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Research Article

Male Remating Success and the Frequency of Copulatory Plugs in the Green Lynx Spider *Peucetia viridans* (Araneae, Oxyopidae)

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Peucetia viridans males were allowed to mate with three virgin females and most matings resulted in live spiderlings, even when males lacked palpal paracymbial processes. Among females, the presence of copulatory plugs was inconsistent, and when present, their condition was not uniform; broken-off male paracymbia were often found in epigynal orifices. There was no size effect in a male's ability to completely plug a female's genital orifices, as well as no significant change in the pattern of plug production over consecutive mating trials. Among mated, field-collected females, the presence of plugs and paracymbia was variable, with females from some sites possessing neither structure. Field-collected females with no plugs were in significantly better condition than those with two plugs and in nearly significantly better condition than those with two paracymbia. Females in the best condition may excel at resisting the emplacement of genital obstructions and/or voiding such structures, potentially enabling them to mate with multiple males. Enhanced prey access mediated by increased water availability may be why females at two sites were in relatively better condition. If plugs help prevent sperm desiccation in inseminated females, this may have contributed to the absence of plugs from females at these two moister sites.

1. Introduction

The green lynx spider *Peucetia viridans* (Hentz) (Araneae: Oxyopidae) is the largest member of its family, with a distribution throughout the southern United States, Mexico and Central America [1]. It is a diurnal, visual hunter that forages on plants, especially on flowers, where it lies in ambush for potential prey (pers. obs., [2]). Although little studied up to 1960, *P. viridans* has been the sole or partial focus of at least 25 papers since then [3], making it one of the best characterized hunting spiders in North America.

While much is now known about the reproductive biology of *P. viridans*, two details of its mating behavior are still unresolved, specifically the success of copulations with multiple females by individual males and the frequency of copulatory plugs. *P. viridans* is one of only two oxyopids (the other is *P. longipalpis* F. O. P.-Cambridge) which are known to produce copulatory plugs [4, 5], structures which are commonly thought to delay and/or reduce the probability of female remating [6]. In addition, a portion of the male palp often breaks off within the female during mating in *P. viridans*, a mechanism which has been found to impede sperm transfer by subsequent males in other spiders [7–9].

Brady [1] was the first to note the presence of copulatory plugs in *P. viridans*, as he found that the two openings of a female's epigynum were usually plugged in preserved specimens with a hard, black material; often the distal portion of the paracybium of a male palpus was also embedded in this material (see [10] for detailed illustrations). Brady stated that the black material must be deposited during

or immediately after insemination, a suggestion perhaps corroborated by Whitcomb and Eason's [11] observation of a large drop of shiny liquid on the epigynum of an individual female immediately following copulation, one which later disappeared. He reasoned that the plugging of the female epigynum and the loss of the male paracymbial process should prevent further mating by both female and male (although since males possess two palpi, an individual male could potentially mate twice). However, in a laboratory study of *P. viridans* mating behavior, Whitcomb and Eason [11] showed that each mating episode involves numerous copulations between female and male with both palps being inserted alternately into the epigynal openings. Males mated freely on successive days and one male mated with three different females over three days. In contrast, each female mated with only one male and actively rejected subsequent male suitors.

Since the paracymbial process acts as an orientation device to guide the embolus into an epigynal opening [10], its loss from both palpi would be expected to prevent repeated copulations by males. Hence, the results of Whitcomb and Eason [11] suggest that the presence of the paracymbial process may not be absolutely necessary for copulation. However, they did not report on the production of egg sacs and live young by their mated females; so it is not known whether the copulations they observed actually resulted in successful fertilization.

Whitcomb and Eason [11] also reported on the frequency of copulatory plugs, as did Exline and Whitcomb [10], who also provided data on the frequency of inserted paracymbia. Whitcomb and Eason [11] found copulatory plugs in all the mated females they examined, but not in any virgin females. In contrast, Exline and Whitcomb [10] found that not all mated females had plugs and noted that plugs can be easily removed and may sometimes be lost during egg-laying. They found that in approximately 20 mated females, 10 had at least one male paracybium embedded in the plug. Among the remaining 10, the plug was either missing altogether or contained neither paracybium. While decades old, these uncertainties concerning the success of copulations with multiple females by individual males and the frequency of copulatory plugs have remained unresolved.

Recently, Ramirez et al. [3] genetically documented cases of multiple paternity in field-collected *P. viridans* broods, indicating that females sometimes remate in the wild. There are a number of reasons why a species which is known to produce copulatory plugs may nonetheless exhibit multiple mating; these are often connected with a lack of uniformity in plug efficacy [12]. For example, with the funnel web spider *Agelena limbata* Thorell (Araneae: Agelenidae), the epigynum has a cavity (atrium) where both insemination ducts open [13]. In a laboratory study, Masumoto [14] found that the copulatory plugs of some males filled the atrium completely (complete plugs), while those produced by others (especially smaller males) did not (incomplete plugs). With incomplete plugs, second males were often able to pry off the plug and inseminate the female, resulting in both first and second males siring offspring, underscoring the potential significance of the size contrast between sexual partners for plug presence. In contrast, with the orb weaver *Leucauge mariana* (Taczanowski) (Araneae: Tetragnathidae), the male's palp deposits small blobs of white paste on the female's epigynum near the openings of her insemination ducts; only if the female contributes, a clear liquid to these blobs will be retained as functional copulatory plugs [15]. Moreover, specific features of male copulatory courtship increase the likelihood that the female will contribute to the formation of a functional plug [16], highlighting the potential for female participation in determining the fate of copulatory plugs.

To improve our understanding of the success of copulations with multiple females by individual males and the frequency of copulatory plugs in *P. viridans*, this study reports on the outcome of a series of matings where individual *P. viridans* males were given the opportunity to copulate with three virgin females. Following each mating, the presence or absence of a copulatory plug (and its condition if present) and the subsequent production of fertile or infertile eggs was noted for each female, as was the presence or absence of the paracymbial process of each palp for males. The epigynal condition was also surveyed for females collected from various sites in southern California and the influence of female body condition on epigynal condition was assessed. Females across a range of taxa often attempt to impede or remove obstructions to their genital tract (e.g., [17–20]) and females in better condition may be more successful at doing so, hence our analysis of body and epigynal condition in *P. viridans*.

2. Materials and Methods

2.1. Mating Trials. We collected subadult *P. viridans* ($n = 21$ females, 7 males) from six sites in Los Angeles, Riverside, and San Diego Counties, California, in July-August 2004 and reared them to adulthood individually to ensure virginity. Collection sites and identification numbers of all participants are as follows: *Los Angeles Co*.—Ernest Debs Regional Park (♂ 1); Loyola Marymount University (♀ 1, 3, 4, 7, 8, 9, 10, 11, 12, 14, 19, 20); *Riverside Co*.—Along Cajalco Rd., east of Eagle Canyon Rd. intersection (♀ 21; ♂ 7); Davis Rd., east edge of Lake Perris State Recreation Area (♀ 13, 17; ♂ 3, 4, 5); *San Diego Co*.—Crest Canyon Preserve, Del Mar (♀ 15); Carmel Valley Road, Del Mar (♀ 2, 5, 6, 18; ♂ 2, 6). The collection site for ♀ 16 is uncertain due to the accidental loss of locality data from its rearing jar, although it was derived from one of these six populations. Spiders were maintained in glass apothecary jars (8.5 cm diam × 21.5 cm tall) and were fed bees and other flying insects every few days. Water was added periodically with an eye dropper.

Once the spiders reached adulthood as indicated by fully developed female epigyna and male palps, each male was allowed to mate with three randomly chosen females. For each mating trial, a virgin female was removed from her rearing jar and was placed on the flower head of a potted buckwheat (*Eriogonum* Michaux spp.) branch sitting in the center of a large bell jar (22 cm diam × 30 cm tall). After she had settled quietly on or under the flower head, the male was

placed in the bell jar near the base of the buckwheat branch. A lid was then placed on the bell jar and the spiders were left together until the next morning, at which time they were separated and their genitalia were examined microscopically. Specifically, the female epigynum was inspected for copulatory plug material and male paracymbial processes, while the presence or absence of the paracymbial process was noted for each male palp. Plug condition was scored as "complete" if it completely filled the funnel-shaped epigynal orifice, or "partial" if it was not large enough to occlude the opening at the base of the "funnel" or was positioned in such a way that it did not physically block this orifice. Thereafter, the male and female were returned to their rearing chambers. The female was fed on subsequent days in anticipation of egg sac production. To ensure the absence of potential chemical residues, the bell jar was washed out between mating trials and fresh buckwheat branches were used for each trial.

Since *P. viridans* males require 12–16 hours to recharge their palpi [11], males in our study were offered females every other day until they had been given access to three females. Insemination of a female was considered successful only if living, active spiderlings emerged from her egg sac(s) (e.g., [21]). Following the conclusion of the study, the adult males and females were preserved in 80% ethanol and their sizes (carapace width (mm)) were determined using a dissecting microscope with a calibrated ocular micrometer. We were unable to obtain the weights of these specimens due to unexpected abdominal deterioration following their preservation.

2.2. Field-Collected Females. We collected adult female *P. viridans* ($n = 54$) found guarding egg sacs from five sites in Los Angeles and San Diego Counties, California, in october–November 2004 (population abbreviation and sample size are indicated in parenthesis): *Los Angeles Co*.—Kenneth Hahn State Recreation Area (HSR, 34); Ernest Debs Regional Park (DEB, 7); Loyola Marymount University (LMU, 9); *San Diego Co*.—Crest Canyon Preserve, Del Mar (CCN, 2); Carmel Valley Road, Del Mar (CVR, 2). Each female was microscopically examined and the presence and condition of copulatory plugs and retained male paracymbial processes were recorded. We also measured female size (carapace width, mm) and weight (mg). Size and weight values were ln transformed and the computational procedures of Jakob et al. [22] were used to generate the residual index (*RI*, the residuals of body mass on body size), a nondestructive measure of body condition, for each spider. Individuals with higher residual index scores are heavier for their size [23] and scores can be reliably compared among conspecifics regardless of sex, age, reproductive state, geographical population, or date of capture [24]. While the assessment of body condition (overall energy balance [25]) using the residual index has been a topic of debate (e.g., [26] versus [27]), Ardia [28] and Schulte-Hostedde et al. [29] empirically revalidated its use as a measure of body condition and found that it was superior to major alternatives which have been proposed.

2.3. Statistical Analyses. Unless otherwise specified, all analyses were carried out using the StatView 5.0.1 [30] statistical analysis program.

2.3.1. Mating Trials. To assess the cause of variation of copulatory plug presence among the mating trial females which mated ($n = 18$), we first assigned them to two groups based on their respective postmating epigynal condition (e.g., [14]): "both" if the left and right epigynal orifices were completely blocked by plug material, or "incomplete" if this was not the case. For females assigned to each group and their respective male partners, we then compared male carapace width, female carapace width, and relative male to female size (male carapace width/female carapace width) using Mann-Whitney U tests. While pseudoreplication may be a concern given the reuse of individual males in multiple trials, each female was used only once and thus each male-female pairing was unique. In addition, to assess whether the pattern of plug production varied among trials, we used a contingency table analysis in which the columns were the different plug combinations and the rows were the three trials. However, since a standard asymptotic chi square test of this table would have been questionable given the small overall sample size [31], we instead used an exact permutation inference approach developed for small-sample categorical data sets by Rugg [32] and implemented in his TableSim program. For each of these analyses, we report a G^2 value and its associated exact p.

2.3.2. Field-Collected Females. To assess whether the pattern of plug and paracymbia production varied among sites, we used contingency table analyses in which the columns were the different plug or paracymbial combinations and the rows were the five sites. However, since some cells of these tables had values of less than 5, we again used TableSim [32] to obtain exact permutation inference results. In addition, while we attempted to test for variability among site-specific plug $\times$ paracymbia contingency tables, comparing these tables using TableSim was not successful, as the program would abort prematurely, probably due to an excessive number of cells with values of 0. For this reason, the plug and paracymbia data were treated separately in the analyses of intersite differences.

We used one-way analysis of variance (ANOVA) to test for differences in means of the residual index for females grouped by epigynal condition. There were three groups based on plug combination (two: complete plugs present in both left and right orifices; one: a single complete plug present in the left or right orifice; none: complete plugs absent from both orifices) and three based on paracymbium combination (two: paracybia present in both left and right orifices; one: a single paracymbium present in the left or right orifice; none: paracymbia absent from both orifices). We also used ANOVA to test for differences in means of the residual index among the five sample sites (HSR, DEB, LMU, CCN, CVR). Since only a single female was found with a partial plug (HSR-49) and another was found with two (HSR-52), these spiders were excluded from the ANOVA analyses to

Table 1: Mating trial results for male *Peucetia viridans* with consecutive female partners (A, B, C). Postmating condition of pairs is as follows: *For Females*: plug in epigynum (left: L, right: R, orifices): C, complete; Pr: partial; N: absent (male paracymbial processes (p) are noted when present); female fertile (= live young from resulting egg sac(s)), yes or no; *For Males*: palp (paracymbial) process missing, left (L), right (R) palp. Source and identification numbers of spiders are given in the text.

Male #1	Post-Mating Outcomes	Female A #1	Female B #5	Female C #9
	Plug in epigynum (L/R)	N/N(p)	N(p)/N	Pr/N
	Palp process missing	R	L/R	L/R
	Female fertile	yes	yes	yes
#2		#2	#13	#17
	Plug in epigynum (L/R)	N/N	C(p)/C(p)	C/C
	Palp process missing	none	L/R	L/R
	Female fertile	no[1]	yes	yes
#3		#3	#7	#11
	Plug in epigynum (L/R)	C(p)/C(p)	C/C	C/Pr
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#4		#4	#8	#12
	Plug in epigynum (L/R)	N/N	N/N	N/N
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	no[2]	no[3]
#5		#6	#10	#15
	Plug in epigynum (L/R)	C(p)/C(p)	N/N	Pr/C
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#6		#14	#16	#18
	Plug in epigynum (L/R)	N(p)/N(p)	C/C	C/C
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#7		#19	#20	#21
	Plug in epigynum (L/R)	N/C(p)	Pr/C	C/C
	Palp process missing	R	L/R	L/R
	Female fertile	no[4]	yes	yes

[1] Two egg sacs produced, both with undeveloped eggs.
[2] No egg sac produced.
[3] Two egg sacs produced, first with undeveloped eggs, second with no eggs.
[4] One egg sac produced, filled with undeveloped eggs.

avoid having groups made up of single individuals. The remaining females ($n = 52$) were therefore used in all the ANOVA tests. In cases where a significant difference was indicated by ANOVA, Fisher's protected least significant difference (PLSD) test [33] was used to conduct post hoc pairwise comparisons among the means.

3. Results

3.1. Mating Trials. The males were generally successful in inseminating their female partners: of the 21 females involved in mating episodes, 17 later spun egg sacs from which live spiderlings emerged, and of these, 12 were second and third partners in the mating sequence (Table 1). Of these 12 females, 9 were inseminated by males which had lost both their paracymbial processes, demonstrating that the presence of this palpal structure is not essential for successful insemination and mating with multiple partners.

When one or both paracymbial processes were lost, they were generally found in the epigynal orifices of the female partner (7 of 9 cases) (Table 1), though there were two exceptions. The presence of a paracymbial process in a female orifice was sometimes accompanied by an epigynal plug and sometimes not. In all three cases in which a single process was transferred to a female during a mating episode, it was found in the epigynal orifice on the corresponding side (left or right) of the female.

Among the 17 females that produced fertile eggs, the epigynal plug was quite variable: 8 (47%) females had a complete plug in both epigynal orifices; 5 (29.4%) females had no plug in either orifice; 3 (17.6%) females had a complete plug in one orifice and a partial one in the other orifice; 1 (5.9%) female had a partial plug in one orifice

Table 2: Means (± SE) of physical characteristics for laboratory pairs of *Peucetia viridans* who mated ($n = 18$) by postmating epigynal condition of females. Epigynal condition was classified as both if the left and right epigynal orifices were completely blocked by plug material, or incomplete if this was not the case. The results of Mann-Whitney U tests for differences between the groups are also shown.

	Epigynal condition			
Physical characteristics	Both	Incomplete	Mann-Whitney	
$n =$	8	10	z	P
Male carapace width (mm)	3.493 (0.128)	3.393 (0.089)	−0.622	0.534
Female carapace width (mm)	3.992 (0.142)	3.998 (0.103)	−0.178	0.859
Body size ratio (male/female)	0.876 (0.015)	0.852 (0.028)	−1.200	0.230

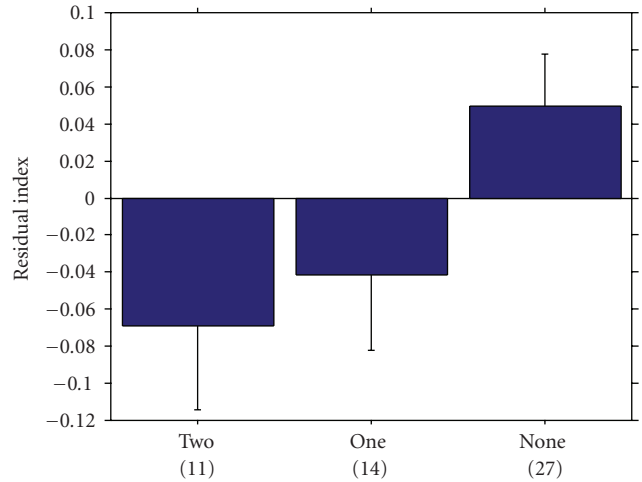

Figure 1: Means (± SE) of the residual index for field-collected *Peucetia viridans* females grouped by combinations of copulatory plugs in their epigyna. Combinations are defined in the text and the number of spiders in each group is indicated in parentheses.

and no plug in the other (Table 1). In addition, one female (#19) produced a single egg sac with infertile eggs, even though she possessed a complete plug in one orifice (as well as a paracymbial process). Hence, of the 18 females that had certainly engaged in copulations with their partners, 13 (72%) had at least a partial plug in one epigynal orifice.

Finally, for mating pairs which included the 18 females that had engaged in copulations and which were grouped by plug condition as defined in Section 2.3.1, male size, female size, and the body size ratio (male/female) did not significantly differ for the Both and Incomplete plug groups (Table 2). Moreover, the pattern of copulatory plugs organized by mating trial for these females (Table 3) showed no significant difference among trials ($G^2 = 11.686$, exact $P = .306$).

3.2. Field-Collected Females. The epigynal plug was also quite variable among the field-collected females (see Table 1 in supplementary Material available online at doi:10.1155/2010/602897). Of the entire sample ($n = 54$), 50% had no plug in either orifice and 20.4% had a complete plug in both epigynal orifices; the remaining females had combinations of partial and complete plugs (Supplementary Table 1(a)). As for the presence of paracymbial processes, 72.2% of the entire sample had no process in either orifice, while approximately equal numbers of the other 15 females had either one or two processes present in their epigynal orifices.

Comparison of the field-collected female data for the individual sites (Kenneth Hahn State Recreation Area (Supplementary Table 1(b)); Ernest Debs Regional Park (Supplementary Table 1(c)); Loyola Marymount University (Supplementary Table 1(d)); Crest Canyon Preserve (Supplementary Table 1(e)); Carmel Valley Road (Supplementary Table 1(f))) reveals considerable interpopulation variation. For example, while the percentage of females with no plug in either orifice was somewhat less than that for the entire sample (50%) at both the Hahn (35.3% (12/34)) and Debs (42.9% (3/7)) sites, no plugs of any sort were found in the females from Loyola Marymount University and Carmel Valley Road, while a single plug was found in a female from Crest Canyon Preserve (Table 4(a)). Similarly, while the representation of females with no paracymbial processes in either orifice was less than that for the entire sample (72.2%) at both Hahn (67.7% (23/34)) and Debs (42.9% (3/7)), no Loyola Marymount University, Crest Canyon Preserve or Carmel Valley Road females were collected with processes in her orifices (Table 4(b)). Surprisingly, this interpopulation heterogeneity was only marginally nonsignificant for plugs ($G^2 = 22.110$, exact $P = .051$) and was not significant for paracymbia ($G^2 = 13.253$, exact $P = .102$). However, since these results may have been affected by the minimal sample sizes ($n = 2$) for the adjacent Del Mar, San Diego Co. sites (Crest Canyon Preserve, Carmel Valley Road), we combined them into a single Del Mar sample ($n = 4$) and reran these analyses with TableSim. This time, inter-population heterogeneity was significant for both plugs ($G^2 = 20.384$, exact $P = .033$) and paracymbia ($G^2 = 13.253$, exact $P = .040$).

The field-collected females grouped by copulatory plug combination differed significantly for the residual index (Supplementary Table 2, Figure 1). Both the Two and One plug females had negative mean *RI* values while the None (no plug) females exhibited a positive mean, with posthoc tests (Supplementary Table 2) showing that only the Two and None females differed significantly ($P < .05$), though the difference between the One and None females approached significance ($P = .067$). When these females were grouped based on combinations of inserted paracymbia, heterogeneity for the residual index approached significance ($P = .061$), with the Two females exhibiting a negative mean *RI* and the One and None females positive means (Figure 2), echoing the relative difference between the Two and None groups based on plug combination (Figure 1). Thus, females with two copulatory plugs were in significantly poorer body condition than those possessing no plugs; females with

Table 3: Copulatory plug combinations for laboratory *Peucetia viridans* females who mated by order of mating trials.

Mating Trials	Copulatory Plugs					
	Two Complete	One Complete	Complete + Partial	One Partial	Both Absent	$n =$
First	2	1	0	0	3	6
Second	3	0	1	0	2	6
Third	3	0	2	1	0	6
$n =$	8	1	3	1	5	18

Table 4: Combinations of copulatory plugs and paracymbial processes in the epigyna of field-collected *Peucetia viridans* females by sample site. Site abbreviations are defined in the text.

(a) Plugs

Site	Copulatory Plugs					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	$n =$
HSR	8	12	1	1	12	34
DEB	3	1	0	0	3	7
LMU	0	0	0	0	9	9
CCN	0	1	0	0	1	2
CVR	0	0	0	0	2	2
$n =$	11	14	1	1	27	54

(b) Paracymbia

Site	Paracymbial Processes			
	Two	One	None	$n =$
HSR	4	7	23	34
DEB	3	1	3	7
LMU	0	0	9	9
CCN	0	0	2	2
CVR	0	0	2	2
$n =$	7	8	39	54

two paracymbia appeared to be in similarly poor condition relative to their peers.

Finally, field-collected females grouped by sample site differed significantly for the residual index (Supplementary Table 3, Figure 3). Loyola Marymount exhibited a large positive mean, one which was significantly greater than the negative means for Hahn, Debs, and Crest Canyon (posthoc tests, Supplementary Table 3). Conversely, Crest Canyon displayed a large negative mean, one which differed significantly from that for Hahn. In addition, Crest Canyon was also nearly significantly different ($P = .068$) from Carmel Valley Road, the other site with a positive mean. In sum, females from Loyola Marymount were in better body condition than those from Hahn, Debs, and Crest Canyon, as was also nearly true for Carmel Valley Road females compared with those from Crest Canyon. Crest Canyon females were also in poorer condition than those from Hahn.

4. Discussion

4.1. Mating Trials. The mating trial results conclusively demonstrate that *P. viridans* males have the potential to successfully inseminate multiple female partners, even if they have lost both their paracymbial processes. These trials also showed that when these male structures do break off, they are not always transferred to the female; yet when they are lodged in an epigynal orifice, they can do so even if a plug is absent. Furthermore, the mating trial results validate Exline and Whitcomb's [10] observation that the probable manner of palpal insertions into the two openings of a female's epigynum during copulation is ipsilateral (right in right, left in left; also known as equilateral [34]).

Since the paracymbial process is not essential for effective sperm transfer, its role during reproduction is unclear. Exline and Whitcomb [10] stated that the breakage of the paracymbium occurs when the male withdraws the embolus from the epigynal orifice and Brady [1] observed that this event "insures fertilization of the female," although he did not elaborate. If the paracymbial process is an orientation device for the palpal embolus during copulation as stated by Exline and Whitcomb [10], males with and without paracymbial processes may differ in the amount of time it takes for them to insert their emboli into the epigynal openings of their female partners, potentially allowing males that position themselves more quickly (those with processes?) to transfer greater amounts of sperm during a copulation [35]. If the paracymbial process does serve to facilitate sperm transfer, it may be that its loss is partly the result of sexual

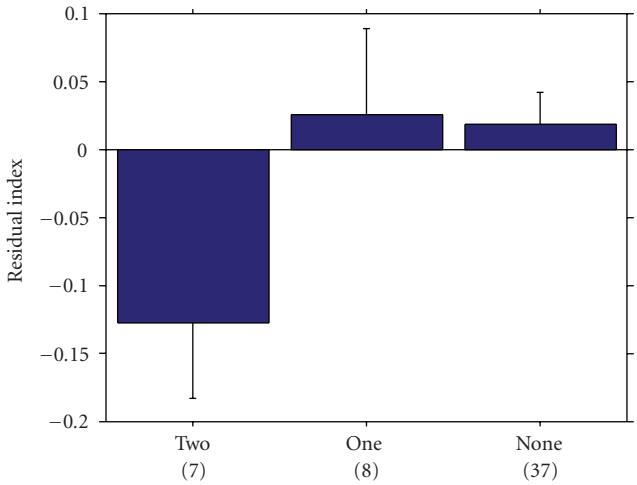

Figure 2: Means (± SE) of the residual index for field-collected *Peucetia viridans* females grouped by combinations of paracymbial processes in their epigyna. Combinations are defined in the text and the number of spiders in each group is indicated in parentheses.

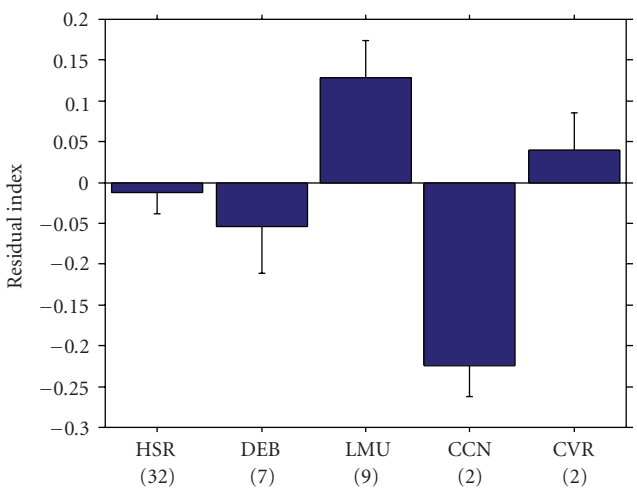

Figure 3: Means (± SE) of the residual index for field-collected *Peucetia viridans* females by sample site. Site abbreviations are defined in the text and sample sizes are indicated in parentheses.

conflict over when to terminate copulation, as has been suggested for the curved tip of the male palpal conductor in the orb weaver *Nephila plumipes* (Latreille) (Araneae: Tetragnathidae), which physically attaches a copulating male to a female until her attempts to dislodge him frequently result in its breaking off [36]. Finally, as noted earlier, paracymbial processes may function as plugs when lodged in a female. Experiments have shown that palpal structures in the female genital tract do act as effective plugs in some spider species but not in others [37, 38]. As for *P. viridans*, Ramirez et al.'s [3] study of multiple paternity in field-collected broods led them to conclude that inserted paracymbial processes (and copulatory plugs) were not associated with a reduction in female remating, though their sample size was limited. Since paracymbial processes were so often absent from freshly mated females in the mating trials (Table 1), we agree that this structure may not represent an effective plug in *P. viridans*. Nevertheless, even structures which only occasionally delay or prevent female remating will be favored as male adaptations for sperm competition (W. Eberhard, pers. comm. [39]). Clearly, a more detailed investigation will be needed to clarify the reproductive nuances of copulations when the paracymbial process is present or absent from one or both male palpi as well as the reproductive significance of its presence or absence in the female epigynal orifice.

As with the paracymbial process, the presence of copulatory plugs was inconsistent among the freshly mated *Peucetia viridans* females (Table 1), and when present, their condition was not uniform. In addition, the presence of plug material was not an absolute indicator that successful insemination had occurred. Since the origin of the copulatory plug in *P. viridans* is unknown [10], it is difficult to assess the significance of the variation in plug presence documented for the mating trials. Masses of material (copulatory plugs) that form within or near the entrance of the female reproductive tract as a result of copulation occur in a diversity of taxa, including spiders [4, 40], and they have long been considered devices which interfere with female access to further sexual partners [12, 41, 42]. As reviewed by Eberhard [13], the material that forms the mating plug in spiders may be deposited by the male, by the female, or by both partners, and for males, may be generated by glands in the abdomen, palps, and/or mouthparts. The drop of liquid on the epigynum of a freshly mated female observed by Whitcomb and Eason [11] could be this material in *P. viridans*, though they were uncertain as to whether it was an actual plug precursor and they did not know its source. In the future, the analysis of video recordings of *P. viridans* copulatory activities, coupled with the fixation of spiders in copula and the examination of serial sections of the copulatory organs in physical contact, may help identify male and/or female contributions to plug formation (e.g., [15, 16, 34, 43]).

In whatever manner plugs are formed in *P. viridans*, many factors could potentially influence their presence and condition in mated females (reviewed in [5, 12, 13]). While a thorough assessment of these factors was beyond the scope of this study, we did evaluate whether relatively smaller males may have greater difficulty in completely filling a female's genital orifices (e.g., *Agelena limbata* [14]) and whether multiple mating by males may produce of a greater proportion of partial or absent plugs in their later mates, due to a decrease in male plugging ability and/or contributions (e.g., *Oedothorax retusus* (Westring) [6]). However, our analysis of epigynal condition and the male/female size ratio of sexual partners found no evidence for a size effect in the ability of male *P. viridans* to completely plug a female's paired genital orifices. Moreover, we also saw no significant change in the pattern of plug production over the course of the three mating trials, as might be expected if the males were declining in their ability to promote plug formation. Of course, given the limited number of males and females involved in the trials, these findings should be considered preliminary. Nonetheless, these results may indicate that

plug presence and persistence in *P. viridans* is largely due to differences among individual females, a possibility which seems consistent with findings for the field-collected females.

4.2. Field-Collected Females. The presence of plugs and paracymbia in the epigyna of mated *P. viridans* females varied widely among individuals and among sites. Particularly notable was the contrast between the varied mix of both structures in females from Kenneth Hahn State Recreation Area and Ernest Debs Regional Park, and the absence of plugs from females at Loyola Marymount University and Carmel Valley Road, as well as the absence of paracymbia from these same two sites, along with Crest Canyon Preserve (Supplementary Table 1). Obviously, the minimal representation of these structures at Loyola Marymount University, Crest Canyon Preserve and Carmel Valley Road could be largely the result of limited sampling at these sites. However, the fact that the small Debs Regional Park sample ($n = 7$) contained females with various combinations of both plugs and paracymbial processes (Supplementary Table 1(c)) suggests that the absence of these structures from the Loyola Marymount University sample ($n = 9$) may not be simply due to limited sampling but may in fact have a biological basis, as we discuss later.

Our analysis of the influence of body condition on epigynal condition showed that mated females with no copulatory plugs were in significantly better condition than females with two copulatory plugs, and that females with two paracymbia appeared to be in similarly poor condition. These results suggest that females in the best condition may be the most able to avoid the emplacement of obstructions to their genital openings during copulation and/or to void such structures thereafter, potentially enabling them to mate with another male(s). There are many potential benefits and costs for females which mate multiply (reviewed in [17, 44]); though since female remating has been widely described in general [45] and among spiders ([5], including *P. viridans* [3]), it appears that females typically gain from remating in terms of increased reproductive fitness [46]. Correspondingly, female efforts to prevent (e.g., [15, 47]), dissolve (e.g., [48, 49]), and/or physically expel (e.g., [50, 51]) genital obstructions are also well known, though to the best of our knowledge, the energetic costs of these activities have never been quantified. However, among anopheline mosquitoes whose males produce copulatory plugs which act as physical barriers to remating [52, 53], Giglioli [54] found that well fed *Anopheles melas* Theobald females were able to dissolve mating plugs more quickly than poorly fed females. Similarly, since a spider with a positive residual index score is fatter than one that has a negative score [22], *P. viridans* females in the best condition presumably had the most acquired resources available for allocation to important tasks, perhaps including keeping their epigyna obstruction-free. While accurately measuring the energetic costs of behaviors can be empirically challenging, techniques such as flow-through respirometry and the measurement of organismal glycogen and lipid levels have facilitated the energetic assessment of mating activities in other invertebrates [55–57] and their use with *P. viridans* might help clarify the relationship between female body condition and epigynal condition detected in this study.

Finally, the fact that body condition also varied significantly by site might be explained by *P. viridans* females experiencing different levels of foraging success depending on their location. Since there are a multitude of factors which can influence insect diversity and abundance in the seasonally dry Mediterranean climate of southern California [58], the availability of insect prey likely differed among the geographically disparate sites sampled for this study. Among these factors, moisture is thought to play a key role in driving insect abundance in this arid region, via its impact on plant primary productivity [59–61]. In this regard, while the Loyola Marymount site is a bluff face with natural coastal sage scrub vegetation [62], it receives surface water runoff from an irrigation system located along the blufftop, given its location on the edge of a university campus. Since *P. viridans* of all instars were concentrated along the blufftop in fall 2004, they were situated in well-watered vegetation and so may have had access to significant numbers of insect prey. This situation may prevail to a lesser degree at the Carmel Valley Road site, which is a larger patch of similar vegetation surrounded by the backyards of urban homes and road edges, with some irrigation systems in place along the road. Thus, enhanced access to insect prey mediated by increased water availability beyond normal rainfall may be one reason why females at Loyola Marymount were in such better condition than their counterparts at the three natural preserves, Hahn, Debs, and Crest Canyon. This may also be why body condition differed between the adjacent Carmel Valley Road and Crest Canyon Preserve sites.

The increased water availability at Loyola Marymount and Carmel Valley Road may have potentially played a more direct role in facilitating the absence of plugs from females at these sites. Specifically, since the genital orifices of female spiders are otherwise always open to the surrounding air unless sealed by copulatory plugs, Huber [63] suggested that their presence may help resist sperm desiccation. Plug frequency may therefore partly reflect the dryness of a population's setting, particularly if they are produced by females. While we are not aware of empirical support for Huber's hypothesis and while we lack the environmental data to thoroughly assess its applicability to *P. viridans*, it is possible that better body condition and an absence of copulatory plugs at the Loyola Marymount and Carmel Valley Road sites may have both been facilitated by anthropogenically enhanced moisture levels. Clearly, a detailed study of abiotic factors, plant and arthropod phenologies, and spider dietary intake (e.g., [64–66]) at nearby mesic and xeric sites over the course of a year will be needed to explicitly evaluate the influence of site-specific environmental parameters on the body and epigynal condition of resident spiders. Indeed, since copulatory plugs are often externally visible when they are present in the epigynum of *P. viridans*, if adult females were inspected for the presence of plugs over time at these sites (e.g., [67, 68]) prior to their eventual collection for measurement, it might also improve our understanding of the frequency and persistence of this structure and its

relationship to female body condition and environmental parameters.

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Supplementary Table 1. Epigynal condition of mated *Peucetia viridans* females collected from sites in Los Angeles and San Diego Counties, California, in October-November 2004. For each female, the presence of a male paracymbial process and/or an epigynal plug were noted for each of her epigynal orifices; following Table 1, plugs were scored as complete or partial if present. Since each female has paired orifices, "two" in the tables below denotes presence in both left and right orifices, while "one" denotes presence in the left or right orifice only. For each combination of paracymbial process and plug condition, the number of females with that configuration is indicated. Row and column totals (*n*) are also provided.

A. All samples (*n* = 54)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	7	0	0	0	0	7
One Present	2	5	0	0	1	8
Both Absent	2	9	1	1	26	39
n =	11	14	1	1	27	54

B. Kenneth Hahn State Recreation Area, Los Angeles Co. (*n* = 34)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	4	0	0	0	0	4
One Present	2	4	0	0	1	7
Both Absent	2	8	1	1	11	23
n =	8	12	1	1	12	34

Supplementary Table 1. Continued.

C. Ernest Debs Regional Park, Los Angeles Co. (*n* = 7)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	3	0	0	0	0	3
One Present	0	1	0	0	0	1
Both Absent	0	0	0	0	3	3
n =	3	1	0	0	3	7

D. Loyola Marymount University, Los Angeles Co. (*n* = 9)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	0	0	0	0	0	0
One Present	0	0	0	0	0	0
Both Absent	0	0	0	0	9	9
n =	0	0	0	0	9	9

E. Crest Canyon Preserve, Del Mar, San Diego Co. (n = 2)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	0	0	0	0	0	0
One Present	0	0	0	0	0	0
Both Absent	0	1	0	0	1	2
n =	0	1	0	0	1	2

Supplementary Table 1. Continued.

F. Carmel Valley Road, Del Mar, San Diego Co. (*n* = 2)

Paracymbial Processes	**Epigynal Plugs**					
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	*n* =
Two Present	0	0	0	0	0	0
One Present	0	0	0	0	0	0
Both Absent	0	0	0	0	2	2
n =	0	0	0	0	2	2

Supplementary Table 2. Means (± SE) of the residual index for field-collected *Peucetia viridans* females grouped by combinations of copulatory plugs and paracymbial processes in their epigyna. As noted in the text, two females with one and two partial plugs, respectively, are not included here, hence the sample size difference between Table 4 (n = 54) and this table (n = 52). The results of one-way analysis of variance (ANOVA) for differences among groups are also shown, as are the results of post-hoc comparisons using Fisher's PLSD test when there was a significant F for ANOVA.

Epigynal Object		**Combination**			**ANOVA**	
		Two	One	None	F	P
Copulatory plugs	n =	11	14	27	3.278	0.046*
	$\overline{X}$ =	-0.069	-0.041	0.050		
	SE =	0.045	0.041	0.028		
		Post-hoc comparisons:		Two vs One		0.643
				Two vs None		0.029*
				One vs None		0.067
Paracymbia	n =	7	8	37	2.961	0.061
	$\overline{X}$ =	-0.127	0.026	0.019		
	SE =	0.056	0.063	0.023		

*$p < 0.05$.

Supplementary Table 3. Means (± SE) of the residual index for field-collected *Peucetia viridans* females by sample site. This table includes the sample set of females (n = 52) as Supplementary Table 2. The results of one-way analysis of variance (ANOVA) for differences among sites and the results of post-hoc comparisons using Fisher's PLSD test are also shown. Site abbreviations are defined in the text.

Site	**Residual Index**			**ANOVA**	
	n	$\overline{X}$	SE	F	P
HSR	32	-0.013	0.025	3.456	0.015*
DEB	7	-0.054	0.057		
LMU	9	0.128	0.047		
CCN	2	-0.224	0.038		
CVR	2	0.040	0.046		
Post-hoc comparisons:			HSR vs DEB		0.484
			HSR vs LMU		0.012*
			HSR vs CCN		0.046*
			HSR vs CVR		0.614
			DEB vs LMU		0.014*
			DEB vs CCN		0.140
			DEB vs CVR		0.411
			LMU vs CCN		0.003**
			LMU vs CVR		0.430
			CCN vs CVR		0.068

*$p < 0.05$, **$p < 0.01$.