# ORIGINAL ARTICLE

# SPATIAL AND TEMPORAL DEMOGRAPHIC VARIATION DRIVES WITHIN-SEASON FLUCTUATIONS IN SEXUAL SELECTION

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Our understanding of selection in nature stems mainly from whole-season and cross-sectional estimates of selection gradients. These estimates suggest that selection is relatively constant within, but fluctuates between seasons. However, the strength of selection depends on demographics, and because demographics can vary within seasons, there is a gap in our understanding regarding the extent to which seasonal fluctuations in demographics may cause variation in selection. Here we use two populations of the golden orb-web spider (*Nephila plumipes*) that differ in density to examine how demographics change within a season and whether there are correlated shifts in selection. We demonstrate that there is within-season variation in sex ratio and density at multiple spatial and temporal scales. This variation led to changes in the competitive challenges that males encountered at different times of the season and was correlated with significant variation in selection gradients on male size and weight between sampling periods. We highlight the importance of understanding the biology of the organism under study to correctly determine the relevant scale in which to examine selection. We also argue that studies may underestimate the true variation in selection by averaging values, leading to misinterpretation of the effect of selection on phenotypic evolution.

**KEY WORDS:** Demographic variation, *Nephila plumipes*, selection gradients, sexual selection.

The strength and direction of selection pressures acting on heritable traits can predict the evolution of phenotypic distributions (Fisher 1930; Lande and Arnold 1983; Kingsolver et al. 2001). The advent of statistical methods to quantify phenotypic selection (Arnold and Wade 1984a,b; Lande and Arnold 1983; Brodie et al. 1995) has led to a better understanding of how selection shapes phenotypes through time. By using estimates of selection gradients derived from cross-sectional sampling or average fitness (longitudinal estimates) across a breeding season, a number of studies have provided information on variation in the strength of selection on a variety of traits associated with fitness (see Kingsolver et al. 2001 for a review). Comparisons of spatially separated populations (e.g., Carroll and Salamon 1995) or the same populations over time (Cockburn et al. 2008; e.g., Grant and Grant 2002; Wilson et al. 2006) have led to a better understanding of how demographic factors such as the operational sex ratio (OSR) and/or population density (e.g., Blanckenhorn 1998; Jann et al. 2000; Pröhl 2002) correlate to changes in the strength and direction of selection.

Recent research has shown that selection can vary between seasons due to factors such as female choice (Chaine and Lyon 2008), predator abundance (Losos et al. 2006), or environmental variation (Cockburn et al. 2008), demonstrating that the time frame over which selection is measured can have significant effects on estimates of selection, and values averaged over long periods can mask fine-scale variation that will affect selection on phenotypic distributions (Hoekstra et al. 2001; Losos et al.

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2006; Cockburn et al. 2008). However, these studies have been performed on long-lived species that usually have relatively synchronized, single reproductive events within a season. This limits our understanding of the association between population demographics and selection, and how small temporal variation in demographics can alter selection as only single estimates can be made within a season. Species that mate multiply, or have a short life span relative to the breeding season, offer an interesting opportunity to examine the association between population demographics and selection as reproductive opportunities can change within a breeding season. Such an approach will reveal the relevance of within-season fluctuating demographics in estimations of selection, and how selection and population demographics interact at smaller scales (Kokko and López-Sepulcre 2007).

In this study, we used the golden orb-web spider (*Nephila plumipes*) to examine (1) how female density and the OSR vary throughout the breeding season at different spatial scales, and (2) whether selection (Lande and Arnold 1983; Brodie et al. 1995) on male body size and weight varies throughout the season along with demographic variation. *Nephila plumipes* is an excellent species to address these questions because of the ease with which male phenotypic traits and demographic variables can be assessed in the field, and also because published paternity studies allow accurate estimates of male fitness in nature based on relative positions of males in females' webs (Schneider and Elgar 2001; Elgar et al. 2003). Furthermore, due to the extreme sexual size dimorphism seen in this species (Vollrath 1980; Elgar and Fahey 1996), males and females mature at different rates (Austin and Anderson 1978; Higgins 1992; Higgins 1993), which will likely result in variation in female density and the OSR throughout the season. Such demographic variation is likely to cause changes in the competitive challenges males are likely to encounter at maturity.

For example, adult male *N. plumipes* face two main selection pressures at maturity. First, males are often clustered on female's webs (Vollrath 1980; Kasumovic et al. 2007), and competition with rivals selects for larger size (Vollrath 1980; Elgar and Fahey 1996) as larger males displace smaller males from the hub (center) position resulting in mating priority (Vollrath 1980; Elgar and Fahey 1996). Second, mating first may ensure the highest paternity (Schneider and Elgar 2001; Elgar et al. 2003), which may select for protandry in the presence of sexually mature females (e.g., Kasumovic and Andrade 2006). Variation in OSR and female density will likely alter the relative importance of intrasexual competition versus protandry, and therefore, the strength of selection for the traits associated with maximizing success in the particular challenge males will encounter.

To examine how selection pressures vary through one breeding season, we surveyed two separate sites containing *N. plumipes* in New South Wales, Australia every two weeks (three surveys:

early, mid, and late-season). With this information, we were able to estimate density and OSR at three different scales: (1) within the aggregation, (2) within 5 m of the focal aggregation (local), and (3) within each site. We estimated male reproductive success based on known relationships between the position on a female's web and paternity (see methods), and estimated selection gradients (Lande and Arnold 1983; Brodie et al. 1995) on male size and weight at each time frame within each population to determine whether selection fluctuated along with changes in demographic variables.

## *Materials and Methods*
### NATURAL HISTORY
*Nephila plumipes* is a univoltine species that breeds over two months of the Australian summer (January and February). Females build webs either solitarily or as part of aggregations with females of different ages (Elgar 1989; Herberstein and Elgar 1994). Aggregations are defined as groups of webs that share support strands (Elgar 1989) where females remain throughout the breeding season (Higgins 1990; Herberstein and Elgar 1994). Males mature on their own web in or near the aggregated webs of females (Higgins 1990), leaving their web upon maturity in search of available females, preferentially taking residence on the webs of penultimate instar or adult females (Vollrath 1980; Higgins 1990; Kasumovic et al. 2007), and waiting for an opportunity to mate. During settlement, males may have to compete against up to three rivals for access to the female (Kasumovic et al. 2007). Although information on the maximum number of males with which a female will mate is not available, a laboratory study demonstrated that females mate with up to three males (Elgar et al. 2003), and vary in the length of time after which they remate (1–15 days, Schneider and Elgar 2001).

*Nephila plumipes* males are limited to a single mating due to a high frequency of fatal sexual cannibalism (∼60%) and a high injury rate while mating (Elgar and Fahey 1996; Schneider and Elgar 2001; Schneider et al. 2001). Even if males survive their first mating, a 76% mortality rate during mate searching (Kasumovic et al. 2007) makes matings with further females unlikely. Thus, to maximize fitness, males must take full advantage of mating with a single female, and this is likely to be most strongly affected by their success in direct or scramble competition rather than in avoiding cannibalism (Elgar 1992; Schneider and Elgar 2002). Although females cannibalize males, there is no evidence that males provide a nutritional benefit to females in this species (Schneider and Elgar 2002).

### MALE COLLECTION
We surveyed two field sites containing *N. plumipes* in Bicentennial Park, Pymble in Sydney, NSW, Australia. The two sites

were approximately 5500 m² (North Pymble; low-density site) and 1700 m² (South Pymble; high-density site) in size and were separated by a mowed, grassy field (170 m wide). As previous research demonstrated that males do not cross gaps (Kasumovic et al. 2007), we considered the two sites independent of one another. Both habitats consisted mainly of shrubs and eucalypts. Each site was surveyed every two weeks throughout the breeding season for a total of three surveys (early, mid, and late-season). Surveys were completed between 1000h and 1400 h. For each survey, we located all *N. plumipes* webs at each site. We found aggregations in the same location during each survey as females are mostly stationary, continually adding to their webs (Higgins 1990; Herberstein and Elgar 1994). Web elevation varied from low to the ground in shrubs and saplings, to the upper canopy of trees (Herberstein and Elgar 1994). We surveyed all webs that were below 2 m in elevation. Only two aggregations above 2 m were observed.

We collected all males found during these surveys, noting their distance to the hub of the web (in cm), whether there were any other adult males found on the web or within the aggregation, and the age of the female on whose web they were found (juvenile, penultimate, and adult). We aged females using the coloration and shape of their copulatory opening, the epigyne. Adult females have a protruding epigyne that has two clear openings, penultimate females have the same protrusion, but the openings are covered, and juvenile females lack a protrusion (Higgins 1992; Kasumovic et al. 2007). We also examined the age structure of females in all aggregations and within a 5 m radius of each focal aggregation. We used these data to calculate three separate estimates of female density and the OSR: (1) aggregation, (2) local (5 m radius), and (3) site level. For all statistical analyses, the scale of the examination was the aggregation rather than the individual male to avoid pseudoreplication. Unless otherwise stated, female density was calculated as the number of preferred females (see results) within an aggregation, and the OSR was calculated as the ratio of sexually mature males to preferred females.

All collected males were brought into the laboratory where they were immediately weighed and measured using the average length of the patella-tibia of the two front legs as a measure of male size. None of the males collected were released back into the monitored field sites as male life span is short (5 days on average, Vollrath 1980) and it is unlikely that males collected at that time would remain on the web for the next collection.

### PATERNITY AND FITNESS ESTIMATES

We estimated paternity for *N. plumipes* males found on female's webs using known sperm use patterns from two- (Schneider and Elgar 2001) and three-male trials (Elgar et al. 2003). For these estimates, we used only males that were found with females. In doing so, we assumed that mate searching did not impose

significant selection on male size or weight in *N. plumipes*. This assumption is consistent with the literature in this and similar species (Vollrath 1980; Andrade 2003; Foellmer and Fairbairn 2005b; Kasumovic et al. 2007). Males closest to the hub mate first (Elgar and Fahey 1996), and in double-mating trials, gain an average of 54% paternity (Schneider and Elgar 2001). However, in mating trials involving three males, the third male gains an average of 23% paternity, diluting the paternity of the first and second males (45%, 32%, respectively; Elgar et al. 2003). Thus, when we found two males together, we assigned 54% paternity to the male closest to the hub, and 46% paternity to the second male. If a third male was present, we assigned paternity as 45%, 32%, and 23% to the first, second, and third male, respectively. We assigned 0% paternity to males that were the fourth furthest from the hub and 100% paternity to a male if he was the only male on the web. We used estimated paternity as our measurement of fitness. We calculated standardized linear (β ± SE), nonlinear (γ ± SE), and correlational selection gradients on male size and weight (Lande and Arnold 1983). We doubled the values of our quadratic selection gradients to accurately reflect how nonlinear selection is functioning (Stinchcombe et al. 2008).

To examine the effect our paternity estimates had on our estimates of selection, we also performed a sensitivity analysis that involved biasing paternity toward either the first or last mating male by a standard deviation (see supplement for greater details). Selection gradients calculated in our sensitivity analysis, were within the standard error of our initial estimates, and in most cases were almost identical. (See online supplementary Fig. S1.)

To test whether there were significant overall patterns of selection, and to determine whether there were significant differences in the strength of selection between sampling time and sites, we used partial *F*-tests (Bowerman and O'Connell 1990; Chenoweth and Blows 2005). We first fit a model with only site as a fixed effect (model 1). We then added the two linear terms as covariates (model 2), and then added the linear by site interactions (model 3). To test for the overall significance of selection gradients, we estimated a partial *F* by comparing model 2 against model 1. To test for overall interactions between sampling time and site, we estimated a partial *F* for model 3 against model 2. This was performed separately for linear and nonlinear selection gradients.

## Results

We collected a total of 327 males throughout the breeding season at both sites. Eight of these males were found on their own webs outside of aggregations, and seven were collected on webs from which the resident female was missing and had likely been depredated (webs were partially destroyed with large central holes). All our analyses used only the males collected from webs on which

females could be identified ($N = 312$) as males on their own webs or otherwise empty webs would have no current opportunity to mate. The number of males and females peaked at midseason at both sites. We collected 182 males from North Pymble (low-density site, early = 28, mid = 79, late = 75) and located 454 females' webs (early = 88, mid = 195, late = 171). We collected 130 males from South Pymble (high-density site, early = 43, mid = 53, late = 34) from 334 females' webs (early = 91, mid = 133, late = 110). Of the 312 males collected, 113 males (36.2%) were found with at least one other male, suggesting that competition for females may play an important role in determining male fitness.

We used a two-way analysis of variance (ANOVA) to test whether there were differences in male size and weight (dependent variables) between sites and at each sampling time (fixed independent variables). There were significant effects of site ($F_{1,306} = 6.92, P = 0.009$) and collection time ($F_{2,306} = 16.25, P < 0.0001$) on male size with larger males occurring earlier in the season and in the lower density site. There was no interaction between collection time and site ($F_{2,306} = 0.006, P = 0.99$). Male weight decreased through time ($F_{2,305} = 29.21, P < 0.0001$). There were no differences in male body condition between sites ($F_{1,305} = 2.60$, $P = 0.11$) or between sites through time (site × time: $F_{2,305} = 0.33, P = 0.72$).

**PREFERRED FEMALES**

Across the two sites, the majority of males were collected from webs of penultimate- and adult-instar females (males found on webs of: juveniles = 40, penultimates = 142, adults = 130; $\chi^2 = 34.2$ df = 2, $P < 0.0001$). Although previous studies have demonstrated that males prefer settling with adult and penultimate-instar females (Higgins 1990; Schneider et al. 2001; Kasumovic et al. 2007), examining male preference with a chi-square using season-wide patterns assumes equal availability of females of each age throughout the breeding season. To demonstrate male preference for females of different ages in our sample, we have to examine male choice with respect to the females available at each collection period because the age structure changed significantly throughout the breeding season at each site (low: $\chi^2 = 153.0$, df = 2, $N = 454, P < 0.0001$; high: $\chi^2 = 73.6$, df = 2, $N = 334, P < 0.0001$; Fig. 1). Thus, to determine whether female availability played a role in male choice, we used a subsampling method to compare observed patterns to a distribution based on random choice (Manly 2007) at each sampling period (see online Supplementary material for further information). Male settlement patterns did not depend on the relative frequency of each instar, rather, there was a primary preference for adult females, and a secondary preference for penultimate females at each sampling period in the low-density site (Early: $\chi^2 = 13.38$, df = 2, $P = 0.001$, Mid: $\chi^2 = 19.80$, df = 2, $P < 0.0001$, Late: $\chi^2 = 27.38$,
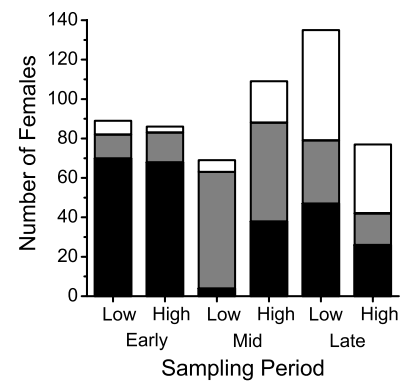


**Figure 1.** The number of adult (white bars), penultimate instar (one moult prior to adulthood, gray bars), and juvenile (more than one moult prior to adulthood, black bars) females in the low and high-density sites throughout the breeding season.

df = 2, $P < 0.0001$) and high-density site (Early: $\chi^2 = 16.28$, df = 2, $P < 0.0001$, Mid: $\chi^2 = 13.80$, df = 2, $P = 0.001$, Late: $\chi^2 = 3.85$, df = 2, $P = 0.15$) (see online Supplementary Fig. S2). As there was differential preference for females of each age, we only used the preferred age classes (penultimate and adult females) to estimate female density and OSR throughout the season.

**DENSITY ESTIMATES**

For our estimate of female density at each site, we calculated the number of preferred females per 100 m². North Pymble had a lower density of penultimate and adult females throughout the season (= low-density site; early = 0.35, mid = 2.18, late = 2.02) than did South Pymble (= high-density site; early = 1.18, mid = 5.29, late = 4.18). As the density of available females is likely to alter selection on males, we used two separate general liner models (GLM) with each measurement scale (aggregation or local) as the dependent variable and site density and time as fixed variables to examine whether the site and survey date affected the density of females encountered by males at the two scales (Fig. 2). We used the aggregation as the level of the analysis ($N = 245$). At the level of the aggregation, males at each site encountered similar average female densities overall ($F_{1,239} = 0.56, P = 0.46$), although the average female density increased as the season progressed ($F_{2,239} = 13.34, P < 0.0001$). There was no interaction between site and time ($F_{2,239} = 1.16, P = 0.32$). In contrast, at the local level, average female density was higher in the high-density site ($F_{1,239} = 41.58, P < 0.0001$), and density changed significantly between sampling times, peaking at mid season ($F_{2,239} = 9.79, P < 0.0001$). There was also a significant time by site interaction ($F_{2,239} = 9.31, P < 0.0001$).

We examined whether there was a difference in variance in female density between sites as this could affect optimal searching or mating strategies for males (Carroll and Corneli 1995), or the adaptive value of plasticity in time to maturity (Kasumovic and
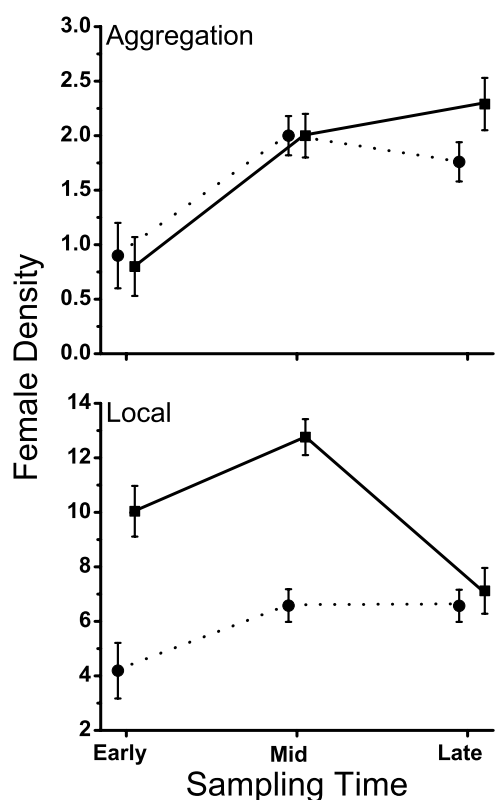
**Figure 2.** The average density of preferred (penultimate- and adult-instar) females within aggregations (top) and within 5 m of the focal aggregation (local scale; bottom) at three survey times in two sites (high density: squares with a solid line; low density: circles with a dashed line).



**Figure 3.** The untransformed operational sex ratio (male:female) within aggregations (top) and within 5 m of the focal aggregation (local; bottom) at three survey times in two sites (high density: squares with a solid line; low density: circles with a dashed line). The horizontal line signifies an equal OSR.

Andrade 2006). Variance in female density was affected by the scale of measurement both between and within sites. There was no difference in the variance in female density between sites at the level of the aggregation (high-density site: $\sigma^2 = 2.40$, low-density site: $\sigma^2 = 1.78$; Bartlett's test: $\chi^2 = 2.74$, $P = 0.10$). At the local level however, variance in female density was more than four times higher in the high-density site ($\sigma^2 = 37.72$) than in the low-density site ($\sigma^2 = 10.09$; Bartlett's test: $\chi^2 = 51.60$, $P < 0.0001$). The measurement scale (aggregation or local) also affected variance in density as it was higher in the high-density site (Bartlett's test: $\chi^2 = 148.93$, $P < 0.0001$) compared to the low-density site (Bartlett's test: $\chi^2 = 96.21$, $P < 0.0001$).

### SEX RATIO ESTIMATES

The overall OSR became female-biased later in the season in both the low- (early $= 1.68$, mid $= 1.23$, late $= 0.91$) and the high-density site (early $= 2.44$, mid $= 0.75$, late $= 0.78$). We also used a GLM (as above) to examine whether the OSR differed between sites and sampling times at each spatial scale (Fig. 3). In 27 cases, males settled in aggregations where there was no penultimate or adult female, making an estimate of OSR impossible. Thus, as males specifically chose juvenile females in these cases, we added
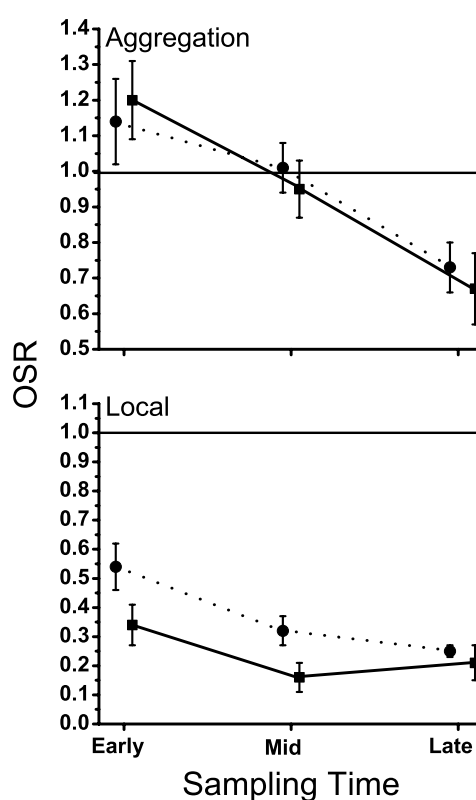
the number of juvenile females to our calculation of preferred females to allow calculation of the OSR. We transformed the OSR to normalize the data ($\sqrt[4]{\text{OSR}}$). At the level of the aggregation, the OSR was similar between sites ($F_{1,239} = 0.28$, $P = 0.60$), although it did change throughout the breeding season ($F_{2,239} = 14.55$, $P < 0.0001$). There was no significant interaction between time and site ($F_{2,239} = 0.52$, $P = 0.60$). At the local level, there was a significant difference between sites ($F_{1,239} = 20.23$, $P < 0.0001$), between sampling times ($F_{2,239} = 3.78$, $P = 0.024$), and a significant site by time interaction ($F_{1,239} = 3.40$, $P = 0.035$).

We examined variance in sex ratio as a function of spatial scale, time of season, and site using a Barlett's test as above. There was no difference in the variance in OSR between sites at the aggregation (high-density site: $\sigma^2 = 0.021$, low-density site: $\sigma^2 = 0.018$; $\chi^2 = 0.70$, $P = 0.40$) or local level (high: $\sigma^2 = 0.016$, low: $\sigma^2 = 0.020$; $\chi^2 = 1.44$, $P = 0.23$). There was also no difference in variance between scales in either site (high density: $\chi^2 = 1.84$, $P = 0.18$; low density: $\chi^2 = 0.40$, $P = 0.52$).

### FITNESS AND SELECTION ESTIMATES

Linear selection gradients on size and weight fluctuated between positive and negative in both sites for both traits as the breeding
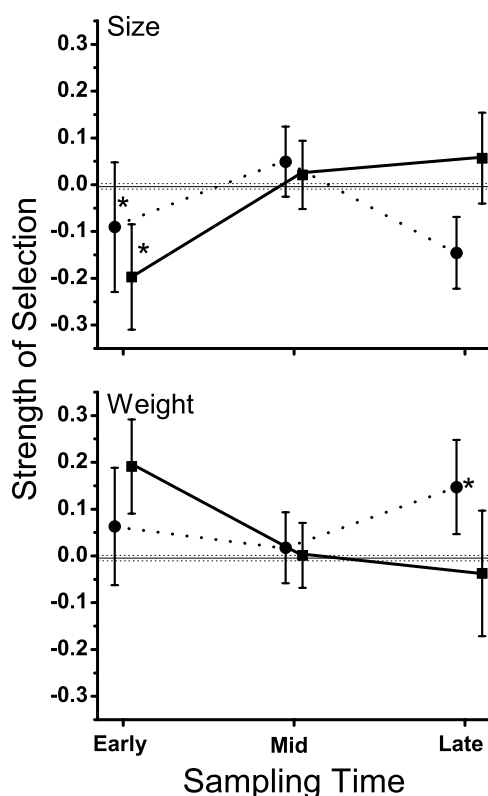
**Figure 4.** The strength of selection on male size (top) and weight (bottom) during three surveys in two sites (high density: squares with a solid line; low density: circles with a dashed line). The average selection gradients calculated from all the data summed across the three survey periods are also shown (solid horizontal line). Asterisks denote a statistical significance level of *P* < 0.10.

season progressed (Figs. 4 and 5). Furthermore, these estimated selection pressures varied from the average selection gradients calculated for all the data collected over the entire season (size: $-0.034 \pm 0.036$, $F_{1,309} = 0.88$, $P = 0.35$; weight: $0.029 \pm 0.036$, $F_{1,309} = 0.62$, $P = 0.43$; Figs. 4 and 5). There were trends toward significant negative selection on male size at early-season in the high-density site ($F_{1,42} = 3.07$, $P = 0.087$) and at late-season in the low-density site ($F_{1,72} = 3.58$, $P = 0.063$). There was also a trend toward significant positive selection on weight in the early-season sample in the high-density site ($F_{2,42} = 3.62$, $P = 0.064$). No selection gradients were significant. We tested for differences between standardized linear ($\beta \pm$ SE) selection gradients on male size and weight between sites between sampling times using a Partial *F*-test. Although there was no significant overall linear selection ($F_{2,305} = 2.23$, $P = 0.11$), selection gradients were significantly different between sites through the season ($F_{6,299} = 3.73$, $P = 0.0013$, Fig. 5).

None of the nonlinear selection gradients were significant (Table 1), even though the nonlinear selection gradients at each sampling time in each site varied from the average nonlinear

selection gradients calculated for all the data collected over the entire season (size: $-0.040 \pm 0.051$, $F_{1,305} = 0.62$, $P = 0.43$; weight: $-0.022 \pm 0.049$, $F_{1,305} = 0.20$, $P = 0.66$). There was also no significant correlational selection at either site at any time in the season (Table 1). We also used a Partial *F*-test to test for differences in nonlinear selection gradients. There was no evidence for significant nonlinear selection ($F_{5,302} = 1.15$, $P = 0.33$), and there was no significant difference between the strength of nonlinear selection at either site between sampling times ($F_{15,287} = 1.34$, $P = 0.18$).

## *Discussion*

We demonstrate that both the OSR and population density differed between sites and fluctuated within sites throughout the breeding season (Figs. 2 and 3). Despite the fact that the number of males and females changed similarly throughout the breeding season, the OSR became progressively female biased (Fig. 3). This progressive shift toward a female biased OSR can either be a result of: (1) our male collections, or (2) sex differences in changes in site demographics. It is unlikely that our collection influenced male density because the absolute number of males we collected peaked in mid-season, matching the pattern of female density and clustering. If our collection influenced the absolute number of males, we would expect male density to decrease over time, and to differ from female patterns as females were not collected. Furthermore, a male's tenure on a web is short under natural circumstances and it is likely the majority of males perish or are cannibalized within five days of arriving on a female's web (Vollrath 1980), or die while searching for other females if they abandon the web (Kasumovic et al. 2007). Thus, the males that we collected would not have been present at the next collection period two weeks later. Our sampling regime thus allowed for an accurate assessment of density, OSR, and male traits throughout the season, while still allowing male and female development to progress naturally between collections.

The variation in demography is therefore likely due to several natural processes. Sex differences in movement (Wauters and Dhondt 1993; Matter and Roland 2002), development (Forster and Kingsford 1983; Foellmer and Fairbairn 2005a), and/or mortality (Gwynne 1987) rates are all potential factors that could lead to observed biases in the OSR. Changes in immigration and emigration rates are unlikely to explain the variation in OSR in this species as a previous study has shown that males do not move between isolated populations (Kasumovic et al. 2007). This is also likely the case with females as they are sedentary once they begin web building (Foelix 1982). The progressive female bias seen in *N. plumipes* is likely due to several factors. (1) There are sex differences in maturity rates with females requiring more time to mature than males (Higgins 1992). (2) Adult survival is likely sexually
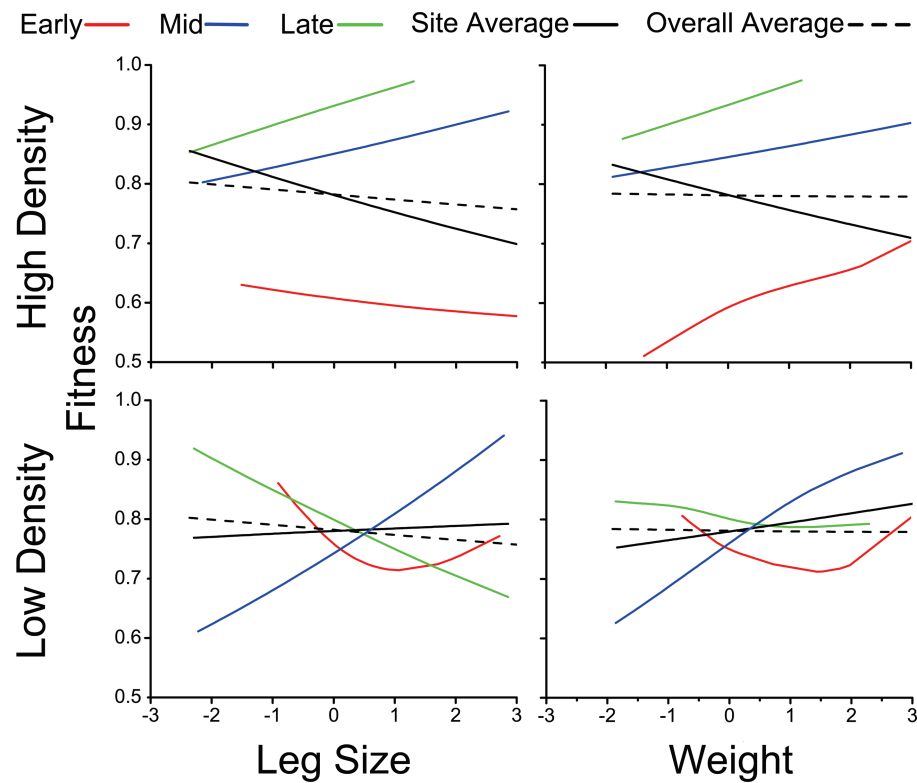
**Figure 5.** Cubic splines of linear selection on male size and weight in the high and low-density sites at the three different sampling times. The overall average cubic spline plotted on each graph allows comparison between sites.

dimorphic with higher mortality rates in males due to mate search (Kasumovic et al. 2007). (3) Female *N. plumipes* are known to sexually cannibalize males before the end of the copulation (Elgar and Fahey 1996; Schneider and Elgar 2001), and multiply-mating cannibalistic females (Elgar et al 2003) could skew the OSR as seen in other cannibalistic species (Vollrath 1980; Zimmermann and Spence 1992; Hurd et al. 1994; Maxwell 1998).

This natural shift in site demographics will alter the opportunity for selection on males within a season as it alters the number

of available females and competing males at different temporal and spatial scales. However, despite the female-biased sex ratio, there were still males clustering on webs of individual females in our study, similar to results in *Argiope aurantia,* another orb-web spider (Foellmer and Fairbairn 2005a). These studies suggest that males may be limited in their ability to find suitable females, and that a female biased OSR may not necessarily relax selection on male traits if clustering leads to intense competition (Fromhage et al. 2005). Although estimates of OSR and density at the site

**Table 1.** Nonlinear univariate and correlational selection gradients (±SE) on size and weight in both sites.

| Sampling time | Selection | | High density | P | Low density | P |
|---|---|---|---|---|---|---|
| Early | Nonlinear | Size | 0.204±0.130 | 0.13 | 0.448±0.232 | 0.07 |
| | | Weight | −0.156±0.112 | 0.17 | −0.196±0.169 | 0.26 |
| | | Correlational | −0.680±0.606 | 0.27 | 0.280±0.644 | 0.67 |
| Mid | Nonlinear | Size | −0.010±0.096 | 0.91 | −0.066±0.078 | 0.40 |
| | | Weight | 0.040±0.090 | 0.65 | 0.202±0.084 | 0.82 |
| | | Correlational | −0.436±0.254 | 0.22 | −0.372±0.528 | 0.48 |
| Late | Non-linear | Size | −0.216±0.130 | 0.11 | −0.044±0.052 | 0.39 |
| | | Weight | 0.206±0.202 | 0.31 | 0.063±0.076 | 0.41 |
| | | Correlational | 0.232±1.132 | 0.83 | 0.744±0.510 | 0.15 |
| Seasonal | Nonlinear | Size | −0.002±0.064 | 0.96 | −0.030±0.050 | 0.54 |
| | | Weight | 0.026±0.062 | 0.66 | 0.020±0.052 | 0.69 |
| | | Correlational | −0.270±0.294 | 0.36 | 0.418±0.278 | 0.13 |

level could aid the understanding of how selection varies (Kokko and Monaghan 2001; Kokko and Rankin 2006), local variance in either factor due to clustering effects within a site may weaken correlations between population level demographics and estimates of the strength and direction of selection.

This variance in demography brings into question the relevance of these demographic measures in determining traditional sex roles and predicting the direction of selection (Kokko and Monaghan 2001; Kokko and Johnstone 2002). Although our dataset does not allow us to determine whether OSR is a good predictor of the strength and direction of selection, it does raise other areas of concern regarding the dependence on OSR in predicting the direction of selection. Population-wide estimates of OSR and density may not accurately depict what is occurring at a more local level if such scaling is relevant to the system under study. For example, population-wide estimates of OSR may be irrelevant if individuals only interact on a more local scale and do not encounter the majority of the population (e.g., Andrade 2003; Foellmer and Fairbairn 2005a). It is therefore important to examine the proper scale specific for the species of study. In *N. plumipes*, the relevant scale of female density and the OSR is most likely the aggregation as males mature either within, or nearby aggregations of females (only 8 of 320 males collected were found on their own web outside of aggregations). Thus, assessing selection on male phenotypes as a function of larger scale estimates could lead to incorrect interpretations regarding the importance of site demographics in this species.

Along with the variation in population demographics, there were significant changes in the direction and strength of linear selection in each site between sampling times (Fig. 5). Microspatial variation at levels below the population may thus cause biologically relevant variation in selection pressures. This means that some studies may underestimate the true variation in the strength and/or direction of linear and correlative selection by averaging values throughout the season, multiple years, and/or populations (Jann et al. 2000; Kingsolver et al. 2001). Furthermore, fluctuations in the strength and direction of selection, such as those measured here, have the potential to dilute the net influence of selection, reducing the response to selection on heritable traits at the level of the population (e.g., Wilson et al. 2006).

Although the available data will not always allow examinations of spatial variation in selection pressures within seasons, it is nonetheless critical to consider the existence of such fluctuations. This is especially important in species that have multiple reproductive periods within a breeding season resulting in rapid demographic shifts (e.g., Blanckenhorn et al. 1999; Jann et al. 2000), and where individual life span is shorter than the breeding season and individuals would not experience a seasonal average (Bradshaw 1965) (e.g., sexually cannibalistic species: Andrade 1996; Maxwell 1998). In such species, variation in selection may

result in the evolution of plasticity in development and/or behavior. The direction that evolution takes will depend on the cues that are available to developing males. For example, the availability of cues of conspecific density and the sex ratio during juvenile stages could lead to the evolution of developmental plasticity in response to demographic cues that determine the strength and direction of selection (e.g., Kasumovic and Andrade 2006; Walling et al. 2007). Cues of the strength and direction of selection available after maturity may lead to the evolution of behavioral plasticity in response to the different competitive challenges that males encounter (e.g., foraging: Sih 1988; competition: Stoltz et al. 2008; mating: Su and Li 2006) or may lead to the evolution of protandry as that may be a secondary means of maximizing fitness (Candolin and Voigt 2003). In contrast, in multiply-mating or long-lived species, a seasonal average estimate of strength of selection, or an estimate of selection during the timing of synchronous reproduction may be the only estimations necessary (e.g., birds: Chaine and Lyon 2008; Cockburn et al. 2008; large mammals: Wilson et al. 2006). However, even in species with synchronous reproduction, if there is variation in arrival times within the sexes, individuals arriving earlier will have fewer rivals for access to territories or the limiting sex, and therefore, may encounter different selection pressures.

Within-season comparisons of effects of selection on long- and short-lived species and species with multiple and single reproductive events are necessary to test these predictions about the relevance of fluctuations in selection to phenotypic evolution. Species that show variation between populations in these characteristics, particularly those amenable to longitudinal study or experimental evolution, may be key to these tests. For example, such comparisons should be made between populations of the same species that differ in geographic location. Populations found at higher latitudes would have a shorter breeding season, likely resulting in a single synchronous reproductive event. In contrast, populations found further south should have longer, continuous breeding seasons with multiple breeding attempts. Comparisons of phenotypic distributions and patterns of selection between such populations would allow us to determine the relevance of fluctuating demographics and selection pressures towards phenotypic evolution, which may also lead to insights regarding the evolution of developmental plasticity.

Overall, we demonstrate that multiple competitive contexts can exist within a single population when examined at different temporal and/or spatial scales as a result of natural fluctuations in population demographics (e.g., male clustering in different areas of a population, differences in maturity rates between the sexes). Coupled with the idea that heritability and selection vary depending on environmental quality, potentially limiting evolution (Wilson et al. 2006), spatial and temporal variation in selection within a population may help explain why estimates of the strength

of selection examined over an entire breeding season seem low (Hoekstra et al. 2001; Kingsolver et al. 2001).

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## *Supporting Information*

The following supporting information is available for this article:

**Appendix S1.** Supplemental Methods and Results.
**Figure S1.** The strength of selection on male size (top) and weight (bottom) during three surveys in two sites (high density: squares; low density: circles).
**Figure S2.** The observed and expected frequencies of females chosen based on the availability of females at each sampling period in each site.

Supporting information may be found in the online version of this article.

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