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WHY STUDY SPIDER SEX: SPECIAL TRAITS OF SPIDERS FACILITATE STUDIES OF SPERM COMPETITION AND CRYPTIC FEMALE CHOICE

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ABSTRACT. I argue that several unusual aspects of spider sexual biology make them extremely promising subjects for future research on sperm competition and cryptic female choice, and outline promising lines for future research. The important traits include: double, bilaterally symmetrical genitalia (allowing the use of the same animal as experimental and control and thus providing unusually complete controls for experimental manipulations); isolation of male ejaculates in pure form during sperm induction (allowing experimental determination of the effects of sperm and male accessory glands on female reproductive physiology, and separation of their effects on the female from those of copulatory courtship and copulation); frequent venter-up orientation and genitalic meshes in which most of the male genitalia is outside rather than inside the female (allowing unusually complete observations of male genital behavior during copulation); immobile sperm (allowing confident deductions about male and female movement of sperm without complications from motility of the sperm themselves); a huge data set on female as well as male genitalic morphology from previous taxonomic studies (enabling, in combination with studies of the fit between male and female genitalia, studies of the details of how rapid genitalic divergence occurs). Studies of spider sex should be in the forefront of the next generation of studies of sperm competition and crytpic female choice.

Keywords: Sperm competition, cryptic female choice, sexual selection, genitalia

Ever since the path-breaking study of sperm competition by Parker (1970), it has been apparent that sexual selection can act on the abilities of males to influence reproductive processes that occur after the initiation of copulation. In Darwin's original treatment of sexual selection (1871), he only considered events leading up to copulation. Perhaps because of cultural strictures that were imposed by his own ideas or those of his wife or daughter (Birkhead 2000) of what was proper for a Victorian gentleman to discuss, he missed the possible significance of the more intimate details of male—female interactions that occur after copulation begins. Parker's attention originally focused on processes corresponding to Darwin's category of direct male-male conflict or intrasexual sexual selection (''sperm competition''), but it later became clear that processes corresponding to Darwin's other category, female choice or intersexual selection, could also occur in addition after copulation has begun (''cryptic female choice''; Thornhill 1983; Eberhard 1985, 1996). Because the crucial events occur within the female's own body, cryptic female choice may have been a more important evolutionary process than sperm competition (Eberhard 1996), but to date it has been less studied.

Recognition of the possible importance of sperm competition and cryptic female choice has forged new connections with the fields of reproductive morphology and physiology, which had developed in relative isolation from sexual selection theory (Eberhard 1985; Eberhard & Cordero 1995). It brings into focus the possible evolutionary consequences of much otherwise arcane information, such as the morphological details of how male and female genitalia function during sperm transfer, how sperm are handled within the female prior to fertilization (e.g., Burger et al. 2003), and the effects of male seminal products on female reproductive physiology, including control of ovulation, oviposition, sperm storage, and induction of resistance to further copulations (Chen 1984).

Sperm competition and cryptic female choice are currently active fields of research, as testified by recent book-length summaries

(Birkhead & Møller 1998; Simmons 2001). These summaries show that the amount of attention that has been given to spiders has, as usual, been relatively minor compared to that paid to insects. I will argue in this paper that this bias should be reduced in the next generation of studies, and that spiders should be in the vanguard of work on these topics due to several peculiar aspects of their sexual biology which facilitate study. My aim is not to present a complete review of work on sperm competition and cryptic female choice in spiders, but rather to show why spiders are particularly well suited for studies regarding sperm competition and cryptic female choice, in the hope of encouraging further study. Because of this objective, my citations of previous work are incomplete and biased toward more recent studies so as to give entries into the published literature.

Morphology of genitalia and sperm.—The genitalic structures with which male spiders introduce sperm into the female are unique, and although studies of their functional morphology are still only fragmentary, their morphology offers many special advantages for the study of sperm competition and cryptic female choice (Table 1). The male's palpal bulb includes: a reservoir which stores sperm that have been emitted from the male's primary genital opening on his abdomen; more or less complex sclerites which brace each other and couple the palp to the female's genitalia; and sclerites which introduce the sperm into her reproductive tract. The bulb is apparently derived embryologically from the tarsal claw of the pedipalp, and, not surprisingly, is devoid of both neurons and muscles (Eberhard & Huber 1998a). Its movements during copulation are somewhat limited in scope, as they are produced by hydraulic pressure that inflates membranous sacs and causes sclerites to move in complex patterns with respect to each other and to the female (Gering 1953; von Helsdingen 1965, 1969; Grasshoff 1968, 1973; Blest & Pomeroy 1978; Huber 1993, 1995a, b). The highly sclerotized rigid nature of both male and female genitalia, the lack of sensory structures on at least the external portions of the female genitalia as well as on the male's genitalia, combined with the limited movements of male genitalia, mean that functional accommodation of male and female forms can be deduced relatively clearly (com-

Table 1.—Special traits of spiders which facilitate study of sperm competition and cryptic female choice and could make spiders leaders in future studies of these phenomena.

1. Sperm are encapsulated when transferred, so sperm displacements within female during copulation can be attributed with confidence to either male or female transport rather than sperm mobility. If sperm do not become decapsulated soon after insemination, clumping may occur in storage.
2. The more or less independent sclerites in the palpal bulb lack nervous connections, thus allowing ablation experiments in which possible sensory effects can be ignored.
3. Movement of palpal sclerites occurs via expansions of membranous sacs (haematodochae) rather than muscles. Most movements are relatively stereotyped and occur on the female's external surface. This allows direct observation of male genitalic movements, and precludes cryptic movements inside the female that cannot be observed directly.
4. External isolation of pure seminal products occurs during the process of charging pedipalps, thus allowing experimental separation of the effects on the female of copulation per se, and of seminal products.
5. The external female genitalia are rigid and often complex, and lack sensory structures on the external surface. The male genitalia must mesh physically with them, allowing relatively easy deductions regarding functional significance of male genitalic structures, by freezing and sectioning spiders during copulation; mechanical functions of male genitalia are especially likely to be important.
6. Paired male and female genitalic structures make it possible to experimentally modify one side of the animal and leave the other left intact, giving unusually complete experimental controls.
7. The forms of both male and female genitalia are easily determined because they are generally strongly sclerotized, and there is already a huge taxonomic literature that documents genitalic structures in females as well as in males. Data is thus already available for broad comparative studies tracing coevolutionary patterns in males and females.
8. Experimental manipulation of male feeding of the female during copulation (e.g. *Argyrodes*) should be easy.

pared with many other animals) by freezing copulating pairs and then sectioning them (e.g,, Huber 1993, 1995a, b; Uhl et al. 1995; Knoflach 1998 and references therein). The general finding from these studies is that many portions of complex rigid male genitalic structures function to contact and brace against the female's rigid and sometimes structurally complex genitalia, or to brace other male sclerites so as to facilitate contact with the female (Eberhard & Huber 1998a). These are functions that are relatively easily determined directly from morphology. It appears that the male genitalia of spiders are seldom used to physically seize the female or directly stimulate her; these are probably common functions of the genitalia of some major groups of insects (Eberhard 2004).

Possibly as a consequence of the lack of neurons in the male palpal bulb that might provide feedback between palps and behavior, male spiders which have lost the entire palp or the intromittent organ (embolus) nevertheless court females and go through normal movements of copulation (Rovner 1966, 1967; Snow & Andrade in press). This makes it possible to experimentally separate the responses of the female that are triggered by stimulation from the male's genitalia and their products from her responses to all the other stimuli normally associated with copulation (pre-copulation courtship, copulatory courtship). Possible behavioral responses of female (e.g., her receptivity to further copulations, willingness to oviposit, clutch size, etc.) that have been ''mated'' by a modified male can be compared with those of females mated with intact males. To my knowledge this exciting possibility has only been exploited in two species, and only with respect to a single female response. Aisenberg et al. (2002) found that in the lycosid *Schizocosa malitiosa* Tullgren 1905 a recently mated female's lack of receptivity to further copulation is apparently due to the semen itself, rather than to the elaborate male courtship before and during copulation. First they sealed the tip of the male's pedipalp soon after he molted to maturity, and thus prevented uptake of semen. These males nevertheless performed apparently normal courtship and copulation behavior (although it is possible that details may not have been identical—see Costa 1998). When a female mated with a male that could not transfer semen, she was much more likely to remate than were control females that had received semen from normal males. Rovner (1966) obtained apparently normal tendencies to court in palpless males of the lycosid *Rabidosa rabida* (Walckenaer 1837), and saw at least approximately normal copulation behavior by palpless males of the linyphiid *Linyphia triangularis* (Clerck 1757); females of this species that had not received semen were more receptive than normally mated females. The conclusion from both studies is that seminal products, either the sperm itself or other products, or perhaps stimuli associated with normal intromission, lower female sexual receptivity. Similar prevention of sperm uptake (by the simpler and more powerful technique of removing the sperm droplet from his sperm web, which leaves the male's genitalia unaltered) showed that male products in the last of the several droplets which male *Theridion* take up during sperm induction were crucial to the formation of the mating plug, and also affected the male's own copulation behavior (Knoflach 1998).

The design of the female reproductive tract in many species also facilitates study. The external female genitalia are often rigid (the epigynum), as are her spermathecae and their ducts, so they offer the advantage that their forms are readily accessible for study. These traits help make comparative functional morphology and detailed studies of stages in the rapid divergent evolution of genitalia particularly feasible in spiders. The long-standing tradition in taxonomic studies of using both male and female genitalia means that there is already a huge data base on both male and female designs in closely related species. With a detailed understanding of the mesh between male and female structures in chosen species, and the phylogenetic relations within a particular group, it should be possible to trace in fine detail both how and why male and female genitalic traits have co-evolved. Even though in some groups it may be necessary to carefully examine females for cryptic complexity (Uhl & Gunnarsson 2001), tracing coevolution would be especially interesting if it were combined with studies of intraspecific variation in different male and female structures (a type of data not traditionally emphasized in taxonomic studies). Comparisons would be of even greater interest if this type of study were

coupled with attempts to correlate intraspecific variation in genital morphology with different variables that could affect copulatory success such as paternity success, female delay to oviposition, female receptivity to remating, or female attractivity to males (pheromone production). Have new male forms led to the evolution of new female forms, or vice versa? If new male forms take the lead (as expected under traditional female choice hypotheses), what are their original consequences during copulation? Have novel male forms evolved to overcome species-specific female defensive structures, as supposed by the currently popular sexually antagonistic coevolution hypothesis (Chapman et al. 2003)?

Spider genitalia are readily visible during copulation, in contrast with those of many other animals. Because many spiders hang upside down in their webs, male and female genitalia can be observed in great detail when copulating pairs are placed under a dissecting microscope (use of a mirror also allows detailed observations of species without webs). Much of the movement of the male's genitalia is observable because it occurs outside the female. The new generation of digital video cameras makes it possible to film behavior easily through a microscope, by simply holding the camera to the eyepiece and shooting. Males of different species perform quite different genitalic behavior, including groping and hammering against the female, rhythmic expansions of haematodochae, twisting, and vibrating or quivering, and repeated insertions and withdrawals of their intromittent structures (Huber 1998). To date, nearly all descriptions are only qualitative (for an exception, see Schäfer & Uhl 2002), and the few available detailed comparative analyses show that genitalic behavior is sometimes complex and species-specific (Rovner 1973, 1974; Stratton et al. 1996; Knoflach 1998). This behavior probably offers useful characters for distinguishing closely related species in some groups. Although the behavior of spiders' genitalia is relatively easy to study, research on this topic has only barely begun.

In spiders, the male's sperm are normally encapsulated when they are transferred to the female. This trait represents an advantage for study of sperm transfer, because it means that any movements within the male or the female can be confidently attributed to male and female effects, and the possibility of sperm mobility can be ignored. In the araneid *Micrathena gracilis* (Walckenaer 1805), copulation involves two separate processes, sperm release and sperm storage (Bukowski & Christenson 1997a). In groups such as *Latrodectus*, in which the male's genitalia can reach the spermatheca, it may mean that even a plug that only partially occludes the insemination duct (enough to detain a subsequent male's intromission) can nevertheless be effective in biasing paternity (Snow & Andrade pers. comm.). Sperm encapsulation may also make sperm precedence studies using irradiated males more useful in understanding natural processes, since sperm mobility is not important, at least in these early stages. The complete lack of disadvantage of sperm from irradiated males in competition with non-irradiated sperm in the pholcid *Physocyclus globosus* Taczanowski 1873 (Eberhard et al. 1993; Peretti pers. comm.) and the theridiid *Latrodectus hasselti* Thorell 1870 (Snow & Andrade pers. comm.) supports this idea. In *L. hasselti*, irradiated sperm were just as competitive in fertilizing the eggs in the female's fourth clutch following copulation as those in her first clutch (Snow & Andrade in press). At least in *Pholcus phalangioides* Fuesslin 1775, sperm activation may occur only shortly before oviposition (G. Uhl pers. comm.).

The paired nature of both male and female genitalia and the alternating use of sides allow one to use the same animal to assume the roles of experimental subject and control in the same experiment. If, for example, one modifies one of the male's palps and then checks insemination success or plug removal success, the other intact palp can serve as an unusually sophisticated control, in which all other variables (male precopulatory and copulatory courtship, male and female size, duration of pairing, etc.) are equal for both experimental and control treatments. This should make studies of experimental modifications of male and female genitalic form unusually powerful and sensitive in spiders, compared with most other animals. The conclusions would be especially interesting if the behavior of the intact palp is not affected (an interesting topic in itself). I know of only one study that has used this experimental design. In the tetragnathid *Leucauge mariana* Keyserling 1881, the effects on insemination and copulatory

plug removal are being tested by cutting off the tips of either the conductor hook, or both the hook and the conductor tip on one palp, but leaving them intact in the other (Mendez & Eberhard unpub. data). The male was then allowed to mate with either a female which had a copulatory plug in her epigynum from a previous mating, or a virgin female. Preliminary data indicate that the conductor hook is important in both plug removal and sperm transfer, while the conductor tip improves insemination. Snow & Andrade (in press, pers.comm.) exploited the female's bilateral design and interruptions of first copulations after only one side was inseminated in *Latrodectus hasselti* to determine the effects of possible plugs and of sperm in different spermathecae on paternity. They also showed that the tip of the embolus functions to facilitate intromission. Watson (1991) mentioned possible palpal damage due to use in the linyphiid *Neriene litigiosa* Keyserling 1886, but gave neither morphological details nor observations on the consequences. Clearly much further work could be done in this area; manipulative studies would be especially feasible with larger species, and with species in which the male intromits only once into each side of the female.

Other manipulations could also illuminate the dynamics of utilization of sperm from different spermathecae, by allowing one male to inseminate one side of the female, and another to inseminate the other, and then checking the paternity of the offspring. Do the contents of the paired spermathecae move simultaneously and in equal numbers into the oviduct for fertilization? Do spiders' multiple sperm storage organs result in biased use of sperm from different males, as has been hypothesized (Hellreigel & Ward 1998; Simmons 2001)? To my knowledge this experiment has been done with only one spider, *Latrodectus hasselti*. When each spermatheca contained sperm from a different male, the paternity success of the two males did not change from the female's first to fourth clutch, suggesting similar use of sperm from the two sides with each clutch (Snow & Andrade in press). When each male inseminated the same side of the female, sperm from the first male were more likely to fertilize her eggs than when each copulated with a different side (Snow & Andrade in press). Because females of this species sometimes allow a male to inseminate only one side (12.5% of observed copulations), this means that females can affect male paternity success. Males also play active roles in this species, as they show a strong tendency to inseminate the side of the female not inseminated by a previous male (Snow & Andrade in press).

Austad (1984) noted that the internal morphology of the female reproductive tract may have an influence on sperm precedence patterns when a female mates with more than one male, and may thus result in a female-determined ''passive preference'' (Wiley & Posten 1996) for males with certain traits. Austad contrasted groups in which there is a single duct associated with each spermatheca and in which the sperm of the last male to mate may be better placed (closer to the exit of the spermatheca) to fertilize eggs; and groups with two spermathecal ducts, in which the first male's sperm may be better positioned (near the fertilization duct). In particular, a strong first male paternity advantage of species in the second group could explain the striking and otherwise puzzling tendency for males of in many species of spiders to seek out sexually immature, penultimate instar females in preference to mature females (Jackson 1986; Eberhard et al. 1993). Some additional studies of sperm precedence and male behavior have found further cases of first male precedence in species with both insemination and fertilization ducts (Bukowski & Christenson 1997a; Snow & Andrade in press). There are also exceptions to this and several researchers have noted intermediate morphologies in some species (Uhl & Vollrath 1998; Elgar 1998; Uhl 2002). It is nevertheless possible that there may prove to be general trends. Many details, including sperm mixing (and lack of mixing) in the female, and the effects of intermediate spermatheca designs remain to be determined for many species (Elgar 1998).

Fertilization in at least one theridiid species does not occur near the mouth of the duct from the spermatheca, as often assumed, but farther up the oviduct (Suzuki 1995), and this could affect sperm usage patterns. Fertilization near the mouth of the fertilization duct apparently occurs during or just after oviposition in a different species of the same family (Berendonck & Greven in press), and also in two other families (Linyphiidae, Pholcidae),

(Uhl & Gunnarsson 2001; G. Uhl pers. comm.). These possible differences add another level of complexity in determination of paternity. The degree of sperm mixing in storage can be easily determined in species with multiple clutches by checking whether paternity values change in different clutches (lack of change would indicate complete mixing). The erratic changes in sperm precedence in successive clutches of the pholcid *Pholcus phalangioides* (Uhl 1992) strongly suggest sperm clumping rather than mixing. These spiders are unusual in storing sperm in an outpouching of the oviduct (the ''bursa'') rather than in discrete spermathecae (Uhl 1992), and the sperm remain encapsulated and embedded in a female secretion, details that suggest clumping. In *Latrodactus hasselti*, in contrast, lack of changes in paternity in successive clutches strongly suggests sperm mixing (Snow & Andrade in press). Decapsulation of sperm soon after copulation, however, in *Nephila clavipes* (Linnaeus 1767) (Brown 1985) and *Leucauge mariana* (Taczanowski 1881) (Eberhard & Huber 1998b) also suggests the possibility of sperm mixing. Further studies of sperm precedence patterns in successive clutches in species with different spermathecal designs, and with different timing of decapsulation would be of great interest.

Isolation of semen.—Male spiders deposit a droplet of semen from their primary gonopore on a small silk web, and then take up the semen in their pedipalps. This means that, in contrast with other animals, obtaining precise counts of sperm at this stage is unusually simple in spiders (just place the droplet on a slide, dilute it and count). If the male removes all sperm from the web, as seems to be common, and if his palps are empty after a copulation, as is true in at least some species such as *Nephila clavipes* (Christenson 1990) (but not in others such as *Anyphaena accentuata* (Walckenaer 1802) Huber 1995b, and *Lycosa malitiosa* Tullgren 1905 Costa 1998); then sperm counts in semen droplets may give unusually precise estimates of ejaculate size. Clearly, this will need to be checked in other species. Do males modulate ejaculate size according to male or female size, to previous sexual experience of the male or female, or according to the likelihood that the female will remate, as occurs in some other groups (e.g., Gage 1995)? Do ejaculate sizes often vary among related species, as seems to be the case in some species of *Theridion* (Knoflach 1998)? If so, then why? Does the fraction of the ejaculate that is stored by the female vary according to differences between males? There is an entirely unexplored field of study, comparative ejaculate sizes and usage, for which spiders are ideal subjects.

A further possible type of experiment, in which seminal products are injected directly into the female, has apparently never been attempted in a spider. Such experiments have been performed with many species of insects and ticks using relatively crude techniques such as injecting or implanting entire glands or their extracts into females. Deductions from similar experiments with spiders should be much more convincing, because they can be done without any contamination of the male's seminal products. Such experiments in spiders will presumably also permit separation of the possible physiological effects on the female of seminal substances that reach her body cavity from the possible effects of sperm in her storage organs (unless injected sperm are able to migrate through her body to the spermathecae). They could thus contribute to answering questions regarding the possibility that spider seminal substances affect sperm usage by the female (e.g., Snow & Andrade 2004). The high frequency with which seminal products have been found to influence female remating and oviposition rates in insects and ticks (Chen 1984; Eberhard 1996; Simmons 2001) makes experiments of this sort with spiders especially promising.

Function of males feeding females during copulation.—Male feeding of the female during copulation has arisen in several families, including Linyphiidae, Theridiidae, Pholcidae, and Pisauridae (summaries in Lopez 1987; Elgar 1998; Vanacker et al. 2003), and is associated in some groups with elaborate male morphology (Lopez 1987). In at least some species the small amounts of material transferred by the male suggest that the male gains from influencing cryptic female choice, rather than from benefiting the female nutritionally (Elgar 1998), a trend also found in the seminal products of many insect groups (Vahed 1998). Experimental manipulation of the male's ability to transfer material to the female (for example, by sealing the openings of the ducts on his cephalothorax, or altering the amount that

he can transfer by manipulating his previous copulatory history) should be especially easy in the spiders which transfer small amounts (e.g., *Argyrodes* spp.); to my knowledge this has never been attempted.

Function of copulatory plugs.—Masses of material (copulatory plugs) at or near the entrance of the insemination ducts are probably very common in spiders (Jackson 1980; Suhm et al. 1996). In some species, the material is apparently deposited entirely by the male, as in the salticid *Phidippus johnsoni* Peckham & Peckham 1883 (Jackson 1980), the agelenid *Agelena limbata* Thorell 1897(Masumoto 1993), and the linyphiid *Dubiarana* sp. (Eberhard 1996). In some others the plug is a combination of male and female products, as in the theridiid *Theridion varians* Hahn 1833 (Knoflach 1998) and some plugs of the tetragnathid *Leucauge mariana* (Mendez 2002). In still others it is apparently produced only by the female (some plugs of *L. mariana* Mendez 2002). Male copulatory plug material comes from glands in his abdomen (Knoflach 1998), his palps (Suhm et al. 1996), or his mouthparts (Braun 1963), and is sometimes composed of more than one type of material (Suhm et al. 1996).

Some copulatory plugs clearly impede subsequent attempts at intromission (Masumoto 1993; Knoflach 1998; Mendez 2002). But females can influence the effectiveness of a plug in preventing intromission in at least three different ways. In *Theridion* spp. and *L. mariana* female products are necessary for the formation of a functional plug (Knoflach 1998; Eberhard & Huber 1998b; Mendez 2002). In *L. mariana* the female sometimes (often, when she is young) fails to add her part, causing the male's attempts to form a plug to fail. Female *L. mariana* also sometimes physically impedes a male's attempt to remove a plug by pushing his palp away from the epigynum with her legs. To my knowledge, no one has ever checked for possible differences between between males which might correlate with such female decisions to facilitate or impede plug deposition or removal.

A more subtle way in which the female's own morphology can influence whether a plug is effective was illustrated in *A. limbata* by Masumoto (1993). The epigynum of this species has a cavity (the atrium) where both insemination ducts open. When a male mates, he first inseminates the female, then deposits a brown liquid in her atrium which soon hardens into a plug. Some plugs fill the atrium and cover the openings of both insemination ducts completely (''complete plugs''); others cover only a portion of the atrium (''incomplete plugs''). Incomplete plugs were common (38% of 50 lab matings involving virgin males and females). Smaller males more often made incomplete plugs, and when the ratio of male's size compared with that of the female was larger, complete plugs were more frequent. After depositing a plug, males in the field usually left the female within a day, presumably to search for other mates.

If a second male *A. limbata* subsequently attempted to mate with a plugged female, he first used his palp in attempts to hook the plug and remove it. Complete plugs were not removable, but incomplete plugs were often dislodged (11 of 15), in which case the male then inseminated the female and deposited a plug of his own. In these pairs, the second male fertilized on average 62.9% of the female's eggs. Masumoto concluded that the design of the female's epigynum (and in particular, of the atrium) enables her to bias the fertilization of her eggs in favor of larger males. If the atria of larger females are larger (this has never, to my knowledge, been investigated), the effects of this bias on male reproduction could be magnified because larger spider females tend to lay a larger numbers of eggs (e.g. Turnbull 1973).

While copulatory plugs in some species thus appear to function in sperm competition and cryptic female choice, important mysteries still remain. Some plugs in spiders are deep within the female (A. Danielson-François pers. comm. on *Tetragnatha*) and so large that they appear difficult to remove, but others do not impede intromission by subsequent males (e.g., *Phidippus johnsoni* (Jackson 1980)). Some especially flimsy plugs are apparently produced by the female, not the male in *L. marina* (Mendez 2002). Perhaps plug material is produced by the spermathecal glands, which are widespread, and which vary in number, location, and cell types; their function or functions need further study (Danielson-François 2002; Uhl & Gunnarsson 2001). Experimental removal of a plug in *L. mariana* is sometimes immediately followed by the female exuding a liquid from inside the insem-

ination ducts; a crust quickly hardens into a thin, weak, scab-like plug on the surface, and the liquid is then withdrawn (W. Eberhard, unpub. data). Todd Bukowski (pers.comm.) has proposed the intriguing idea that some plugs may function to prevent genitalic infections in females; another possibility is avoidance of desiccation (Huber 1995a). Both these ideas fit with the unusual genitalic design of many spiders, in which the openings of the female insemination ducts are embedded in a rigid sclerite (the epigynum), and are thus, unless plugged, permanently open and exposed externally. Predictions of both the infection and desiccation hypotheses that could be easily checked are that plugs should be more common in species with shorter, more direct insemination ducts, relatively larger entrances, or (in the case of desiccation avoidance) that live in drier habitats.

Breakage of the male genitalia within the female occurs in several groups of spiders, and in some cases male sclerites have distinct lines of weakness and seem clearly designed to break. In some araneoids the morphology of the male structure (part of the embolus) suggests that it acts as a plug that probably prevents subsequent intromissions (Levi 1972, 1975 on the araneid genera *Araneus* and *Singa*; Knoflach & van Harten 2002; Berendonck & Greven in press on the theridiid genus *Latrodectus*). However, direct observations of mating and determination of paternity showed that in another species, *Nephila plumipes* (Latreille 1804), the broken male structure (the conductor tip) did not preclude subsequent inseminations (Schneider et al. 2001), and multiple embolus tips in some other *Latrodectus* females also testify to both multiple intromissions and multiple penetrations reaching the spermathecae (Abalos & Baez 1966; Knoflach & van Harten 2002; summary of evidence Uhl 2002). Some female *Latrodectus* may control breakage using a muscle attached to the copulatory duct (Berendonck & Greven in press), although further work is needed to clarify this point (B. Berendonck pers. comm.). Experimental manipulation of male palps in *Latrodectus hasselti* showed that breaking off the embolus tip prior to copulation did not reduce the male's ability to transfer sperm, but breakage within the female did reduce access of subsequent males to her spermathecae (Snow & Andrade pers. comm.). The suggestion is that in other species of this genus in which the male is not sterile after his first mating (as occurs in *L. hasselti*) breakage does not entail elimination of insemination ability. Perhaps broken embolus tips serve as partially effective plugs that only sometimes exclude subsequent males in this genus. Perhaps their incomplete effectiveness is due to male inability to always leave the tip in the most effective site within the female, or variation in the success of subsequent males to overcome this barrier (possible consequences of the lack of innervation of male genitalia?). The significance of palpal breakage in *N. plumipes*, which may occur only if the female performs certain types of rejection behavior, is not yet clear (Schneider et al. 2000).

Progress in techniques.—Recent advances in techniques also contribute to making spiders an attractive group for future studies. Technical problems with counting sperm should be substantially reduced by the techniques recently worked out by G. Uhl (pers. comm.), and Bukowski & Christenson (1997a). It is possible to stain the sperm in a plug while leaving the rest of the plug nearly transparent, using the nuclear stain acetocarmine (Mendez 2002). Another technical problem—combining freeze-fixed specimens with embedding and sectioning of strongly sclerotized genitalia—has been solved by Huber (1993). Huber's discoveries have also laid to rest the mistaken idea that one can understand the functional relations of pedipalpal sclerites by using another technique, simply expanding the male's pedipalp in isolation from the female.

Further mysteries and suggestions for future studies.—One phenomenon which seems simple on the surface but becomes more difficult to understand on closer examination is sperm ''dumping'' by the female. Sperm masses often emerge from females of the pholcids *P. phalangioides* and *P. globosus* during or immediately following copulation (Uhl et al. 1995; Huber & Eberhard 1997; Peretti unpubl. data). In *P. globosus* sperm masses sometimes emerge during copulation when the female has not mated previously. The mystery is that size and presence of dumping seems to have no effect on paternity patterns when the female is mated to two males, at least in *P. globosus* (Peretti unpubl. data). Perhaps sometimes the first male's sperm are emitted, and

perhaps sometimes it those of the second male. Sperm are also emitted immediately following copulation in the distantly related *Pachygnatha clerki* Sundevall 1823. (Tetragnathidae), and are then eaten by the female (Gerhardt 1923). The structure of the female genitalia of the oonopid *Opopaea fosuma* Burger 2002 also suggests the possibility of sperm dumping (Burger et al. 2003). Careful observation of other species to check for sperm dumping, and the circumstances in which it occurs (male smaller, less elaborate courtship, more female resistance) is likely to be interesting.

Another recently discovered and even more mysterious trait is the correlation in the linyphiid *Pityohyphantes phrygianus* (C.L. Koch 1836) between the angle at which a female rests with respect to gravity and the sex ratio in her offspring (Gunnarsson & Andersson 1996; Uhl & Gunnarsson 2001; Gunnarasson et al. 2004) . The mechanism by which this is accomplished in this species may be related to the extraordinarily complex female sperm storage organs, which have three chambers and three valves on each side, and different types of gland cells associated with different chambers. The reproductive significance for this species of biasing sex ratios in nature is not clear.

One vast area of study which is as yet nearly completely unexplored in spiders (as well as in other animals) concerns the effects of male copulatory courtship on the female. Copulatory courtship behavior is quite common in spiders (Eberhard 1994; Huber 1998), but its effects have hardly been studied (but see Schäfer & Uhl 2002). I predict that observation of pairs in which male copulatory courtship varies (or can be altered experimentally), combined with measurements of female reproductive responses such as numbers of sperm transferred to storage sites, fertilization of eggs by different males, numbers of eggs laid, rapidity with which eggs are laid, and readiness to mate with an additional male, will yield further insights. Manipulations of males so as to change their morphological and behavioral abilities to perform different types of copulatory courtship could be especially useful in this context. The apparently widespread nature of copulatory courtship among spiders suggests that there will be a variety of results of such studies.

A fascinating recent discovery by Alfredo Peretti (pers. comm.) of active female participation in copulatory courtship opens still further major research questions. Females of *Physocyslus globosus* (Pholcidae) respond to powerful rhythmic squeezes by the male's palps by stridulating, rubbing ridges on their pedipalps across stridulatory files on their chelicerae. Females produce bursts of squeaky sounds that can be made audible when a microphone is held nearby. Female squeaking occurs more frequently when the male is squeezing her tightly with his powerful palps, and the male appears to relax his squeeze more quickly when she squeaks, so the female message may be ''Ouch, stop that!'' Males that were more ''obedient'', and responded more consistently by relaxing their squeezes when the female squeaked obtained more offspring than less obedient males that mated with the same females. Female stridulatory structures are widespread in some pholcid taxa (B. Huber, pers. comm.), so copulatory dialogues of this sort may be widespread in this family.

Does copulatory communication of this sort occur in other families? In the araneid *Micrathena gracilis*, the female strokes the male on his venter with her legs I and II, apparently to induce him to flip his body over to assume the venter to venter copulation position (Bukowski & Christenson 1997b). Female movements also occur during copulation in species in other groups, such as *Leucauge mariana* (W. Eberhard, unpubl. data), but as yet it is not clear whether they are communicatory in function. As a general rule, female behavior during copulation is very poorly studied (Peretti pers. comm.), and the field is wide open.

Conclusion.—In conclusion, spider sexual biology is of special interest for documenting phenomena related to sperm competition and cryptic female choice. Several properties of spiders make experimental manipulations especially feasible and powerful. Several mysteries have already been discovered, and there are literally thousands of species in different major taxonomic groups whose sexual behavior is almost completely unknown. The opportunities for future studies are legion.

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