Female feeding history impacts gonad development and reproductive timing in the wolf spider *Schizocosa ocreata* (Hentz, 1844)

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Abstract. In mating systems that include semelparous reproduction and/or scramble competition, synchronous maturation of the sexes is vital for success. However, food limitation may alter the onset of maturation or the overall quality of the mature individuals and affect reproductive success. We examined the role of feeding history (well-fed vs. long-term deprivation) on female reproductive timing and its correlation with temporal patterns of receptivity behavior in the wolf spider *Schizocosa ocreata* (Hentz, 1844). We found that feeding history influenced developmental time and delayed maturation in long-term food-limited females. There was no significant difference in relative condition between treatments, yet well-fed females showed higher rates of receptivity. Peak receptivity behavior was correlated with the estimated overall mass of female ovaries/eggs, with females that possess larger ovaries and eggs showing more receptive behavior. This supports the hypothesis that while a food-limited female may attain maturity, the limiting factor underlying reproductive success is gonad maturation.

Keywords: Fecundity, diet, receptivity, egg development

Understanding the interactions between nutrition and the development of reproductive anatomy is important when addressing mate choice, sexual selection, and sexual conflict (Arnqvist & Rowe 2005; Uetz & Norton 2007). For semelparous organisms with well-defined seasonal reproduction, many factors may impact the onset and maintenance of receptivity and courtship within a species (Lehrman 1965; Barth & Lester 1973). This ontogeny may be linked to the natural seasonality of the environment and impose intrinsic control over the ability to acquire food and allocate sufficient energy to reproductive efforts. Throughout the animal kingdom, sexually reproducing species are constrained by the maturation of reproductive structures and the initiation of sex specific behaviors, as these behaviors are often associated with hormones released by the developing gonads (Lehrman 1965; Barth & Lester 1973; Ringo 1996).

Maintenance of physiological condition and gametes is also important to reproductive success, and conflict exists between mating age, egg maturity and egg maintenance (Moore et al. 2007). Research has demonstrated links between female reproductive state, egg maturity, female receptivity and aggression in both solitary and subsocial spiders and some insects (Trabalon et al. 1988,1992; Wilgers & Hebets 2012). In spiders, females have been shown to produce different levels of hormones in relation to egg maturation, which contribute to female tolerance of conspecifics (Trabalon et al. 1988,1992). Even so, no research on spiders has yet examined the condition-dependent nature of female ovary development and its relationship to behavior, or the temporal consequences of diet on reproductive physiology. There is, however, evidence for compensatory development in spiders, based on recovery of body size and mass after nutritional stress (Jespersen & Toft 2003). Additional support suggests that during times of stress (i.e., food deprivation) there should be compromise in physiological processes (Gustafsson et al. 1994; McNamara & Houston 1996). It is well-established that diet has direct consequences for reproductive investment of females (Enders 1976; Simpson 1995; Parker & Begon 1996;

Toft & Wise 1999; Kreiter & Wise 2001). Thus, while much speculation exists about egg development/investment in spiders (Foelix 1996), little else is known about the direct effects of diet on gonad development. By examining the interactions between diet and gonad development, we will better be able to see how physiological limitations imposed by long-term diet restrictions impact mate choice decisions of females.

For many spider taxa, there are age-related differences in mate choice and female receptivity, frequently accompanied by differential male courtship investment (Norton & Uetz 2005; Uetz & Norton 2007; Wilgers & Hebets 2012; Rundus et al. 2015). In the wolf spider *Schizocosa ocreata* (Hentz, 1844), females have a predictable receptivity cycle after maturity, in which they are initially highly resistant to male advances (Week 1) followed by a level of high receptivity (Week 3) and a return to resistance (Week 5 and later); high levels of resistance are also demonstrated after mating (Norton & Uetz 2005; Uetz & Norton 2007). Uetz & Norton (2007) suggested this pattern might be related to potential availability of males, as phenology of maturation in this species is typically asynchronous (i.e., males tend to mature before females, leading to a male-biased sex ratio early in the breeding season). However, long-term observations (Uetz & Roberts, unpubl.) have shown that maturation synchrony does vary from year-to-year with weather factors. Additional research has demonstrated that this cycle is also condition dependent (Moskalik & Uetz 2011), suggesting the possibility that concomitant factors such as gonad development might be driving receptivity. The analysis of egg maturity during phases of this behavioral cycle may therefore shed light on what may be driving female receptive and aggressive behavior.

Here, we tested two hypotheses: (1) diet will affect development of and total investment in reproductive structures of female spiders for both penultimate (follicle number, size) and adult *S. ocreata* (fecundity, egg size, volume, total clutch volume) and (2) female ovary and egg maturation in well-fed spiders will coincide with the previously described
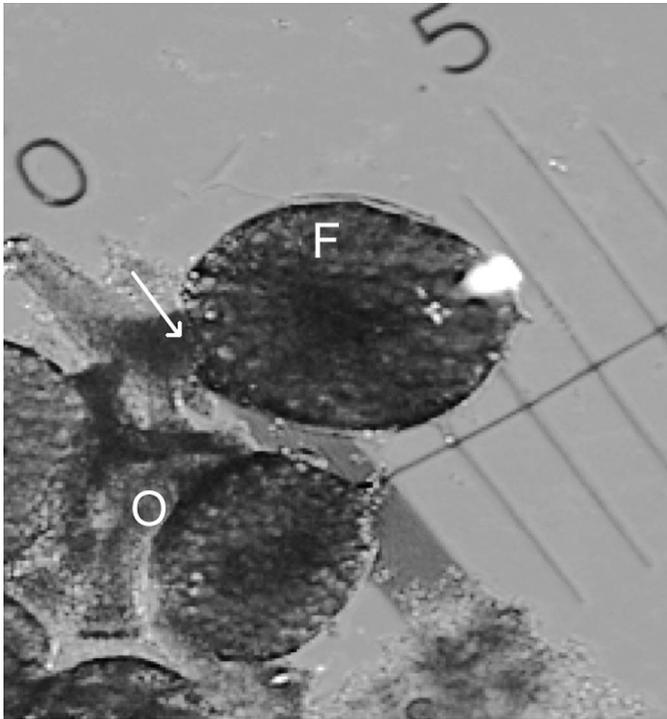
Figure 1.—Image of ovulated follicles (oocytes) from a well-fed ultimate female 21 days mature. F shows an individual follicle, the arrow indicates pedicle/funiculus, and O indicates a fragment of the ovary.
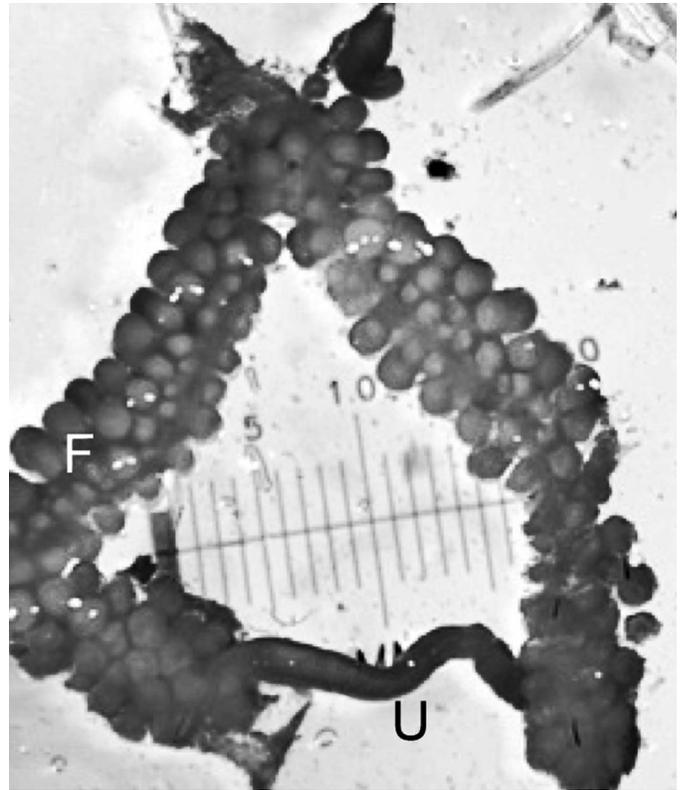

Figure 2.—Biramous ovary with follicular development from well-fed penultimate female. F indicates follicle clusters and U the oviduct/uterine tube.

receptivity curve (Uetz & Norton 2007) and influence behavior in a predictable manner. We predicted that if diet impacts reproductive structure development, then the food-limited group should demonstrate smaller average gonad size and reduced numbers of ovulated follicles and/or mature ova compared to the well-fed group. Also, if female ovary development impacts receptivity, a relationship between egg maturation (size) and previously observed temporal patterns of receptivity will be seen, with female peak receptivity coinciding with the time that eggs become larger because of the accumulation of yolk.

METHODS

Spider maintenance.—Hatchling spiders were obtained from field collected females with egg sacs and maintained in deli-style containers (9 cm height x 5 cm width). Spiders were held in the laboratory on a 14:10 light:dark cycle at 23°C and randomly assigned to well-fed or food-limited dietary protocols (Uetz et al. 2002; Balfour et al. 2003). Both treatments received gut-loaded crickets (*Acheta domesticus*) with well-fed individuals receiving 100% of their mass twice per week and food-limited individuals receiving 50% of their body mass once per week. Diet implementation and growth were followed from hatching to adulthood. Individuals were then randomly assigned to assay groups (penultimate; week 1 post ultimate molt, hereafter mature week 1; week 3 post ultimate molt, hereafter mature week 3).

Assessment of female growth.—We assessed all females and compared mass, cephalothorax width (CW) and developmen-

tal time (total days to maturity and number of instars) between starvation and well-fed treatment groups with a one-way ANOVA. To assess female body condition between diet treatments, we used an ANCOVA of weight x treatment, and adult cephalothorax width (CW) as the covariate (Marshall et al. 2000; Garcia-Berthou 2001; Schulte-Hostedde et al. 2005).

Assessment of female receptivity.—We assessed female receptivity with behavioral assays at three designated times (penultimate, mature week 1, mature week 3) matching those of earlier studies of female behavior (Uetz & Norton 2007). Females were placed with well-fed, lab-reared males in an arena for 5 minutes and observed for receptive behavior and male mounting behavior. The 5-minute duration has been shown to be an effective time frame to determine female choice (Moskalik & Uetz 2011). Males were mature for three to five weeks and in apparently good condition. To control for male variation, if no mating occurred, the first male was removed and a second male was placed in the arena and allowed to court for another 5 minutes. If mounting occurred, the pair were immediately separated and the female was euthanized under CO_2, preserved in AAF fixative (10 ml concentrated formalin: 5 ml glacial acetic acid: 75 ml 96% ethyl alcohol: 10 ml distilled water) and dissected to examine egg development.

To examine the effect of diet treatment on spider development, we compared well-fed and food-limited treatment females ($n = 75$ and 46, respectively) using one-way ANOVA. The effect of treatment on behavior was assessed by using a Chi square comparison of proportion of receptive

Table 1.—Female development. One-way ANOVA analyses of female development. Bold P value indicate significant differences between diet treatments

Source	df	F ratio	P
Mature weight	1	8.534	**0.004**
Mature cephalothorax	1	7.871	**0.006**
Mean instar length	1	0.024	0.877
No. of molts	1	8.762	**0.004**
Total development duration	1	6.513	**0.012**

adult females at two points in time after maturity (Week 1 and Week 3).

Images of eggs and oocytes were taken with an Olympus digital camera (Figs. 1, 2). Measurements of egg development included total egg number and mean egg diameter. Mean diameter was generated by measuring the width of 10 eggs at random or 10% of the clutch, whichever was greater. Using the standard equation for the volume of a sphere, we estimated the volume of the eggs using the mean diameter. A measurement representing total investment (total clutch volume) was derived by multiplying the mean volume by the total number of eggs. Egg number and total estimated clutch volume were analyzed with a two-way ANOVA, with treatment (starved vs. well-fed) and female age (penultimate, mature week 1, mature week 3) as factors.

RESULTS

Spider development.—Stadia did not differ significantly between treatments, but number of instars, total duration of development, cephalothorax width, and adult mass varied significantly with diet (Table 1). Tukey post hoc examinations revealed that mean stadia did not differ significantly between treatments (Well-Fed: 27 ± 4.5 vs. Starved: 27 ± 4.8 days; $F_{1,91} = 0.024$, $P = 0.89$) but there was a significant effect of diet on number of instars, with well-fed females requiring fewer molts to attain maturity (Well-Fed: 6.68 ± 0.14 vs. Starved: 7.32 ± 0.17 molts respectively; $F_{1,91} = 8.76$, $P = 0.004$). Total developmental time varied significantly with well-fed females maturing fastest (Well-Fed: 179 ± 4.9 vs. Starved:196 ± 4.5 days: $F_{1,91} = 6.51$, $P = 0.012$). Well-fed females were also larger and heavier than the long-term starvation groups (Table 1). Results of female body condition indicate that relative condition (i.e., mass scaled to body size) did not vary by treatment when cephalothorax was accounted for ($F_{1,49} = 0.94$, $P = 0.35$).

Behavior.—In week 1, females within each treatment were equally likely to be receptive to males ($n = 20$, $X^2 = 0.02$, $P =$
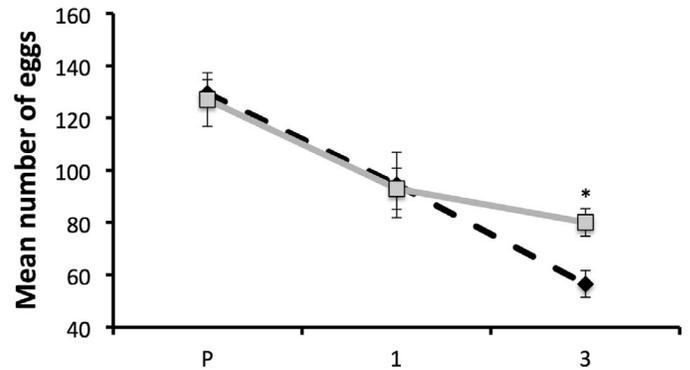

Figure 3.—Graph showing the decline and divergence of mean egg number ± standard error between diet treatments. The gray solid line represents the well-fed group and the dashed line represents food-limited group. * Indicates a significant difference.

0.89; <20% of females). However during week 3, treatment significantly impacted the proportions of females receptive to males ($n = 20$, $X^2 = 6.54$, $P = 0.011$) with 60% of well-fed females showing receptivity, versus 20% of limited females becoming receptive.

Comparisons of egg development.—Egg number (Table 2) per female was normally distributed and was analyzed with a multifactor ANOVA, revealing whole model significance, a significant difference between weeks, and a significant interaction between diet and week (Table 3). Post hoc analyses revealed that egg numbers declined uniformly between treatments but by week 3, low diet treatments were significantly lower than well-fed (Table 2, Fig. 3). Egg diameter was not normally distributed (Shapiro-Wilks W = 0.92, $P = 0.012$) and was examined for outliers. Grubbs outlier test (JMP 8) confirmed two outliers that were removed and the subsequent distribution was then normal (Shapiro-Wilks W = 0.95, $P = 0.19$). The same multifactor ANOVA was applied and indicated whole model significance and demonstrated that week and diet both impacted egg diameter but there was no interaction (Table 4). As expected, well-fed females had larger eggs than food limited females (Tables 2, 4) and egg diameter significantly increased with time (Tables 2, 4). Penultimate females had the smallest diameter with eggs growing successively larger each week (0.13 ± 0.006 mm vs. 0.14 ± 0.003 mm vs. 0.16 ± 0.003 mm: penultimate, weeks 1 and 3 respectively). The total clutch investment showed significant right skew (Shapiro-Wilks W = 0.80, $P < 0.0001$) and was Box Cox Y transformed for best fit (Shapiro-Wilks W = 96, $P = 0.19$; JMP 8). The multifactor ANOVA showed whole model significance and that diet and a diet x week interaction

Table 2.—Egg developmental characteristics based on treatment.

Treatment	Age	No. of egg follicles	Follicle diameter (mean, mm)	Follicle diameter (range, mm)	Estimated clutch volume (mm³, investment)
Well-fed	*Penultimate*	127	0.133	0.125–0.148	0.165
	Week 1	86	0.152	0.137–0.179	0.168
	Week 3	81	0.189	0.157–0.233	0.305
Food-limited	*Penultimate*	123	0.130	0.122–0.138	0.114
	Week 1	91	0.121	0.115–0.127	0.105
	Week 3	51	0.151	0.145–0.157	0.091

Table 3.—Two-way ANOVA analyses of mean number of eggs. Bold *P* values indicate significant differences.

Source	df	F ratio	P
Whole model	5	11.79	**<0.0001**
Treatment	1	2.1011	0.16
Week	2	24.6155	**<0.0001**
Treatment*Week	2	3.9543	**0.03**

Table 5.—Two-way ANOVA analyses of mean total clutch volume. Bold *P* values indicate significant differences

Source	df	F Ratio	P
Whole model	5	9.16	**<0.0001**
Treatment	1	14.99	**0.0006**
Week	2	1.08	0.35
Treatment*Week	2	4.09	**0.03**

significantly impacted total investment (Table 5). Tukey post hoc analyses revealed that initial clutch volume was equal in both groups and there was a divergence for both groups in week 1; in week 3, the divergence increased (Fig. 3).

DISCUSSION

Our results support previous findings that females who were fed more grew faster and produced more eggs. As a consequence, development and maintenance of follicles and eggs was compromised in starvation treatments. The impacts, however, were not significant until later in maturity (week 3). This suggests that female feeding history can impact egg maintenance, which could potentially affect receptivity behavior and subsequent mating or mate choice decisions. As predicted, follicular development mirrored the behavioral tendencies first observed by Uetz & Norton (2007). We observed that a reduced female feeding history significantly reduced the likelihood of female receptivity over time. From these results, we can infer a correlation between female age, diet, ovary/follicle development and receptivity. The initial results suggest that females invest equal amounts of resources into follicles regardless of treatment during their penultimate stage until the onset of adulthood. Then as maturity and adulthood ensue, females diverge in their ability to 1) maintain overall egg numbers and 2) invest in egg nutrition that would subsequently support the post-embryonic spiderling.

This research also highlights the impact that diet has on spider developmental plasticity, as *S. ocreata* demonstrated marked plasticity in developmental time. Well-fed females were able to mature in fewer instars and still attain a population mean adult size. On the other hand, food-limited females were delayed in maturation by the addition of one or two more instars, thus passing through 9–10 post emergent instars, which has been previously reported for this species (Amaya & Klawinski 1996). These late maturing females were significantly smaller than the rest of the laboratory population. However, an ANCOVA based on mass scaled to body size revealed no differences. This finding raises questions regarding the sensitivity of ANCOVA vs. a ratio or residual body condition index (BCI) when examining development and

plasticity within a population subjected to varying food abundance.

Previous research on spider ovary development has described the developmental stages eggs go through as they mature and how these correlate with female ecdysteroid levels in the spiders *Coelotes terrestris* (Wider, 1834) and *Tegenaria domestica* (Clerck, 1757) (Trabalon et al. 1988, 1992). There were several distinct differences between these results and our wolf spider population. Seemingly, the development of follicles in these agelenid spiders occurs after maturation, whereas in our lycosid species it begins during the penultimate stage. Additionally, the growth of the agelenid spiders was very robust, with each individual requiring a fixed number of instars to reach adulthood. Our lycosid spiders, even in the absence of diet variation, showed some plasticity in maturation time and could mature earlier than the expected population maxima (8th instar post emergence).

Research presented here demonstrates the importance of female diet and how it affects growth, behavior, reproduction and fecundity of female *S. ocreata* wolf spiders. While growth shows plasticity, certain other developmental aspects do not. Female follicular ovulation seems to be fixed with respect to the number of initial oocytes generated. There are clearly more oocytes ovulated than can be supported by these females, as even the well-fed group had an intrinsic "rate of decay" within the first week of maturity. The loss of eggs may be a timing event that eventually signals the maximum clutch amount for the female, thus representing an "internal clock". This observation generates many interesting avenues of inquiry that deserve future attention. Female behavior appears to be regulated by feeding; perhaps food limited females have a
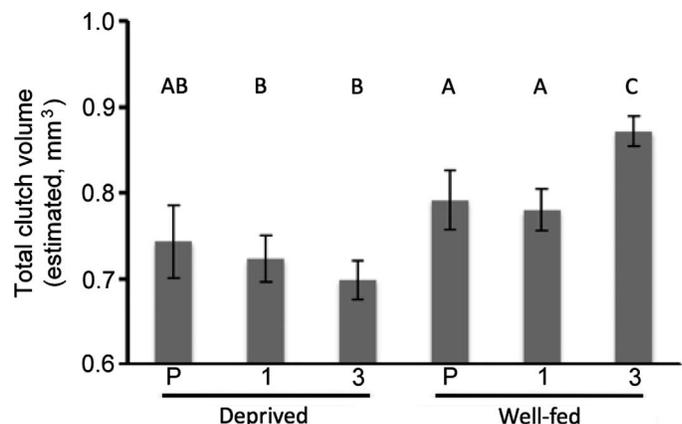
Table 4.—Two-way ANOVA analyses of mean egg diameter. Bold *P* values indicate significant differences.

Source	df	F ratio	P
Whole model	5	12.61	**<0.0001**
Treatment	1	11.26	**0.002**
Week	2	14.97	**<0.0001**
Treatment*Week	2	1.30	0.29

Figure 4.—Estimated total volume of clutch in mm[3] (bars indicate standard error). Letters indicate significant differences within and between treatments [*P* = penultimate instar; 1, 3 indicate weeks post-maturity) based on Tukey post hoc test.

continuous rate of egg loss and ideally benefit from cannibalism, not mating, in order to support fecundity. However once egg loss slows or stops, mating should ensue, regardless of feeding, as maximal fecundity has been reached for that individual. Future studies should address potential trade-offs and physiological relationships between fecundity, survival and maturation timing.

ACKNOWLEDGMENTS

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