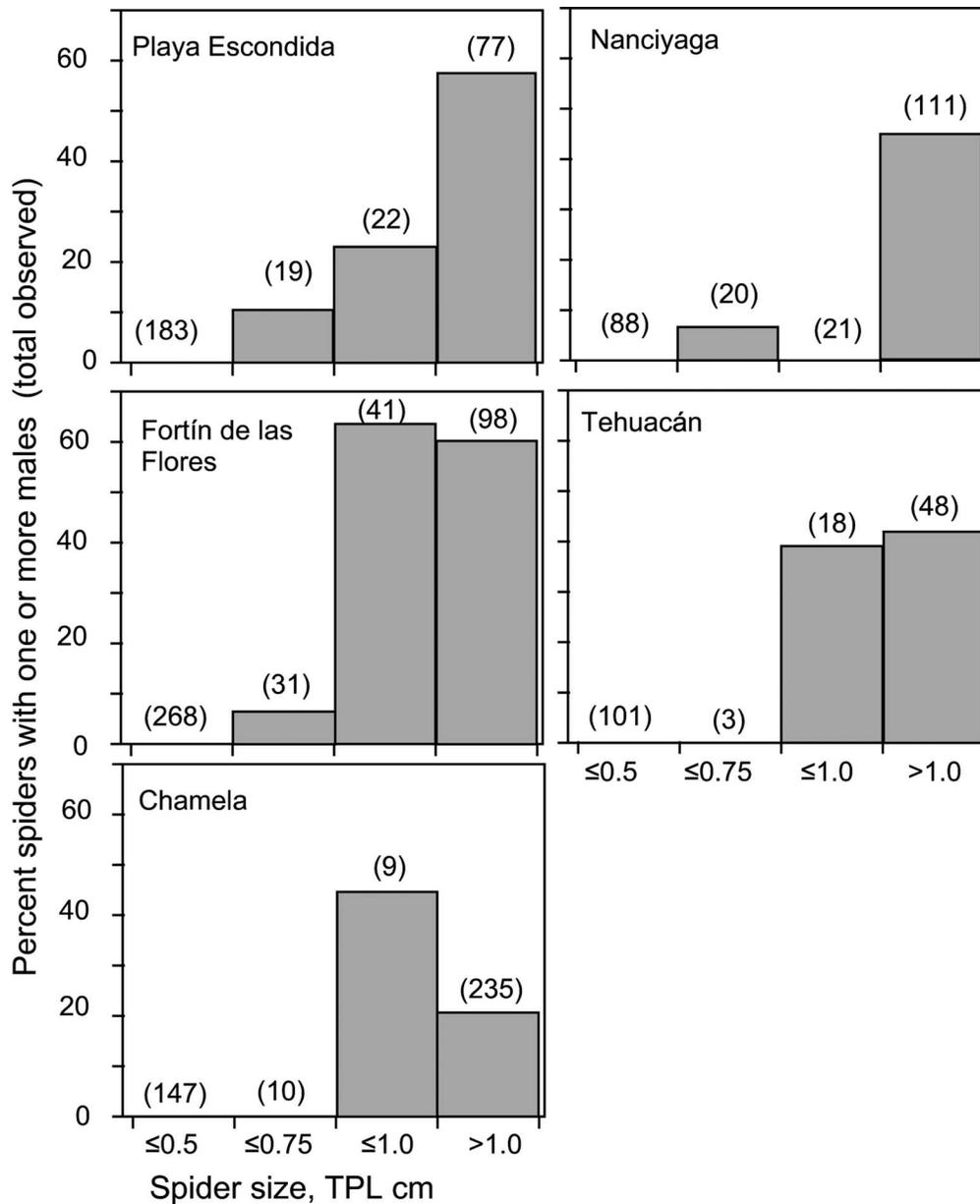


Figure 4.—Percentage of females in each size class that had at least one co-habiting male in five Mexican populations. Males never are found with females smaller than 0.5 cm TPL, and only rarely with females smaller than 0.75 cm TPL.

females increase in size presumably reflects different optimal sizes for the sexes; fecundity selection for females and selection on a variety of sexually-related behavioral factors for males (Lande 1980; Slatkin 1984; Roff 2001; Badyaev 2002; Kuntner et al. 2008, 2019; Moya-Laraño et al. 2009; Corcobado et al. 2010; Danielson-François et al. 2012; Grossi & Canals 2015; Neumann & Schneider 2015; Uhl et al. 2015; Biaggion et al. 2016; Herberstein et al. 2017; Kuntner & Coddington 2020). However, evolution of distinct developmental trajectories may result in asynchrony of maturation between males and females that emerge simultaneously.

To simulate simultaneous emergence in the field, we compared growth and development of siblings emergent from the same egg sac. By rearing spiders on quantitatively distinct diets, we sought to increase the likelihood that rapidly-growing (high food) females would mature synchronously with slowly-growing (low food) males

(Higgins 1993; Higgins & Goodnight 2011). As described by Vollrath (1998), males from all populations developed more slowly than their female siblings, also observed in the araneid spider *Larinioides scolopetarius* (Clerck, 1757) by Kleinteich & Schneider (2010, 2011). However, despite the difference in development rate, males and females did not mature synchronously.

Our results indicate that in univoltine populations, the differences in size will be accompanied by a serious logistical problem: asynchronous maturation. Parallel investigation of post-maturation survival suggests that early-maturing males are unlikely to survive until simultaneously-emergent females reach maturity (this study and Higgins unpublished data). In the field, males cohabit with juvenile females. In the laboratory, mean male age at maturity corresponded to the mean age of the smallest females found in the field with males. This suggests that these juvenile females, rather than mature

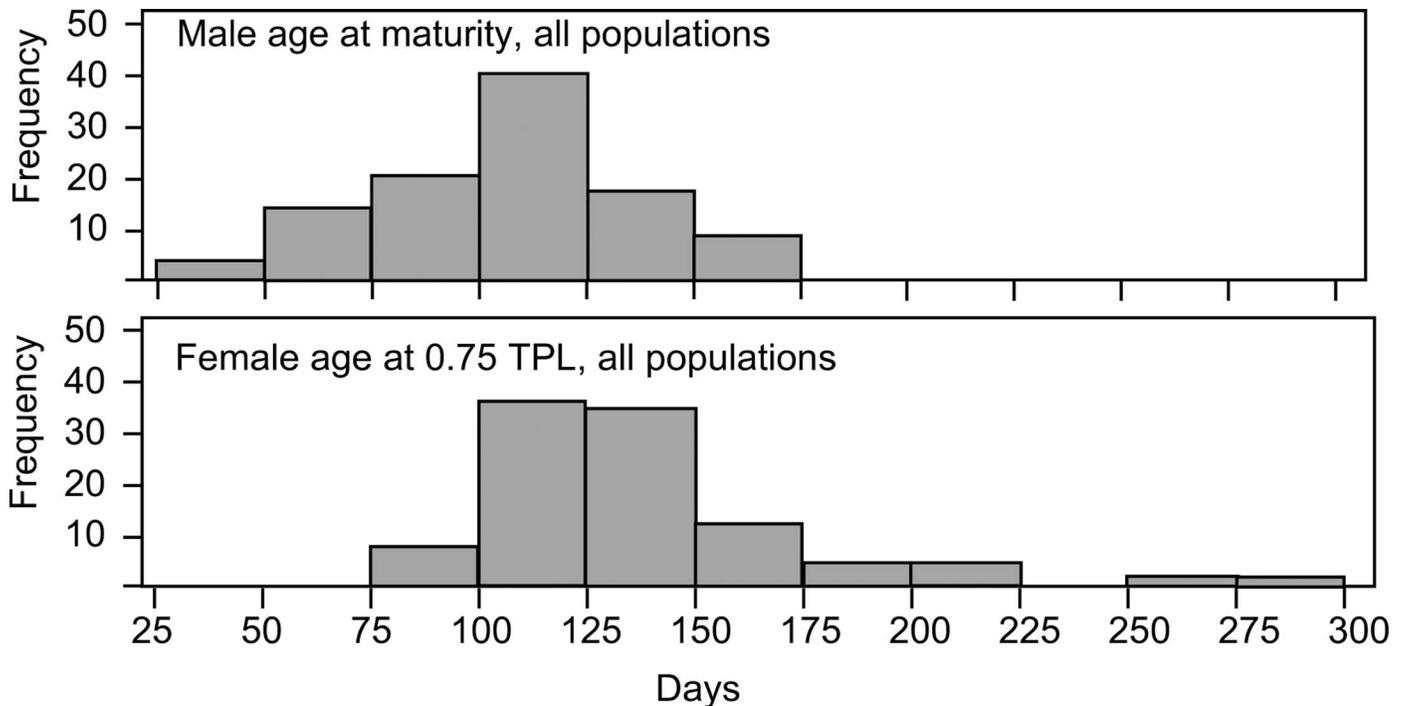


Figure 5.—Distribution of ages for (a) all males reaching sexual maturity and (b) all females reaching 0.75 cm TPL during laboratory rearing. Note that the peak of male maturation coincides with the peak of females becoming large enough to accept a male; however, a large proportion of the males mature before suitable host females would be available.

females, set the limit for earliest maturation of males, and hence the lower limit of male size. However, when the average male reached maturity in the laboratory, only about 50% of the females had achieved the size that, in the field, are found with cohabiting males. Since males do not survive without access to a female's web, these data suggest that early-maturing males are unlikely to reproduce. However, this would only be an issue for univoltine populations at the beginning of the season and earliest maturing egg sacs. Early maturing males later in the season may be able to find adult females that themselves emerged at the beginning of the season. Further, there is an observed asymmetry in male emergence from a single egg sac that may mitigate the protandry effect if at least some males from an egg sac mature late enough to catch the first females. Finally, in some *Trichonephila* the males may show sexually cued anticipatory plasticity in order to synchronize maturity depending on female presence (Neumann & Schneider 2016). To summarize, in univoltine populations with distinct periods of emergence, maturation, and death, many early-emergent males may not survive to mate. This is a here-to-fore undetected consequence of the incidental male protandry (Matsuura 2006) resulting from female gigantism and male dwarfism in univoltine populations. Sexual asynchrony may in turn increase the risk of local extinction (Calabrese et al. 2008). There may be few juvenile females available to support early-emergent males when they mature and, on the other hand, there may be few fertile males available to inseminate late-emergent or slowly-growing females when they mature (contributing to female mating failure; Higgins 1989; Rhoads 2010; Rittschoff et al. 2012; Morse 2013). Clearly, in such circumstance, natural selection should act on synchronizing maturation, favoring late maturation in males, and/or early maturation in females. However, we suggest several processes that may ameliorate the impact of asynchronous maturation in seasonal environments

and maintain this apparently maladaptive trait in univoltine populations of a largely tropical species.

First, *T. clavipes* is a widespread species with the majority of its range lying in the relatively aseasonal tropics. The univoltine populations are found in marginal habitats for the species, and on the climatic edges of the species range in USA. Those populations could at least occasionally be boosted by tropical immigrants. A phylogeographical study indeed suggests that dispersal, even over water, is at least common enough to maintain gene flow in *T. clavipes* among the Caribbean islands (Čandek et al. 2020). It seems reasonable that if the edge population consists of interbreeding local individuals and occasional tropical immigrants, natural selection favoring early males in aseasonal environments may outweigh local selection for synchrony. Second, we suggest that micro-environmentally induced variation in timing of emergence in the spring and in growth and maturation of males and females. These two factors may be key to the maintenance of eSSD in populations inhabiting highly seasonal environments. Nevertheless, it seems likely that this trait that evolved in the relatively aseasonal tropics may be maladaptive in highly seasonal environments and our findings would predict selection to act against sexual size dimorphism in edge habitats. If the effect of protandry is severe, another prediction would be that *T. clavipes* may move north with warming temperature more slowly than similar species that have synchronously maturing sexes.

There are several hypothesized selective advantages of early male maturation or small male size in arthropods (Foellmer & Moya-Larano 2007; Kuntner & Coddington 2020). Models exploring these hypotheses were the subject of a thorough review by Foellmer & Moya-Larano (2007) and Kuntner & Coddington (2020), and here we consider only whether any of them might account for the logistical difficulty described above. We do not consider the cannibalism

hypothesis (that small males avoid cannibalism because they are below the prey-size threshold of females; Darwin 1871) for two reasons. First, males are within the size range of accepted prey by females (Higgins & Buskirk 1992); and second, sexual cannibalism is extremely rare in *T. clavipes* (LEH pers. obs).

All of the hypothesized selective pressures favoring small male size rely to some extent upon the assumptions that females are not aggregated and that low densities cause relaxed male-male competition and favor morphologies enabling males to win the scramble competition for receptive females. All of these models also assume that when males mature, they will immediately start competing for access to mature or subadult females. In essence, these models are proposing that natural selection favoring early male maturation is stronger than male-male competition favoring large male size. However, both access to food as well as social cues can impact timing and size of males at maturation. The proposed selective forces are either costs of post-maturation searching (Vollrath & Parker 1992; Moya-Larano et al. 2002, 2009; Kasumovic et al. 2007), or the energetic requirements of prolonged male development (Ghesilin 1974). However, Quinones-Lebron et al. (2021) finding that social cues can impact male size at maturation indicates another level of plasticity and provides support for both male-male competition favoring large size, and early access to females favoring rapid maturation.

As suggested above, *T. clavipes* phenology varies with habitat, ranging from discrete bivoltinism to strictly univoltine (Higgins 2000). In the univoltine populations that have been studied, the operational sex ratio is male-biased early in the season and female-biased late in the season (Higgins 2000). The shift in sex-ratio suggests that maturation at a small size may indeed be advantageous late in the season when there are many penultimate and mature females and few males (Ghesilin 1974; Vollrath & Parker 1992). Importantly, empirical studies of competing males show both large male and first-male advantage in *T. clavipes*. Using mark-recapture studies, Meraz et al. (2012) found that larger males more rapidly encountered female webs (see also Quiñones-Lebron et al. 2019). When there is direct competition for a female on a web, the largest male is dominant (Christenson & Goist 1979; Rittschof 2011). Paternity tests for pairs of male *T. edulis* (Labillardière, 1799) simultaneously cohabiting with a female show that small and large males fertilize equal numbers of eggs (Elgar & Fahey 1996; Schneider et al. 2000; Schneider & Elgar 2005). However, arrival times of males in the field in *T. clavipes* may differ by hours to days (LEH pers. obs.) and Christensen & Cohn (1988) found strong first-male paternity advantages when several days elapsed between copulations by different males. Taken together, these data support a model of early maturation at small size being favored by scramble competition for females (Schneider et al. 2000; Schneider & Elgar 2005; Danielson-François et al. 2012; Herberstein et al. 2017), sperm competition (Neumann & Schneider 2015), and perhaps by reduced energetic costs of searching in smaller males (Schneider & Elgar 2005; Uhl et al. 2015; Biaggion et al. 2016). This advantage may be augmented by low survival of searching males that minimizes direct competition favoring large males (Vollrath & Parker 1992; De Mas et al. 2009). However, all of these models assume that subadult and mature females are present in the population when males mature. Our experiments suggest that in highly seasonal habitats (edge habitats for *T. clavipes*), early-emergent, early-maturing males will, at best, find juvenile females willing to host them as kleptoparasites. Males that do not locate a host within a few days are unlikely to survive (Kasumovic et al. 2007; Meraz et al. 2012).

To investigate the evolutionary origin of such a counter-intuitive situation, we looked at phylogenies recently published by Su et al. (2011) and Kuntner et al. (2013, 2019). In the most recent evolutionary hypotheses (Kuntner et al. 2019), there is support for the sister relationship between two *Nephila* Leach, 1815 species with the remaining Nephilidae, previously thought to be congeners of *T. clavipes*. The two *Nephila* species are found in regions with nearly aseasonal habitats (*N. constricta* Karsch, 1879 and *N. pilipes* (Fabricius, 1793)). The latter species is well studied. Tropical populations of *Nephila pilipes* have broadly-overlapping generations with males cohabiting and mating with females that are probably from earlier generations (Robinson & Robinson 1973; Higgins 2002). Even in the more seasonal habitat of Taiwan, mature females can be found all year round (although in low abundance in the winter; I. Tso pers. com.). Little is known of the ecology of *N. constricta*, as it is found only in tropical West Africa. The *N. pilipes* observations suggest that in these spiders, female-biased sexual size dimorphism may have evolved initially in aseasonal environments. In such environments, spiders can emerge and grow throughout the year, reducing the developmental asynchrony associated with delayed female maturation. Indeed, the developmental asynchrony may act to reduce inbreeding because matings will generally take place between individuals of different generations.

As an interesting side note, we document through restriction feeding experiments that females are less likely to survive to maturity than males. This may help explain the highly male biased sex ratios typically encountered in the field (e.g., Fromhage et al. 2007).

In conclusion, the eSSD strategy that characterizes several species of the mostly tropical and aseasonal *Trichonephila* presents some challenges for populations that secondarily occupy univoltine habitats such as *T. clavipes* in USA. Here we demonstrate that male protandry resulting from the difference in numbers of instars in males and females (despite relatively delayed maturation of males) incurs a cost as at least some early maturing males early in the season will forgo mating opportunities due to lack of appropriately sized females to host and mate with them. However, variation in maturation times of eggs even from a single egg sac, and likely dispersal from more aseasonal environments likely facilitate the persistence of sexual size dimorphism in populations inhabiting strongly seasonal habitats.

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LITERATURE CITED

Badyaev AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology & Evolution* 17:369–378.

- Biaggion MD, Sandomirsky I, Lubin Y, Harari AR, Andrade MCB. 2016. Copulation with immature females increases male fitness in cannibalistic widow spiders. *Biology Letters* 12:20160516.
- Blackledge TA. 2011. Prey capture in orb weaving spiders: Are we using the best metric? *Journal of Arachnology* 39:205–210.
- Blanckenhorn WU, Dixon AFG, Fairbairn DJ, Foellmer MW, Gibert P, van der Linde K et al. 2007. Proximate causes of Rensch's rule: Does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist* 169:245–257.
- Burnham KP, Anderson DR. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd ed., Springer-Verlag.
- Calabrese JM, Fagan WF. 2004. Lost in time, lonely, and single: Reproductive asynchrony and the Allee effect. *American Naturalist* 164:25–37.
- Calabrese JM, Ries L, Matter SF, Debinski DM, Auckland JN, J. Roland, et al. 2008. Reproductive asynchrony in natural butterfly populations and its consequences for female matelessness. *Journal of Animal Ecology* 77:746–756.
- Čandek K, Agnarsson I, Binford GJ, Kuntner M. 2020. Caribbean golden orbweaving spiders maintain gene flow with North America. *Zoologica Scripta* 49:210–221.
- Christenson TE, Cohn J. 1988. Male advantage for egg fertilization in the golden orb-weaving spider *Nephila clavipes*. *Journal of Comparative Psychology* 102:312–318.
- Christenson TE, Goist KC. 1979. Costs and benefits of male-male competition in the orb-weaving spider, *Nephila clavipes*. *Behavioral Ecology and Sociobiology* 5:87–92.
- Corcobado G, Rodríguez-Gironés MA, De Mas E, Moya-Laraño J. 2010. Introducing the refined gravity hypothesis of extreme sexual size dimorphism. *BMC Evolutionary Biology* 10:236.
- Danielson-Francois A, Hou C, Cole N, Tso IM. 2012. Scramble competition for moulting females as a driving force for extreme male dwarfism in spiders. *Animal Behaviour* 84:937–945.
- Darwin C. 1871. The Descent of Man, and Selection in Relation to Sex. London: John Murray.
- De Mas E, Ribera C, Moya-Larano J. 2009. Resurrecting the differential mortality model of sexual size dimorphism. *Journal of Evolutionary Biology* 22:1739–1749.
- Elgar MA, Fahey BF. 1996. Sexual cannibalism, competition, and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille Araneae: Araneioidea. *Behavioral Ecology* 7:195–198.
- Esperk T, Tammaru T, Nylin S, Teder T. 2007. Achieving high sexual size dimorphism in insects: females add instars. *Ecological Entomology* 32:243–256.
- Foelix RF. 1982. The Biology of Spiders. Harvard University Press, Cambridge MA.
- Foellmer M, Moya-Larano J. 2007. Sexual size dimorphism in spiders: Patterns and processes. Pp. 71–81. In *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. (DJ Fairbairn, WU Blanckenhorn, T Székely, ed.) Oxford University Press, Oxford.
- Fromhage L, Jacobs K, Schneider JM. 2007. Monogynous mating behaviour and its ecological basis in the golden orb spider *Nephila fenestrata*. *Ethology* 113:813–820.
- García E. 1973. Modificaciones al Systema de Clasificación Climática de Köppen. Universidad Nacional Autónoma de México Press, Mexico City.
- Gheslin MT. 1974. The Economy of Nature and the Evolution of Sex. University of California Press, Berkeley CA.
- Goodnight C. 1988. Population differentiation and the transmission of density effects between generations. *Evolution* 42:399–403.
- Grossi B, Canals M. 2015. Energetics, scaling and sexual size dimorphism of spiders. *Acta Biotheoretica*. 63:71–81.
- Harvey JA. 2022. Prey availability affects developmental trade-offs and sexual-size dimorphism in the false widow spider, *Steatoda grossa*. *Journal of Insect Physiology* 136:104267.
- Head G. 1995. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). *Evolution* 49:776–781.
- Herberstein ME, Painting CJ, Holwell GI. 2017. Scramble competition polygyny in terrestrial arthropods. *Advances in the Study of Behavior* 49:237–295.
- Higgins LE. 1989. Effect of insemination on the morphology of the internal female genitalia of the spider *Nephila clavipes* Araneae, Araneidae. *Annals of the Entomological Society of America* 82:748–753.
- Higgins LE. 1992. Developmental plasticity and fecundity in the orb-weaving spider *Nephila clavipes*. *Journal of Arachnology* 20:94–106.
- Higgins L. 1993. Constraints and plasticity in the development of juvenile *Nephila clavipes* in Mexico. *Journal of Arachnology* 21:107–119.
- Higgins L. 2000. The interaction of season length and development time alters size at maturity. *Oecologia* 122:51–59.
- Higgins L. 2002. Female gigantism in a New Guinea population of the spider *Nephila maculata*. *Oikos* 99:377–385.
- Higgins L, Buskirk RE. 1992. A trap-building predator exhibits different tactics for different aspects of foraging behavior. *Animal Behaviour* 44:485–499.
- Higgins L, Goodnight C. 2010. *Nephila clavipes* females have accelerating dietary requirements. *Journal of Arachnology* 38:150–152.
- Higgins L, Goodnight C. 2011. Developmental response to low diets by giant *Nephila clavipes* females. *Journal of Arachnology* 39:399–408.
- Higgins LE, Rankin MA. 2001. Mortality risk of rapid growth in the spider *Nephila clavipes*. *Functional Ecology* 15:24–28.
- Higgins L, Coddington JA, Goodnight C, Kuntner M. 2011. Testing ecological and developmental hypotheses of mean and variation in adult size in nephilid orb-weaving spiders. *Evolutionary Ecology* 25:1289–1306.
- Hormiga G, Scharff N, Coddington JA. 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders Araneae, Orbiculariaae. *Systematic Biology* 49:435–462.
- Kasumovic MM, Bruce MJ, Herberstein ME, Andrade MCB. 2007. Risky mate search and mate preference in the golden orb-web spider *Nephila plumipes*. *Behavioral Ecology* 18:189–195.
- Kleinteich A, Schneider JM. 2010. Evidence for Rensch's rule in an orb-web spider with moderate sexual size dimorphism. *Evolutionary Ecology Research* 12:667–683.
- Kleinteich A, Schneider JM. 2011. Developmental strategies in an invasive spider: constraints and plasticity. *Ecological Entomology* 36:82–93.
- Kuntner M, Cheng R-C. 2016. Evolutionary pathways maintaining extreme female-biased sexual size dimorphism: convergent spider cases defy common patterns. Pp. 121–133. In *Evolutionary Biology: Convergent Evolution, Evolution of Complex Traits, Concepts and Methods*, (P Pontarotti, ed), Berlin: Springer.
- Kuntner M, Coddington JA. 2009. Discovery of the largest orbweaving spider species: the evolution of gigantism in *Nephila*. *Plos One* 4:e7516.
- Kuntner M, Coddington JA. 2020. Sexual size dimorphism: Evolution and perils of extreme phenotypes in spiders. *Annual Review of Entomology* 65:57–80.
- Kuntner M, Elgar MA. 2014. Evolution and maintenance of sexual size dimorphism: aligning phylogenetic and experimental evidence. *Frontiers in Ecology and Evolution* 2:26.
- Kuntner M, Arnedo MA, Trontelj P, Lokovšek T, Agnarsson I. 2013. A molecular phylogeny of nephilid spiders: Evolutionary history of a model lineage. *Molecular Phylogenetics and Evolution* 69:961–979.
- Kuntner M, Čandek K, Gregorič M, Turk E; Hamilton C, Chamberland L, et al. 2023. Increasing information content and diagnosability in family-level classifications. *Systematic Biology* 72:964–971.
- Kuntner M, Coddington JA, Hormiga G. 2008. Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): Testing morphological and ethological homologies. *Cladistics* 24:147–217.
- Kuntner M, Hamilton CA, Cheng RC, Gregorič M, Lupše N, Lokovšek T et al. 2019. Golden orbweavers ignore biological rules: phylogenomic and comparative analyses unravel a complex evolution of sexual size dimorphism. *Systematic Biology* 68:555–572.

- Kuntner M, Zhang S, Gregoric M, Li D. 2012. *Nephila* female gigantism attained through post-maturity molting. *Journal of Arachnology* 40:345–347.
- Lande R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–307.
- Legrand RS, Morse DH. 2000. Factors driving extreme sexual size dimorphism of a sit-and-wait predator under low density. *Biological Journal of the Linnean Society* 71:643–664.
- Matsuura K. 2006. Early emergence of males in the termite *Reticulitermes speratus* Isoptera: Rhinotermitidae: Protandry as a side effect of sexual size dimorphism. *Annals of the Entomological Society of America* 99:625–628.
- Mayntz D, Toft S, Vollrath F. 2003. Effects of prey quality and availability on the life history of a trap-building predator. *Oikos* 101:631–638.
- Meraz LC, Henaut Y, Elgar MA. 2012. Effects of male size and female dispersion on male mate-locating success in *Nephila clavipes*. *Journal of Ethology* 30:93–100.
- Morse DH. 2013. Reproductive output of a female crab spider: the impacts of mating failure, natural enemies, and resource availability. *Entomologia Experimentalis Et Applicata* 146:141–148.
- Moya-Larano J, Halaj J, Wise DH. 2002. Climbing to reach females: Romeo should be small. *Evolution* 56:420–425.
- Moya-Larano J, Vinkovic D, Allard CM, Foellmer MW. 2009. Optimal climbing speed explains the evolution of extreme sexual size dimorphism in spiders. *Journal of Evolutionary Biology* 22:954–963.
- Neumann R, Schneider JM. 2015. Differential investment and size-related mating strategies facilitate extreme size variation in contesting male spiders. *Animal Behavior* 101:107–115.
- Neumann R, Schneider JM. 2016. Socially cued developmental plasticity in web-building spiders. *BMC Evolutionary Biology* 16:170.
- Neumann R, Ruppel N, Schneider JM. 2017. Fitness implications of sex-specific catch-up growth in *Nephila senegalensis*, a spider with extreme reversed SSD. *PeerJ* 5(11):e4050.
- Prenter JR, Elwood RW, Montgomery WI. 1999. Sexual size dimorphism and reproductive investment by female spiders: A comparative analysis. *Evolution* 53:1987–1994.
- Quiñones-Lebrón SG, Gregoric M, Kuntner M, Kralj-Fišer S. 2019. Small size does not confer male agility advantages in a sexually-size dimorphic spider. *PLoS ONE* 14(5):e021603.
- Quiñones-Lebrón SG, Kuntner M, Kralj-Fišer S. 2021. The effect of genetics, diet, and social environment on adult male size in a sexually dimorphic spider. *Evolutionary Ecology* 35:217–234.
- Rhainds M. 2010. Female mating failures in insects. *Entomologia Experimentalis Et Applicata* 136:211–226.
- Rittschof CC. 2011. Mortality risk affects mating decisions in the spider *Nephila clavipes*. *Behavioral Ecology* 22:350–357.
- Rittschof CC, Hilber SA, Tudor MS, St Mary CM. 2012. Modeling male reproductive strategies and optimal mate number in an orb-web spider. *Behavioral Ecology* 23:1–10.
- Robinson MH, Robinson B. 1973. Ecology and behavior of the giant wood spider, *Nephila maculata* Fabricius in New Guinea. *Smithsonian Contributions to Zoology* 149:1–73.
- Roff D. 2001. *Life History Evolution*. Sinauer Associates, Inc., Sunderland MA.
- Schneider JM, Elgar MA. 2005. The combined effects of pre- and post-insemination sexual selection on extreme variation in male body size. *Evolutionary Ecology* 19:419–433.
- Schneider JM, Herberstein ME, De Crespigny FC, Ramamurthy S, Elgar MA. 2000. Sperm competition and small size advantage for males of the golden orb-web spider *Nephila edulis*. *Journal of Evolutionary Biology* 13:939–946.
- Slatkin M. 1984. Ecological causes of sexual dimorphism. *Evolution* 38:622–630.
- Su YC, Chang YH, Smith D, Zhu MW, Kuntner M, Tso IM. 2011. Biogeography and speciation patterns of the golden orb spider genus *Nephila* Araneae: Nephilidae in Asia. *Zoological Science* 28:47–55.
- Teder T. 2014. Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. *Functional Ecology* 28:479–486.
- Thornhill R, Alcock J. 1983. *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge MA.
- Uhl G, Zimmer SM, Renner D, Schneider JM. 2015. Exploiting a moment of weakness: Male spiders escape sexual cannibalism by copulating with moulting females. *Scientific Reports* 5:16928.
- Vollrath F. 1980. Male body size and fitness in the web-building spider *Nephila clavipes*. *Zeitschrift fur Tierpsychologie* 53:61–78.
- Vollrath F. 1998. Sex-linked differences in the growth of *Nephila clavipes*. Pp. 161–166. In *Proceedings of the 17th European Colloquium of Arachnology*, Edinburgh 1997. (Selden PA, editor).
- Vollrath F, Parker GA. 1992. Sexual dimorphism and distorted sex ratios in spiders. *Nature* 360:156–159.
- World Spider Catalog. 2022. World Spider Catalog. Version 23.5. Natural History Museum Bern, online at <http://wsc.nmbe.ch>, accessed on 25 September 2022.

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