ORIGINAL ARTICLE

INTERSEXUAL ARMS RACE? GENITAL COEVOLUTION IN NEPHILID SPIDERS (ARANEAE, NEPHILIDAE)

Matjaž Kuntner,[1,2,3] Jonathan A. Coddington,[2,4] and Jutta M. Schneider[5,6]

[1]Institute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Novi trg 2, P.O. Box 306, SI-1001 Ljubljana, Slovenia

[2]Department of Entomology, National Museum of Natural History, Smithsonian Institution, NHB-105, P.O. Box 37012, Washington, D.C. 20013

  [3]E-mail: kuntner@gmail.com

  [4]E-mail: Coddington@si.edu

[5]Biozentrum Grindel, University of Hamburg, Martin-Luther-King, Platz 3, Hamburg D-20146, Germany

  [6]E-mail: js@gilgamesh.de

Genital morphology is informative phylogenetically and strongly selected sexually. We use a recent species-level phylogeny of nephilid spiders to synthesize phylogenetic patterns in nephilid genital evolution that document generalized conflict between male and female interests. Specifically, we test the intersexual coevolution hypothesis by defining gender-specific indices of genital complexity that summarize all relevant and phylogenetically informative traits. We then use independent contrasts to show that male and female genital complexity indices correlate significantly and positively across the phylogeny rather than among sympatric sister species, as predicted by reproductive character displacement. In effect, as females respond to selection for fecundity-driven fitness via giantism and polyandry (perhaps responding to male-biased effective sex ratios), male mechanisms evolve to monopolize females (male monogamy) via opportunistic mating, pre- and postcopulatory mate guarding, and/or plugging of female genitalia to exclude subsequent suitors. In males morphological symptoms of these phenomena range from self-mutilated genitalia to total castration. Although the results are compatible with both recently favored sexual selection hypotheses, sexually antagonistic coevolution, and cryptic female choice, the evidence of strong intersexual conflict and genitalic damage in both sexes is more easily explained as sexually antagonistic coevolution due to an evolutionary arms race.

KEY WORDS: Cryptic female choice, monogamy, morphological complexity, polyandry, reproductive character displacement, sexual conflict, sexually antagonistic coevolution, sperm competition.

Genital traits are crucial taxonomically: they vary widely and concordantly with species limits. Many closely related taxa exhibit diverse and complex genital morphologies (reviewed in Eberhard 1985). Although such genital complexity and diversity probably function to do more than simply transfer sperm (Hosken and Stockley 2004), underlying selection pressures remain poorly understood (Arnqvist 1998; Huber 2005). Historically, sexual se-

lection studies have tended to focus on precopulatory male–male competition and female choice (for review, see Andersson 1994). However, postcopulatory processes are now known to be just as important (Eberhard, 1996; Arnqvist and Danielsson 1999; Snook 2005; Andersson and Simmons 2006). Postmating sexual selection, for example sperm competition or cryptic female choice, might be responsible for much of the observed variation in
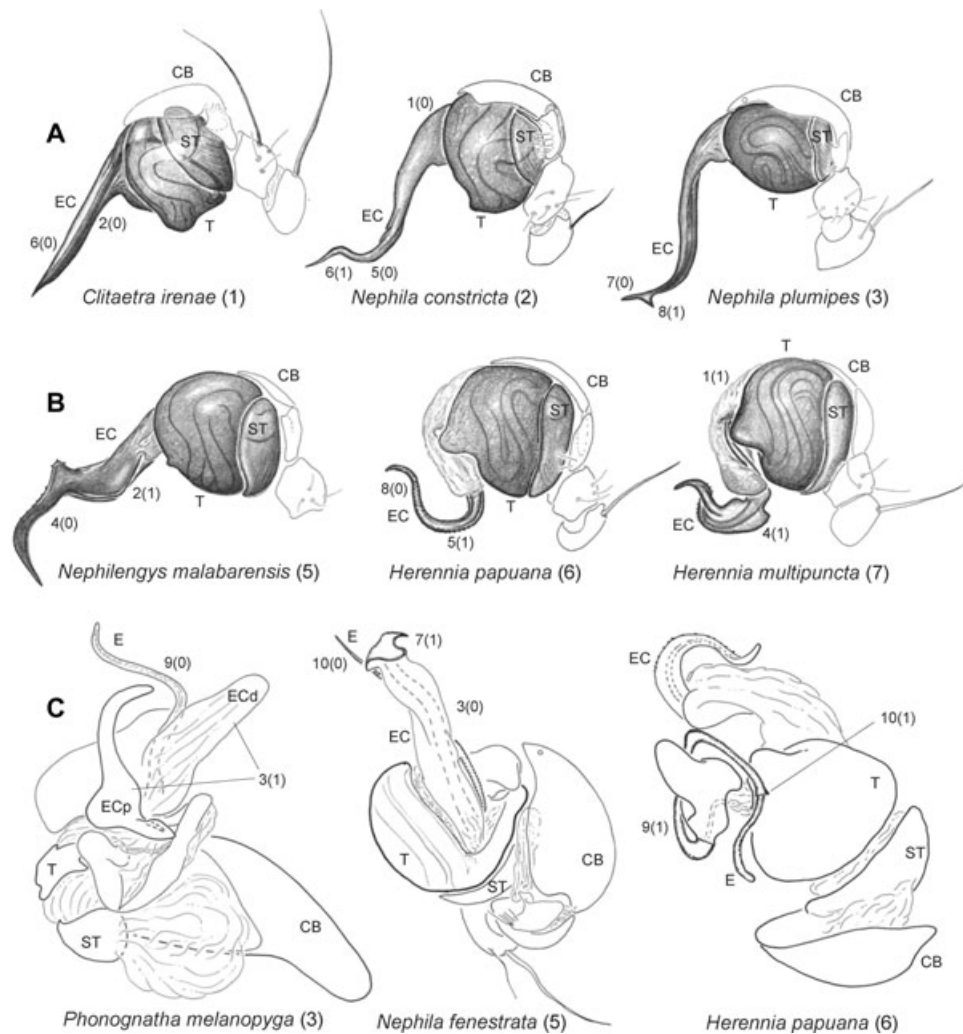
Figure 1. Male genital morphologies relevant to nephilids and outgroups: (A) relatively simple palps as in *Clitaetra* and most *Nephila*; (B) relatively complex palps as in *Nephilengys, Herennia* and *Nephila fenestrata*; (C) expanded palps showing most prominent palpal sclerites (CB, cymbium; E, embolus; EC, embolic conductor; Ecp, proximal part of EC; Ecd, distal part of EC; ST, subtegulum; T, tegulum) and genital complexity features (see Table 1). Total complexity scores for each species in parentheses.

genital morphology (Eberhard 1985, 1996, 2004a; Arnqvist 1998). Sexual conflict promoting antagonistic coevolution has also recently been proposed to cause rapid variation in reproductive traits (Arnqvist and Rowe 1995; Pizzari and Snook 2003; Zeh and Zeh 2003; Arnqvist 2004; Arnqvist and Rowe 2005; Hosken and Stockley 2004; Arnqvist 2006; but see Eberhard 2006). Antagonistic coevolution has been extensively tested in insects (Arnqvist and Rowe 2002a,b; Rowe and Arnqvist 2002), leading to the claim that sexual conflict drives sexual coevolution in many animal clades (for reviews, see Arnqvist and Rowe 2005; Tregenza et al. 2006; Wedell et al. 2006).

Spiders lend themselves to postcopulatory sexual selection studies for several reasons: (1) their genitalia are often the only traits known to vary significantly between species virtually indistinguishable in ecology and life history; (2) genital variation is informative phylogenetically and therefore routinely categorized

into multiple independent variables for phylogenetic inference; (3) genitalic complexity can therefore be quantified on an ordinal scale; and (4) spider genitalia are paired so that one animal can serve as both experiment and control (Eberhard 2004b). The male palp (Fig. 1) exhibits complex sclerites and membranes, which closely interact with the female epigynum (Fig. 2), a sclerotized plate leading to often convoluted ducts and spermathecae. The repeated evolution of extreme sexual size dimorphism also suggests that sexual selection strongly operates in spiders, and the former also correlates positively with polyandry (Coddington et al. 1997; Ramos et al. 2005). Insofar as polyandry ameliorates inbreeding (Tregenza and Wedell 2002) it may be especially important to sessile females such as web-building spiders.

Intersexual conflict in particular spider species is well documented (Elgar 1991; Schneider and Lubin 1998; Schneider and Elgar 2001, 2002; Herberstein et al. 2002, 2005; Fromhage and
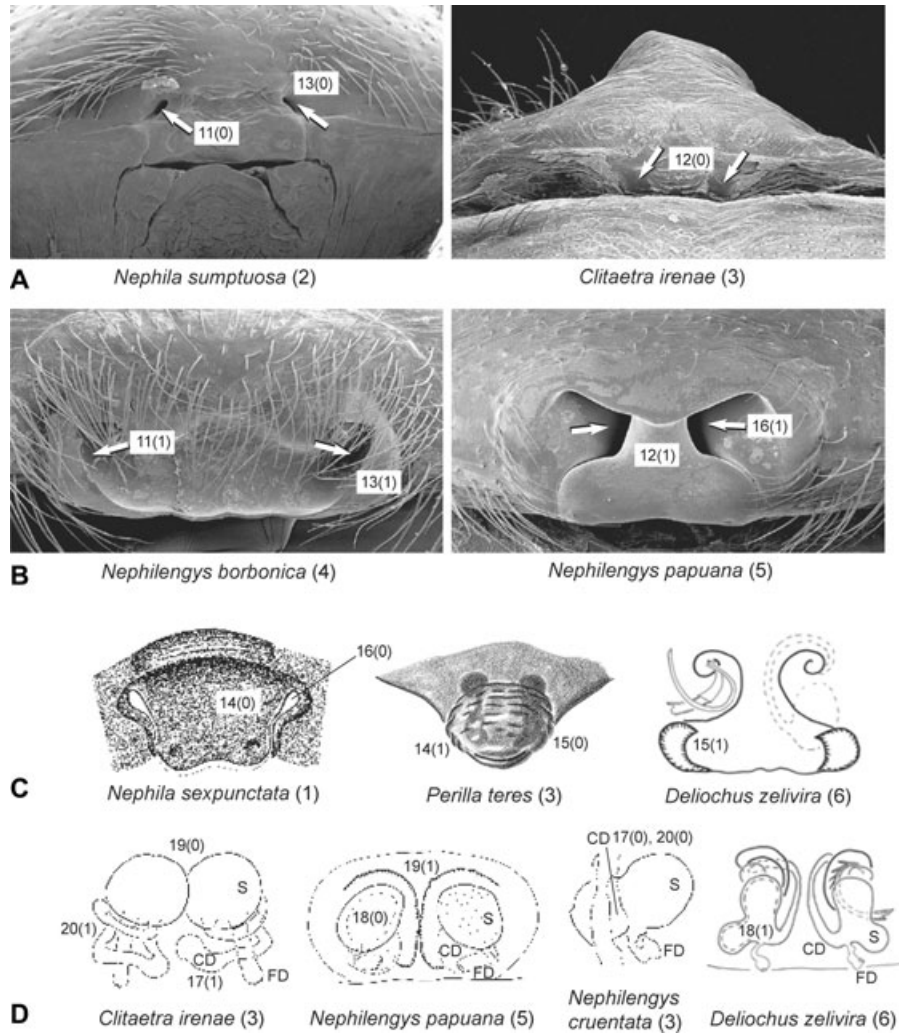
Figure 2. Female genital morphologies (A–C external, D internal) of nephilids and outgroups, illustrating prominent features (CD, copulatory duct; FD, fertilization duct; S, spermatheca) and numbered genital complexity features (see Table 1). Total complexity scores for each species are given in parentheses. Arrows in (A) and (B) point to paired copulatory openings.

Schneider 2005a; Schneider et al. 2006; Vahed 2007), and Miller (2007) has shown that the morphological and behavioral correlates of sexual conflict in spiders covary at broad phylogenetic scales. Within small clades mating strategies can vary from monogamy to extreme polygamy (Fromhage et al. 2005; Kuntner et al. 2008), and their evolution may be largely influenced by population dynamics (Kokko and Rankin 2006). If male and female interests conflict, antagonistic morphological, physiological, and behavioral traits ought to evolve (Arnqvist and Rowe 2005). However, thus far the comparative biology literature in spiders contains little evidence for intersexual genitalic coevolution in general (Ramos et al. 2005) and for sexually antagonistic coevolution in particular (Eberhard 2004a; also Huber 1998).

Nephilid spider biology may constitute a counter-example. The generic revisions of *Herennia, Clitaetra, Nephilengys*, and *Nephila* (Kuntner 2005, 2006, 2007; M. Kuntner, unpubl. ms.) have revisited a stunning array of morphological and behavioral

traits that are likely shaped by sexual selection. Kuntner et al. (2008) put those in a phylogenetic perspective and suggested that male sexual self-mutilation and embolic plugging may represent evolutionary countermeasures to polyandry, which is widespread and probably primitive for araneoids and spiders as a whole. Although sperm competition alone might drive diversification of some male traits, the extraordinary armature of male palps, plugging behaviors, and apparently correlated female traits (Fig. 3), suggests that other selection pressures may be involved, which result in certain evolutionary outcomes that are in the interest of one, and not the other, gender. Kuntner et al. (2009) suggest that sexual conflict shapes the evolution of sex in nephilids, based on different patterns of courtship, mating, and mutilation, as well as differences in mating plug efficiencies and frequencies in *Nephila* and *Herennia*.

Here, we evaluate nephilid sexual biology as intersexual coevolution and attempt to explain the pattern with the recently
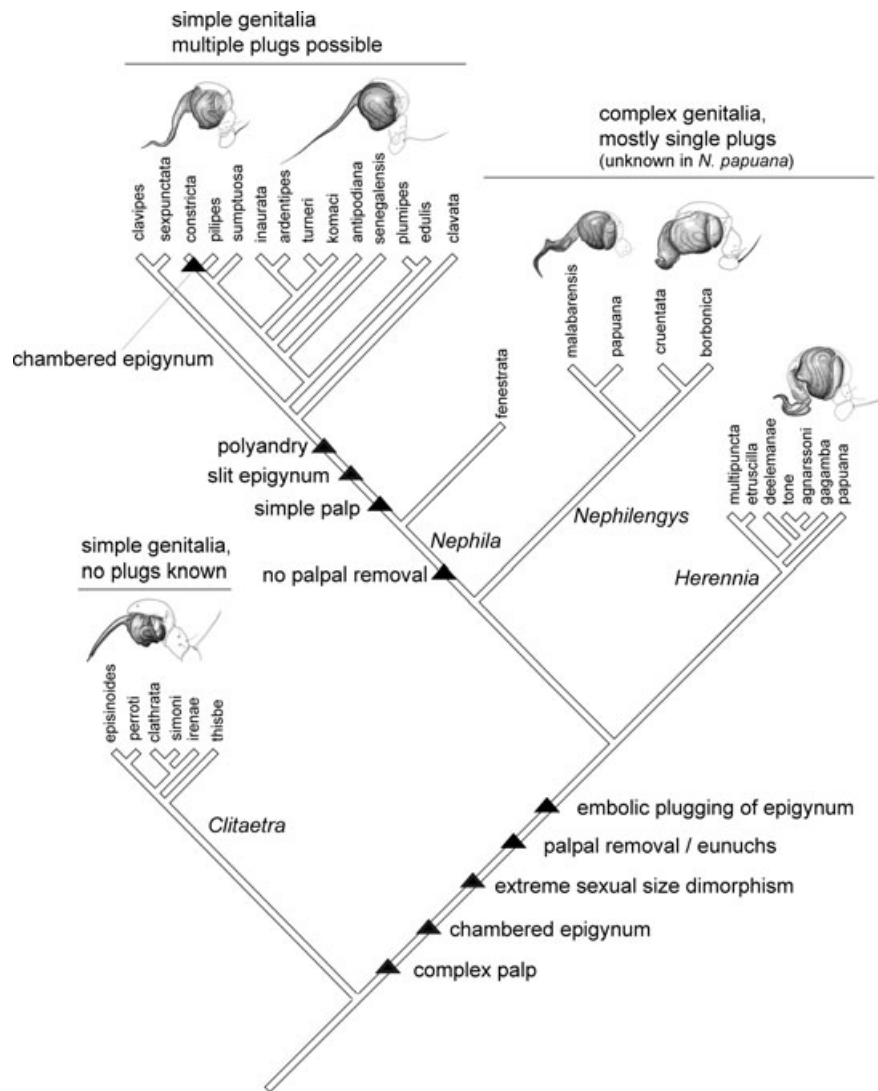
Figure 3. Summary of evolution of genital morphologies and sexual behaviors as implied by nephilid phylogeny.

invoked theories of sexual selection. We review the literature and place the known genital morphologies and sexual behaviors in a phylogenetic, coevolutionary perspective. Our assessment of genital complexity is based on evolutionary homology of sexual organs responsible for plugging and related behaviors. Specifically, we map male and female nephilid genital variation as suites of characters on a species-level phylogeny (Kuntner et al. 2008), synthesize these features into indices of male and female genitalic complexity, and use independent contrasts to show that male and female genital complexity positively covary. We also test, and reject, an alternative hypothesis for evolutionary changes in complexity between sympatric/parapatric sister species, that predicts reproductive character displacement in closely related species.

Methods

We use the most recent species-level phylogeny of nephilids and outgroups (Kuntner et al. 2008). An unweighted cladistic analysis of 231 morphological and behavioral characters scored for 61 taxa yielded four most parsimonious trees, which differed only in some outgroup relationships—irrelevant here-–and distal relationships in *Herennia*, which also do not affect the current analysis. Kuntner et al. (2008) used successive weighting to prefer one fully resolved topology, which we use here. Taxa relevant to this assessment of genital complexity are all known nephilids (*Clitaetra* [6 spp.], *Herennia* [7 spp.], *Nephilengys* [4 spp.], *Nephila* [15 spp.]), and the araneids *Deliochus* and *Phonognatha* as outgroups. The latter taxa were chosen because they also have genitalic apophyses (embolic conductors) homologous to those in nephilids and also use them to plug female genitalia.

Morphological "complexity" is difficult both to define and to measure (Cisne 1974; Bonner 1988; Valentine et al. 1994; McShea 2000; Adamowicz et al. 2008). Spider genitalia are sub-millimeter, highly three-dimensional (3D) structures whose complexity cannot adequately be captured by, say,

morphometric landmark analysis of two-dimensional (2D) images, or even distances measured directly on carefully positioned specimens, because shapes cannot be aligned a priori in homologous orientations, and, in any case, distances do not capture important aspects of complexity such as surface texture, presence or absence of minor features, or sclerite interactions. Male spider genitalia evolve so rapidly that important whole structures come and go within species groups, frustrating methods that require measurements of homologous (i.e., universally present) structures. 2D images of 3D structures distort shape. Although quantitative morphometrics has been successful with large, flat, or silhouette surfaces such as body shape in humans (Brown et al. 2008), cranial shapes in fish (Frederich et al. 2008), the anatomy of large fossils (Wilkinson 2008), and plant morphology (McLellan and Endler 1998; Del Guacchio and Caputo 2008), for tiny, extremely complex, and extremely 3D structures workers have tended to adopt simpler, more direct, and more intuitive measures of complexity, such as counts of serial structures and/or presence/absence of features (Fusco and Minelli 2000; Adamowicz et al. 2008). We adopt a variant of the latter approach here by tabulating 10 comparisons in each sex that exemplify "genital complexity" in these animals, and score each taxon cumulatively for such features (Table 1, illustrated in Figs. 1 and 2). For example, female genitalia that exhibit sclerotized flaps, hooks, projections, and elaborate copulatory openings are more complex than unadorned, two dimensional plates with paired slit openings. Similarly, male genitalia with broad terminal sclerites possessing hooks, flaps, and ridges are more complex than those with simple, thin, finger-like terminal sclerites. The "genitalic complexity" of a taxon, then, is simply the number of complex features it exhibits, and varies potentially from 0 to 10 for each sex.

Genital complexity indices were then analyzed using phylogenetically independent contrasts (PIC) analysis in PDAP version 1.13 (Felsenstein 1985; Midford et al. 2008) as implemented for Mesquite version 2.5 (Maddison and Maddison 2008). PIC requires a resolved phylogeny with specified branch lengths and assumes that evolutionary changes follow a Brownian motion model (Felsenstein 1985; Harvey & Pagel 1998). Statistical conformance with the assumptions of phylogenetically independent contrasts is checked by a series of diagnostic procedures (Midford et al. 2008), although PIC is reasonably robust to violations of branch length assumptions (Garland et al. 1999 and references therein). For cases in which phylogenetic branch lengths must be modeled or inferred, Midford et al. (2008) recommend four possible branch length transformations: equal, contemporaneous tips with internodes set to one (Pagel 1992), contemporaneous tips with internodes set to one less that the number of descendant tip species (Grafen 1989), and contemporaneous tips with internodes set to the log of number of descendant tip species (Nee

in Purvis 1995). We tested for significant correlation under all four transformations. Intersexual coevolution implies reciprocal gains of novelties, i.e. positive correlation between the sexes, thus implying a one-tailed test.

Reproductive character displacement can also explain rapid evolution of male and female genital complexity in closely related species (Brown and Wilson 1956; Marshall and Cooley 2000; Smith and Rausher 2008) and thus constitutes an alternative hypothesis to intersexual coevolution. It predicts greater than average differences in phenotype among sister species whose ranges overlap. Therefore we tested whether closely related (sister), sympatric or parapatric nephilid species pairs exhibit greater differences in genital complexity than the average of all 595 potential pairwise comparisons among the 35 nephilid species studied here.

Results

Table 1 lists and Figures 1 and 2 illustrate all features contributing to genital complexity and the summary index for male and female genitalic complexity for each species. The newly discovered male of *Nephila komaci* is scored here, but *Clitaetra simoni, C. thisbe,* and *Herennia agnarssoni* males are unknown and so receive a "?" for male genital complexity.

Neither the male ($P = 0.08$–0.56) nor the female genital complexity index ($P = 0.13$–0.78) positively correlated with its standard deviation under any branch length assumption (all equal, Grafen, Pagel or Nee, see above). The data therefore meet PIC statistical assumptions. Under all branch length assumptions except Grafen ($P = 0.067$), palpal and epigynal complexity are positively correlated (Figs. 5 and 6; $n = 31$; $r^2 = 0.155$; df $= 30$; one-tailed $P = 0.013$ for branch lengths equal to 1, for Pagel or Nee transformations, $P = 0.009$–0.045).

The data do not support reproductive character displacement. Nine sister species pairs are sympatric or parapatric and thus were predicted to differ more than average in genital complexity: *Clitaetra episinoides* vs. *perroti, C. clathrata* vs. *simoni, Herennia multipuncta* vs. *etruscilla, Nephilengys malabarensis* vs. *papuana, N. cruentata* vs. *borbonica, Nephila inaurata* vs. *ardentipes, turneri* vs. *komaci, clavipes* vs. *sexpunctata,* and *plumipes* vs. *edulis*. The average difference between these pairs was 0.667, whereas the average difference among all 595 pairs of nephilid species was 3.79. Sympatric/parapatric sister taxa differ less, not more, than average, but not significantly (*t*-test, $P < 0.366$).

Figure 3 graphically summarizes the important morphological and behavioral events in nephilid sexual biology. Complex palps, epigynal plugging, and palpal removal all evolve at the same node as extreme sexual size dimorphism (actually female giantism; Coddington et al. 1997). The latter causes a

Table 1. Features of male (1–10, adapted from characters 172–173, 175–181, 192 of Kuntner et al. 2008) and female genital complexity (11–19, adapted from characters 91, 93, 95–97, 105–107, 111 of Kuntner et al. 2008; feature 20 is new), and overall genital complexity for each sex of each species. All features are illustrated in Figures 1 and 2.

Character/taxon	Deliochus	Phonognatha graeffei	Phonognatha melanopyga	Clitaetra episinoides	Clitaetra perroti	Clitaetra clathrata	Clitaetra irenae	Clitaetra simoni	Clitaetra thisbe	Nephila clavipes	Nephila sexpunctata	Nephila constricta	Nephila fenestrata	Nephila senegalensis	Nephila sumptuosa	Nephila inaurata	Nephila ardentipes	Nephila turneri	Nephila komaci	Nephila pilipes	Nephila antipodiana	Nephila clavata	Nephila plumipes	Nephila edulis	Herennia multipuncta	Herennia etruscilla	Herennia deelemanae	Herennia papuana	Herennia tone	Herennia gagamba	Herennia agnarssoni	Nephilengys malabarensis	Nephilengys papuana	Nephilengys cruentata	Nephilengys borbonica
1. Embolic conductor membrane: (0) absent; (1) present.	1	1	1	0	0	0	0	?	?	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	?	?	?	1	1	1	1
2. Embolic conductor shape: (0) finger-like; (1) broad.	1	1	1	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
3. Embolic conductor (division): (0) entire; (1) subdivided into two or more sclerites.	1	1	1	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0
4. Distal embolic conductor flap: (0) absent; (1) present.	0	0	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	?	0	0	0	0
5. Embolic conductor edge: (0) smooth; (1) ridged.	0	0	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	?	1	1	1	1
6. Embolic conductor curvature: (0) more/less straight; (1) sigmoidal/bent distally.	0	0	0	0	0	0	0	?	?	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	?	1	1	1	1
7. Embolic conductor tip: (0) straight; (1) hooked.	0	1	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0
8. Embolic conductor subdistal protuberance: (0) absent; (1) present.	0	0	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	?	?	0	0	0	0
9. Embolus relative length: (0) short to medium (<2×cymbium length); (1) long (>2×cymbium length).	0	0	0	1	1	1	1	?	?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	?	1	1	1	1
10. Embolus: (0) smoothly curved; (1) sharply hooked.	1	0	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	1	?	?	?	?	0	0	0	0

Continued.

Table 1. Continued.

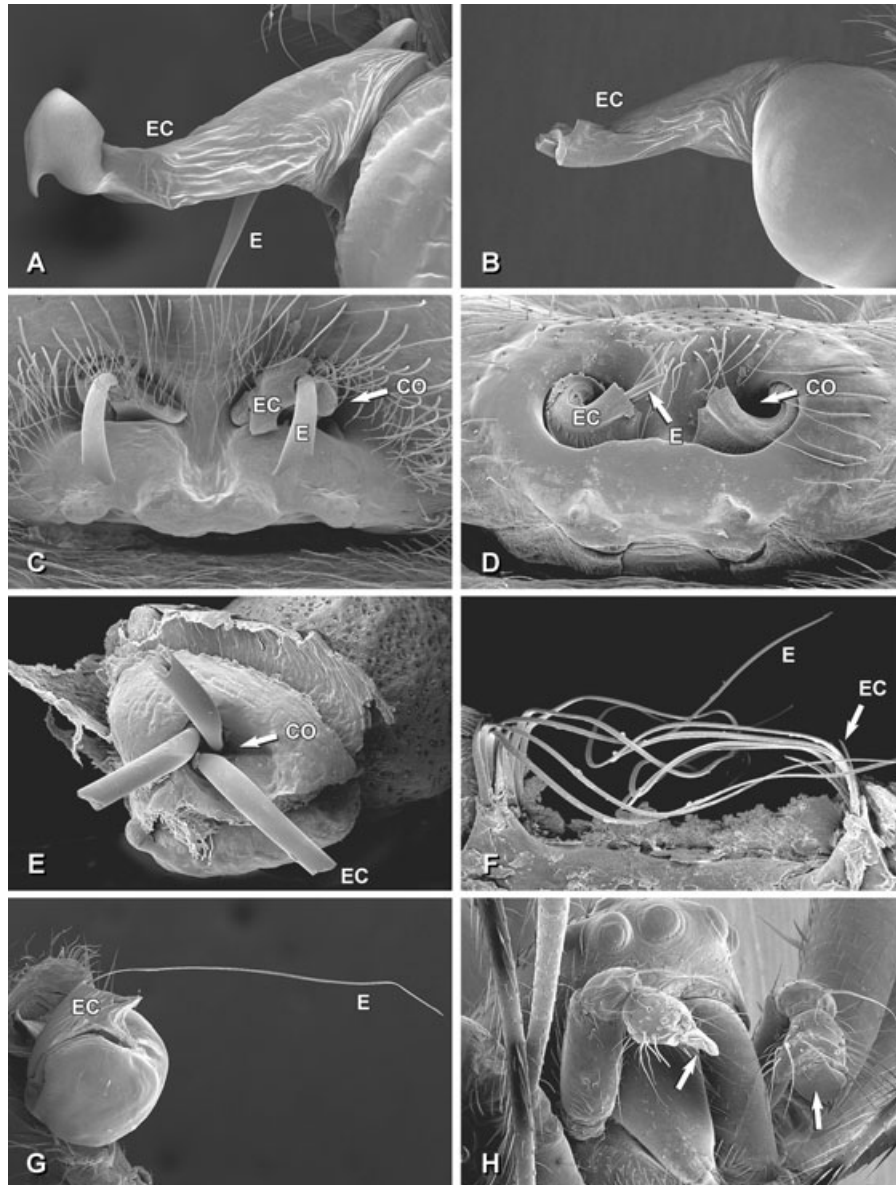| Character/taxon	Deliochus	Phonognatha graeffei	Phonognatha melanopyga	Clitaetra episinoides	Clitaetra perroti	Clitaetra clathrata	Clitaetra irenae	Clitaetra simoni	Clitaetra thisbe	Nephila clavipes	Nephila sexpunctata	Nephila constricta	Nephila fenestrata	Nephila senegalensis	Nephila sumptuosa	Nephila inaurata	Nephila ardentipes	Nephila turneri	Nephila komaci	Nephila pilipes	Nephila antipodiana	Nephila clavata	Nephila plumipes	Nephila edulis	Herennia multipuncta	Herennia etruscilla	Herennia deelemanae	Herennia papuana	Herennia tone	Herennia gagamba	Herennia agnarssoni	Nephilengys malabarensis	Nephilengys papuana	Nephilengys cruentata	Nephilengys borbonica
11. Epigynal openings: (0) superficial; (1) in chambers.	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
12. Epigynal septum: (0) absent; (1) present.	1	01	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	01	1	1	1	1	1	1	1	1	1	0	0
13. Epigynal paired sclerotized pocket: (0) absent; (1) present.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
14. Epigynal scape: (0) absent; (1) present.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15. Paired epigynal flap: (0) absent; (1) present.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16. Copulatory opening form: (0) elongated slit openings; (1) rounded openings.	1	1	1	1	1	1	1	1	1	1	0	1	1	01	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1
17. Copulatory duct morphology: (0) short (wider than long); (1) long (longer than wide).	1	0	1	1	1	1	1	?	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	?	?	1	1	?	1	1	0	1
18. Spermathecae: (0) unlobed; (1) lobed.	1	1	1	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19. Epigynal sclerotized arch: (0) absent; (1) present.	0	0	0	0	0	0	0	?	?	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0
20. Copulatory duct curvature: (0) none or slight; (1) curved or looped.	0	0	1	1	0	?	1	1	0	1	?	1	0	1	1	?	0	1	1	1	1	0	1	0	1	1	?	?	0	?	?	0	0	0	0
Male genitalia complexity index	4	4	3	1	1	1	1	?	2	2	2	2	5	2	2	2	2	2	2	1	2	3	3	2	7	7	6	6	5	5	?	5	5	5	5
Female genitalia complexity index	6	4	6	2	2	2	3	1	2	2	1	5	6	4	2	1	1	2	2	2	2	1	3	2	6	6	3	3	5	4	3	5	5	3	4

Figure 4. Nephilid genital mutilation, epigynal plugging, and emasculation: **(A)** *Nephila fenestrata* male intact embolic conductor (note distal hook); **(B)** *N. fenestrata* mutilated embolic conductor (distal part missing); **(C)** *N. fenestrata* female epigynum with embolic conductors plugging each copulatory opening; **(D)** similarly plugged epigynum in *Herennia multipuncta*; **(E)** multiple male parts plugging copulatory openings of *Nephila constricta*; **(F)** multiple male parts embedded in amorphous epigynal plug in *Nephila pilipes*; **(G)** dysfunctional *Nephila sumptuosa* male palp, presumably mutilated during copulation; **(H)** male eunuch *Nephilengys malabarensis* with no palps (arrows). Legend: CO, copulatory opening; E, embolus; EC, embolic conductor.

male-biased effective sex ratio, male accumulation at female webs, and polyandry (Miller 2007).

Males most commonly plug females by breaking off two connected sclerites, the embolus and embolic conductor (Fig. 4A) inside her genitalia. Such behavior, termed as self-mutilation, emasculation, palpal breakage, or genital plugging, occurs during or when copulation ends (Kuntner et al. 2009). The damaged male palp is rendered dysfunctional (Fig. 4B). *Herennia* and *Nephila fenestrata* males apparently can permanently

plug female openings (Fig. 4C,D; Kuntner 2005; Fromhage and Schneider 2006; Kuntner et al. 2009), and *Nephilengys* may as well (Fig. 3). *Nephila pilipes* females, on the other hand, remate up to at least five times, regardless of male embolic plugs (Fig. 4F; Kuntner et al. 2009). *Nephila* males do not sever the whole palp (Fig. 4B,G), even though mutilation probably renders it dysfunctional. Mutilated *Herennia* and *Nephilengys* males completely castrate themselves by autotomizing the entire bulb, and are termed "eunuchs" (Fig. 4H; Robinson and Robinson 1980;
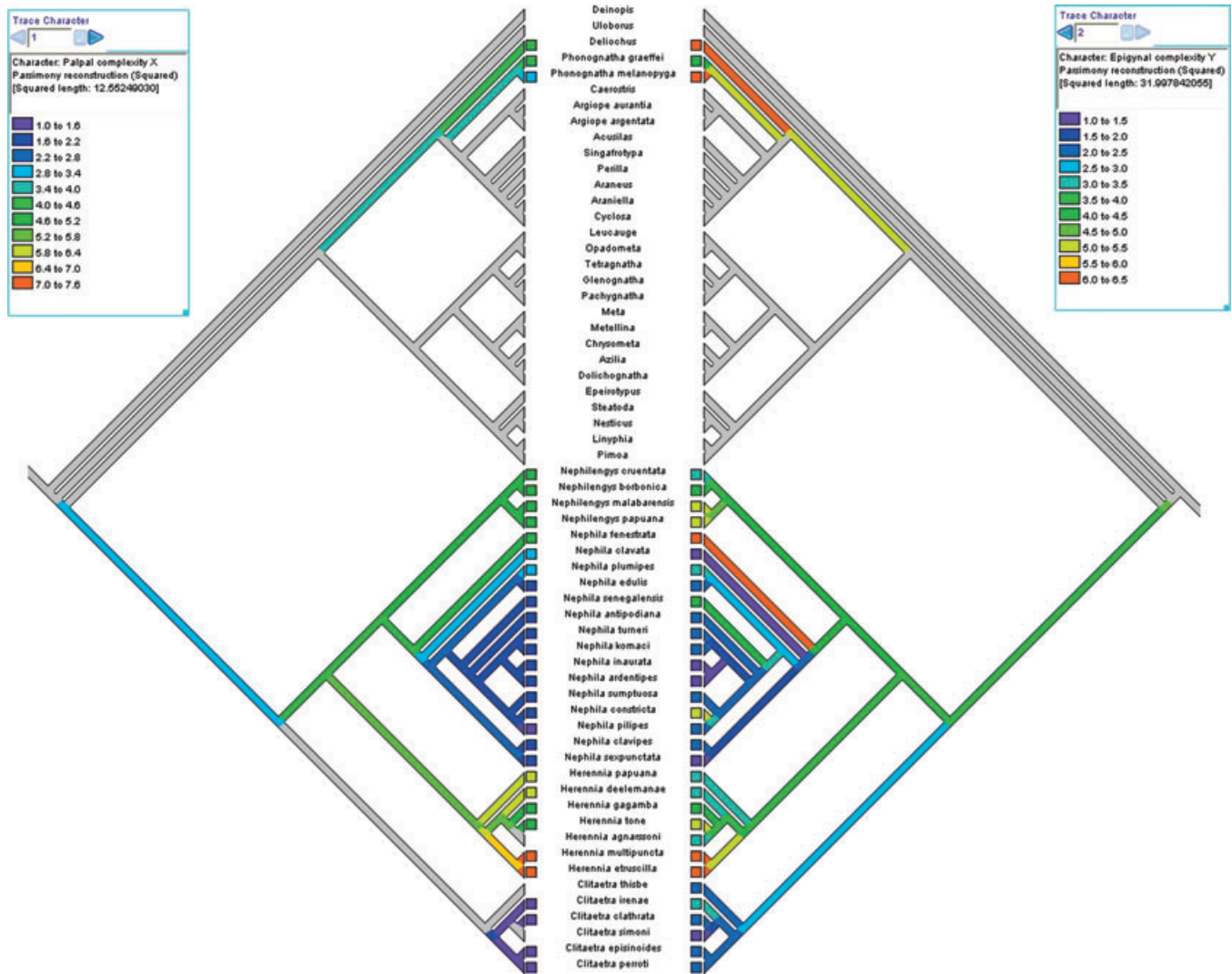
Figure 5. Coevolution of male (left) and female (right) genital complexity indices in nephilid spiders and outgroups.

Kuntner 2005, 2007). Eunuch males remain in or around the female's web and attempt to prevent or interfere with visits of other males (Kuntner et al. 2009).

Male emasculation and eunuchs are unknown in *Clitaetra*, but are common in all *Herennia* and *Nephilengys* species (M. Kuntner pers. obs.; Kuntner 2005, 2006, 2007). *Nephila* species usually emasculate themselves to some degree but are never eunuchs. Because emasculation only occurs as a result of plugging behavior, one can infer from damaged male museum specimens that plugging occurs in all nephilids distal to *Clitaetra*, apart from a few *Nephila* species (see below).

Nephila fenestrata is sister to all other *Nephila* (Kuntner et al. 2008). Their complex palps make large, completely effective plugs (Fromhage and Schneider 2006). The remaining *Nephila* species evolved slender emboli. *Nephila plumipes* (Schneider et al. 2001), *inaurata* (Schneider et al. 2005), *senegalensis*, *komaci* (pers. obs.), *constricta* (Fig. 4E), *pilipes* (Fig. 4F), and *clavipes*

all frequently (rarely in *clavipes*, pers. obs.) have the embolus-conductor complex broken off deep inside the female genitalia, but this does not block subsequent suitors effectively. All these *Nephila* species are polyandrous (Schneider and Elgar 2001, 2005; Schneider et al. 2000, 2001; Fromhage and Schneider 2005a,b, 2006; Kuntner et al. 2009). No evidence was seen for palpal damage and embolic plugs in *N. antipodiana* (6 males/48 females examined), *sexpunctata* (4/20), *ardentipes* (1/4), *edulis* (4/22), or *clavata* (22/36).

Discussion

Male and female genital complexity are significantly and positively correlated in nephilid spiders. Many sexually related features coevolve at the same phylogenetic nodes (Fig. 3), for example in the hypothetical ancestor of *Herennia, Nephilengys* and *Nephila*, and again in the hypothetical ancestor to all *Nephila*
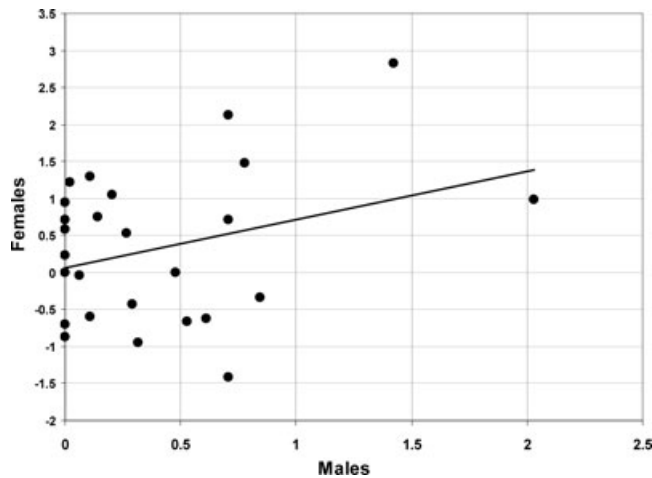
Figure 6. Independent contrasts of male (x) vs. female (y) genital complexity are positively correlated ($n = 31$, $r^2 = 0.155$, df = 30, $P = 0.013$, branch lengths equal).

minus *N. fenestrata*. Plesiomorphic (simple) female genitalia are slit-like and equipped with relatively straight, short ducts. Females subsequently enlarged copulatory chambers and elaborated connecting ductwork tremendously. The male plesiomorphic condition is also simple, followed by the evolution of enlarged, complex palps equipped with hooks, ridges, and twists. In both sexes genitalia ultimately reverse to more simple shapes. Thus, we find strong support for the hypothesis of intersexual coevolution of nephilid morphologies and related behaviors.

The prevalence of damaged male and female genitalia (broken or plugged) suggests sexual conflict. Male embolic breakage, epigynal plugging, pre- and postcopulatory mate guarding, and opportunistic mating are all male-mating strategies. We interpret these traits as male persistence mechanisms (Arnqvist and Rowe 2005). Ultimately they function to minimize sperm competition and, by preventing female access to additional males, oppose female interests. These behaviors and/or morphologies counter polyandry (note, because *Clitaetra* sexual behavior is unstudied, the ancestral state for polyandry at the nephilid common ancestral node is also unknown). The evolution of a chambered epigynum may constitute a female resistance mechanism (Arnqvist and Rowe 2005) that counters male plugging by enlarging the space to permit additional matings, thus facilitating female choice. On the other hand, increased male embolic conductor complexity and size may counter larger chambered epigynal openings. The palpal and epigynal complexity appear to compete in evolutionary time—essentially an arms race. *Herennia* and *N. fenestrata* males show the morphological correlates of and behavioral evidence for successful monopolization of females (Kuntner et al. in press; Fromhage and Schneider 2006). These taxa bracket *Nephilengys* phylogenetically, which also shows the same morphological correlates, and therefore implies that male

monopolization in *Nephilengys* will be confirmed behaviorally. If so, the nephilid clade from *Herennia* through *Nephilengys* to *N. fenestrata* exemplifies successful male persistence mechanisms.

The subsequent evolutionary reversals to extreme female polyandry, simple slit epigyna, and thin, long palpal sclerites (Figs. 1A, 2A, 3, and 4G) may reflect selection for thin sclerites able to bypass existing plugs. Embolic conductor length and flexibility then compete with long, coiled female copulatory ducts. The recurrence of polyandry in the relatively distal *Nephila* (*plumipes*: Schneider et al. 2001; *pilipes*: Kuntner et al. 2009) suggests that female resistance mechanisms have defeated male monopolization.

Mating duration can also reflect sexual conflict (Schneider et al. 2006). Males gain more from prolonged mating than females (Arnqvist and Rowe 2005). Distal embolic conductor hooks (Figs. 1 and 4A) enable dimorphically small males to prolong copulation by entangling themselves in the female genital chambers (Schneider et al. 2001). If so, palpal mutilation may be a side effect of adaptations that prolong copulation. Whether females forcefully terminate copulation (Schneider et al. 2001), or males cannot disengage, palpal damage results. Whether directly selected to minimize polyandry or as side effects of efforts to prolong copulation, mating plugs still constitute sexual conflict and fit broadly into the arms race model.

More research is needed to test whether male plugs in nephilid spiders are voluntary, evidence both conflicts (*N. plumipes*: Schneider et al. 2001; *N. borbonica*: Kuntner et al. in press) and supports this hypothesis (*N. fenestrata*: Fromhage and Schneider 2006). Certainly the reversion to slender emboli that do not plug epigyna effectively is, from a male point of view, paradoxical and difficult to explain. The simplest explanation is that females "recently" evolved mechanisms to reassert polyandry, and that males in these lineages, although continuing to break off genitalia in the females, now fail to interdict female access to additional mates (Fig. 4E,F). If so, the origin of the male plugging mechanism, not its maintenance, deserves a closer scrutiny (Arnqvist 2006). Thus, future studies should focus on studying *Herennia* more than *Nephila* (Fig. 3).

Reproductive character displacement in closely related and sympatric species is an appealing hypothesis, supported in some studies of premating sexual barriers, such as bioacoustics in birds, frogs, and insects (Marshall and Cooley 2000; Gabor and Ryan 2001; Höbel and Gerhardt 2003). However, our data on genital complexity evolution in nephilid spiders did not support it, suggesting that genital morphologies do not evolve rapidly enough at the level of sister species to show the effect.

Two recent hypotheses are compatible with the observed pattern of intersexual coevolution, cryptic female choice (Eberhard 1996, 2006) and sexually antagonistic coevolution (Arnqvist and

Rowe 2002a, 2005). Much recent literature contrasts the hypotheses as exclusive alternatives (Eberhard 2004a, 2006), but, of course, both can be partially correct (Cordero and Eberhard 2005). Both may operate in Nephilidae, but the apparent strong sexual conflict makes sexually antagonistic selection a more straightforward explanation.

Being essentially an overview of pattern, comparative phylogenetic research cannot by itself fully explain sexual selection patterns (Zeh and Zeh 2003). Indeed, genital coevolution and sexual conflict by themselves do not necessarily imply sexually antagonistic coevolution (Lessells 2006). Future studies should focus on testing the predictions of the two hypotheses. Cryptic female choice predicts female benefits by resisting some and not other male phenotypes (Eberhard 1996), whereas sexually antagonistic selection predicts female costs due to male persistence mechanisms.

The most likely biological explanation of the mechanisms of intersexual coevolution in nephilid spiders is as follows. The orb webs of size-dimorphic, solitary, sedentary *Nephila* and *Nephilengys* function as islands or habitat patches (Agnarsson 2003) because courtship and mating only occur in webs or retreats (Robinson and Robinson 1980; Robinson 1982; Kuntner et al. 2008). Sexual size dimorphism results in male-biased tertiary sex ratios because females take longer to reach their relatively enormous size (Higgins 1992, 1993, 2002) and therefore proportionately fewer survive. The relatively abundant, vagrant males accumulate and compete with one another at female webs (Fromhage et al. 2005; Miller 2007). At the same time, male mortality during searching behavior can be extremely high (Vollrath and Parker 1992; Andrade 2003), so the best strategy for males is to monopolize one female (monogamy) via mate guarding, opportunistic mating, and plugging of female genitalia by genital self-mutilation (Fig. 4). Females, on the other hand, evade male monopoly via multiple matings (polyandry), postcopulatory mate choice (Eberhard 1996), and evolution of genitalia that obviate male plugging strategies (Fig. 3). These behavioral and morphological strategies reflect the different interests of each gender, and may represent an evolutionary arms race (Kuntner et al. 2009), which triggers morphological and behavioral coevolution.

Conclusions

Nephilid coevolution appears to be driven at least in part by sexual conflict (Kuntner et al. 2009). We presented, tested, and found significant support for the hypothesis of intersexual coevolution of genital and sexual behavioral traits in orb weaving spiders, but not for reproductive character displacement. We interpret the pattern as an intersexual arms race, although cryptic female choice cannot be excluded by these data. Experimental manipulations of behavior and morphology, including paternity analysis and female fitness consequences of male plugging, might provide supplementary data on specific taxa to test the overall patterns predicted by phylogenetic perspectives.

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Associate Editor: N. Wedell