SHORT COMMUNICATION

Nephila spider male aggregation: preference for optimal female size and web clustering

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Abstract

Sexual size dimorphism theory predicts biased operational sex ratios (OSRs) and an uneven distribution of males among certain females. We studied this phenomenon through a field census of the giant wood spider *Nephila pilipes* (family Nephilidae) in Singapore, a species where females are, on average, 6.9 times larger than males. Specifically, we tested two hypotheses concerning male distribution, given their tendency to aggregate in certain female webs. The *optimal female size hypothesis* predicts that males would predominantly occupy webs of intermediate-sized females. The *web clustering hypothesis* posits that more males would be found in webs closer together compared to those farther apart. Our snapshot census revealed a female-biased OSR (females: males = 1.85) with an uneven distribution of males in female webs. Most males were found in webs of intermediate-sized females aligning with the *optimal female size hypothesis*. Proximity among female webs was indicative of male presence, lending support to the *web clustering hypothesis*. While our study's limited sample size warrants caution, we conclude that in *N. pilipes*, male occupation of female webs is facilitated by the clustering of webs, and males prefer to cohabit with optimally sized, receptive females.

Key words: eSSD, *Nephila pilipes*, operational sex ratios, sexual selection, sexual size dimorphism

INTRODUCTION

Extreme, female-biased sexual size dimorphism, abbreviated as eSSD (Kuntner & Coddington 2020), refers

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to a phenotypic outcome in animal species where females consistently exhibit significantly larger sizes than males (Webb & Freckleton 2007; Cheng & Kuntner 2014). This contrasts with scenarios where males are larger (male-biased sexual size dimorphism) or with the expected biological norm where size differences are minimal (sexual size monomorphism) (Fairbairn 2007). eSSD represents a notable deviation from this norm and raises questions about the underlying selective mechanisms driving such

size differences in animal species. Among terrestrial animals, certain spider groups, particularly the golden orb-weaver family Nephilidae and the genus *Nephila* (Kuntner *et al.* 2019), are best known for eSSD (Foellmer & Moya-Laraño 2007; Kuntner & Coddington 2020).

Operational sex ratio (OSR) is defined as the proportion of sexually competing males to sexually active females in a population at any given time (Hardy 2002). In other words, it reflects the ratio of fertilizable females to sexually active males in a population. Unlike the physical sex ratio, which includes all individuals regardless of sexual activity, OSR focuses on those individuals actively participating in mating competition. Sexual size dimorphism theory predicts that, due to developmental, growth, and ecological constraints, OSRs in field populations of species with giant females and small males will be skewed, that is, will be usually male-biased (Kuntner & Coddington 2020). While this theoretical assumption simplifies the matter by ignoring seasonal variation in OSR (Kasumovic *et al.* 2008), it is nonetheless logical for eSSD species considering sexual dimorphism in developmental biology: To reach adulthood, small males undergo fewer molts, spending much less time and resources compared to large females that undergo delayed maturation (Higgins 2002). Consequently, more males than females are often detected in populations of eSSD spider species (Foellmer & Fairbairn 2005), albeit with exceptions (Vollrath & Parker 1992).

We studied the phenomenon of biased OSR through a field census of *Nephila* in Singapore, an equatorial and nearly aseasonal location. Colloquially known as the giant wood spider, *Nephila pilipes* (Fabricius, 1793) is a commonly encountered orb-web spider inhabiting tropical forests of South, Southeast, and East Asia, Wallacea, New Guinea, parts of Australia, Solomon Islands, and Vanuatu (Harvey *et al.* 2007). With average adult body sizes of 30.4 mm in females and 4.4 mm in males (Kuntner *et al.* 2019), it is one of the most prominent spider species exhibiting eSSD (Kuntner & Coddington 2020), making it an ideal model to test assumptions and predictions from eSSD theory (Reeve & Fairbairn 1996, 2001; Fairbairn 2007).

In our snapshot census, we counted *N. pilipes* males in female webs (Fig. 1a,b) and evaluated their distribution (for detailed methods, primary and additional parameters recorded in the field and the laboratory, raw data, statistical analyses, and code, see Text S1, Table S1, and Data S1, Supporting Information). Based on background knowledge suggesting that males in *Nephila* are commensals in female webs and tend to aggregate in certain, though not all, female webs, we specifically tested two hypotheses regarding the distribution of males in female webs.

The first—the *optimal female size hypothesis*—predicted that most males would occupy webs of intermediate-sized females. *Nephila* males are known to aggregate in female webs (Miller 2007) and exhibit a suite of mate-guarding behaviors (Kuntner *et al.* 2009). While literature and our own observations reveal cases of males that can guard relatively small immatures, receptive adult females, and even the largest females—those that have mated and are about to oviposit—the specific size group of females that receives the most males remains unclear. Unlike most araneoid spiders, *N. pilipes* females continue to grow through additional molts after reaching maturity (Kuntner *et al.* 2012b; Cheng *et al.* 2017), resulting in a substantial size range of females available in equatorial localities. This female size range provides males with a wide choice of preferences. Our hypothesis aligns with sexual selection theory, which predicts that most males would not invest in guarding small, juvenile, or virgin females (Elgar *et al.* 2003; Tuni & Berger-Tal 2012), nor should they prefer the large-bodied, older females that have already mated and are unlikely to continue to be receptive or even have plugged genitals, as is the case in *N. pilipes* (Kuntner *et al.* 2012a). Instead, the *optimal female size hypothesis* predicts that males should prefer to compete for intermediate-sized females, at sizes indicating receptivity of freshly molted, healthy adults in good condition. In essence, males should tend to guard females of sizes and mating status that promise them the highest paternity share and the lowest risk of sperm competition. In line with this prediction, mate choice experiments in widow spiders indicate a male preference for unmated, well-fed females (MacLeod & Andrade 2014).

The second—the *web clustering hypothesis*—predicted that more males would be found in webs of closer proximity compared to those farther apart. *Nephila* webs function as habitat islands, each web exclusively occupied by its female proprietor. Unlike the colonies of its close relatives, *Trichonephila*, *Nephila* webs are never adjacent or continuous (Agnarsson 2003; Kuntner *et al.* 2010, 2019). Therefore, it stands to reason that denser populations of *N. pilipes* females would likely provide more resources to roaming males. Consequently, we hypothesized that webs clustered together would harbor more males compared to more isolated webs. This hypothesis predicts that male spiders would be more prevalent in female webs that are clustered together, reflecting a strategic mating behavior aimed at optimizing reproductive success by increasing encounter rates with potential mates.
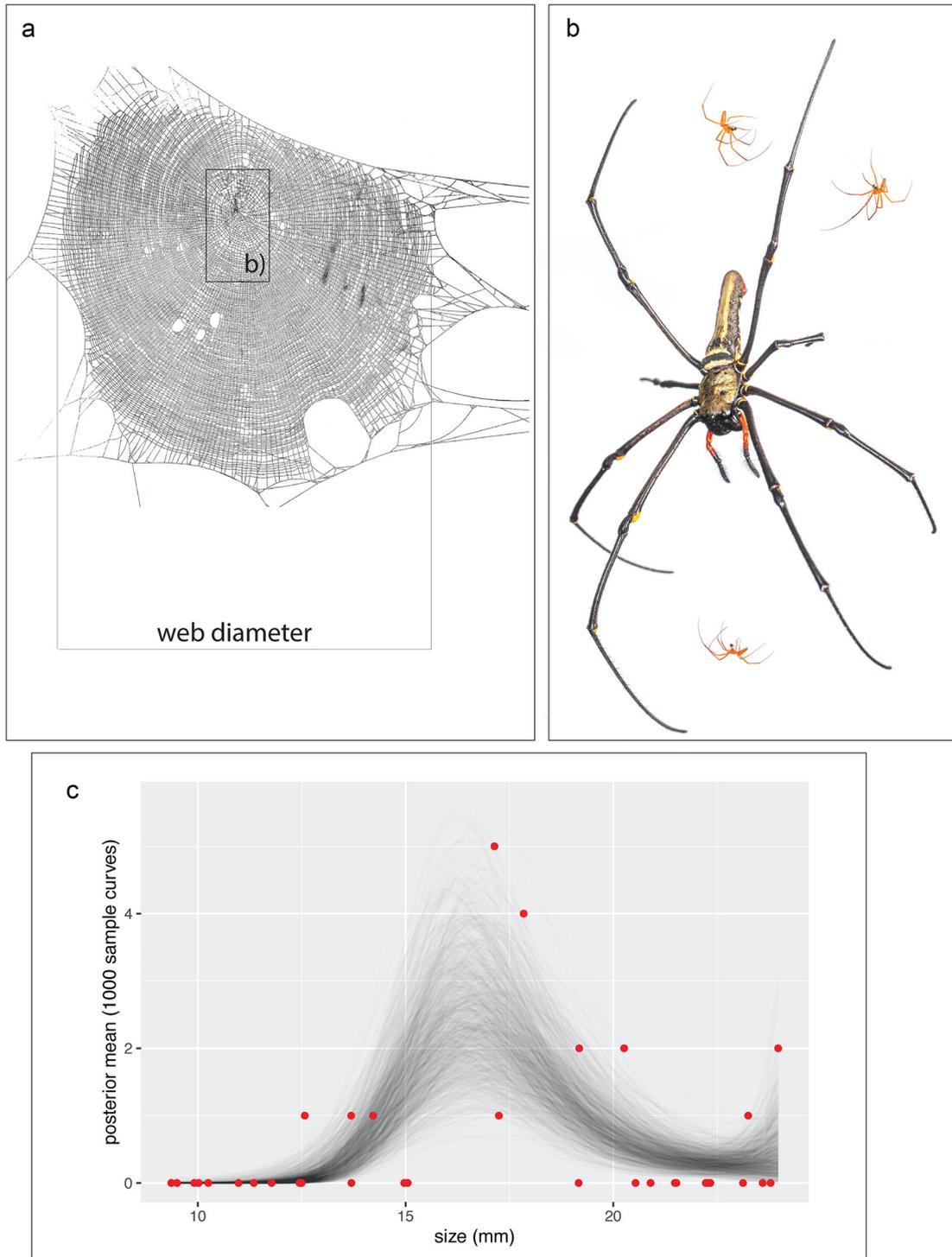
Figure 1 *Nephila pilipes* in Singapore: (a) female web architecture showing web diameter measurement as a web size proxy and with web's hub delimitation; (b) a typical male–female interaction at the web's hub: composite image of a female and three males illustrates extreme sexual size dimorphism (eSSD) and male aggregation in this species; (c) observed and simulated number of *N. pilipes* males in female webs plotted against female size proxy that is first leg tibia + patella length (mm). Red dots, observed males; black curves, simulated posterior mean numbers of males using the best-fitting cubic relationship model.

RESULTS

Among the 37 webs of subadult and adult *N. pilipes* females investigated at three localities in Singapore (Methods described in Text S1, Supporting Information), horizontal web diameters (*d*) ranged from 22.0 to 111.0 cm (1st Qu. = 39.0; Median = 47.0; Mean = 52.7; 3rd Qu. = 66.0). The calculated web areas ranged from 380.1 to 9676.9 cm^2 (1st Qu. = 1194.6; Median = 1734.9; Mean = 2497.5; 3rd Qu. = 3421.2). Female first patella + tibia lengths ranged from 9.362 to 23.967 mm (1st Qu. = 12.486; Median = 17.245; Mean = 17.005; 3rd Qu. = 22.226). In total, the 37 webs contained 20 *N. pilipes* males, which translated to a female-biased OSR of 1.85 females per male. The distribution of 20 males in our survey was uneven: 27 webs contained no males, 5 webs contained a single male, 3 webs contained two males, and 1 web each contained four and five males, respectively (mean number of males in a web = 0.54).

Our snapshot census did not detect the expected pattern of a male-biased OSR in *Nephila* as often implicitly assumed in the SSD literature. Instead, we found a female-biased OSR, with a ratio of 1.85 females to males. The uneven distribution of males among female webs allowed testing of the two hypotheses.

The *optimal female size hypothesis*, predicting that most males will occupy webs of females of intermediate, rather than largest sizes, was supported by the data. A nonlinear (cubic) relationship between female size and the number of males was the best-fitting model (Fig. 1c), better than the intercept-only baseline with 0.83 probability, and all model coefficients for size were statistically relevant (their 99.9% posterior intervals did not contain 0). This model could not be improved upon by including other variables (the cubic model was better with 0.8 probability). However, for more conclusive results regarding the predictive power of size and whether a quadratic or cubic relationship was a better model (the cubic model was better with 0.63 probability), a larger sample size would be required. As seen in Fig. 1c, the observed and simulated numbers of males in female webs plotted against female size proxy show the main peak at intermediate female sizes, aligning with the *optimal female size hypothesis*. It needs to be noted, however, that the best model is not strictly unimodal and that a second, smaller peak is likely at the largest female sizes.

The observed negative correlation between web proximity and the number of males (Pearson's $\rho = -0.361$, 95% CI = $(-0.61, -0.04)$, *P*-value = 0.03) corroborated the *web clustering hypothesis*, which predicted more males in webs closer together. Males also cluster in specific webs (Lloyd's crowding index = 3.6, 95% CI = (1.7, 8.4)).

DISCUSSION

Female-biased operational sex ratio

The observed female-biased OSR diverged from the expected male-biased OSR. The unexpected female bias in OSR in the Singapore *Nephila* population may stem from sampling limitations. Our survey focused on male counts within habitat patches consisting of female webs exclusively, neglecting males roaming in vegetation. Another limitation is the snapshot nature of our sampling, disregarding potential seasonal effects. That said, the detected OSR may also reflect reality. A study of *N. pilipes* demographics in Taiwan, China (Danielson-François *et al.* 2012) namely also detected a slightly female-biased OSR in one population (male: female 0.94 compared to 0.54 in our study), while another Taiwanese population exhibited a strongly male-biased OSR (male: female 1.89; Danielson-François *et al.* 2012), aligning more closely with eSSD theoretical assumptions (Kuntner & Coddington 2020). In *Trichonephila clavipes*, a distal nephilid relative of *Nephila* of comparable eSSD, OSR can also be female-, not male-biased (Vollrath & Parker 1992). Vollrath and Parker showed this phenomenon in a Panamanian population, attributing the skewed sex ratio to differential mortality rates between roving males and sedentary females. In other, more seasonal populations of *T. clavipes*, OSR is male biased early and female-biased late in the season (Higgins 2000; Agnarsson *et al.* 2024).

It seems that OSR in orb-web spiders can fluctuate throughout the mating season (Kasumovic *et al.* 2008), although this should be less pronounced in the equatorial, aseasonal environment of Singapore. Therefore, a male-biased OSR might not *necessarily* result from eSSD demographics. Additionally, the presumed link between eSSD and prolonged female maturation, challenged in several arthropod lineages, including spiders (Blanckenhorn *et al.* 2007), questions the assumption that eSSD necessarily affects OSR.

Male preference for females of intermediate size

Although modeling results suggest the possibility of a modest second peak of male abundance in webs of females of the largest sizes, the main peak is clear at intermediate female sizes (Fig. 1c) suggesting that males predominantly cohabit with intermediate-sized females. The

discrepancy between our unimodal theoretical expectation and the bi-modal nature of the data may warrant some caution, but we believe this to be a stronger test of the prediction than fitting a unimodal function that might falsely yield the expected outcome. More data are required to investigate this further, including the possibility of a confounding variable.

Nonetheless, we interpret our result to support the *optimal female size hypothesis*, predicting that most spider males prefer to occupy webs of intermediate-sized females. This expectation arises from the trade-off male spiders face between the costs and benefits of mating with large, previously mated females versus small, virgin females. Larger females are generally more fecund but are also more likely to have previously mated, potentially resulting in shared paternity for guarding males, low receptivity, or even outright aggression from plugged females (Kuntner *et al.* 2009, 2012a; Zhang *et al.* 2011). This aggression may culminate in sexual cannibalism in eSSD spiders in general (Kuntner & Coddington 2020; Wilder & Rypstra 2008) and in *Nephila* in particular (Kuntner *et al.* 2009, 2012a; Zhang *et al.* 2011, 2022). Consequently, males may benefit from targeting small, virgin females in good physical condition to maximize reproductive success, but since smaller females may face a longer potential mating period, this can lead to increased male–male and/or sperm competition. Our findings suggest that males resolve this trade-off by preferring intermediate-sized females.

Corroboration of the *optimal female size hypothesis* raises new questions that warrant further research. First, do intermediate-sized females signal their receptivity through the strategic use of pheromones, thereby attracting more males? Experimental evidence in other spider species supports this possibility (MacLeod & Andrade 2014). Second, while our study was conducted in a nearly aseasonal equatorial population, it remains unclear whether male preference for intermediate-sized females varies seasonally in non-equatorial regions of the species range. Finally, a study on *T. clavipes* found that male preference for certain-sized females is plastic and dependent on male size (Pollo *et al.* 2019). Therefore, future studies on *Nephila* should incorporate measurements of male size in relation to mate choice.

Male aggregation in clustered female webs

Our findings corroborate the *web clustering hypothesis*, suggesting that in web-building spiders, more males are likely to be found in female webs closer together compared to those farther apart. This hypothesis stems from the notion that male spiders tend to locate female webs that are in close proximity to each other, thereby increasing their chances of successful courtship and mating. Analogous to male distribution, research on the distribution of small spider kleptoparasites in the webs of *T. clavipes* has shown similar facilitation by the clustering of the host webs (Agnarsson 2003). While our hypothesis specifically focused on *Nephila* males, it follows the same rationale. This hypothesis aligns with the concept of mate-searching behavior in spiders, where males actively seek out females for mating. By positioning themselves near several female webs clustered together, males can efficiently locate potential mates and enhance their reproductive success. This behavior may confer advantages in environments where female spiders are dispersed and sit in their webs that resemble habitat islands, but are still within a reasonable distance from each other.

The hypothesis implies that male spiders may strategically position themselves near multiple female webs to maximize their reproductive opportunities. However, our study did not actively census males occupying vegetation away from female webs, thus limiting the scope of our testing. Future studies could address this bias by sampling more inclusively across substrates albeit increasing sampling invasiveness. Additionally, extending the census efforts temporally and geographically across the extensive range of *N. pilipes* would enhance the rigor of future investigations.

DATA AVAILABILITY STATEMENT

All data are provided in Table S1.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Raw data

Text S1 Materials and Methods

Data S1 Source code for the statistical analyses as an R Markdown Notebook

Cite this article as: