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CONSTRAINTS AND PLASTICITY IN THE DEVELOPMENT OF JUVENILE *NEPHILA CLAVIPES* IN MEXICO

Linden Higgins[1]: Centro de Ecología, Universidad Nacional Autonoma de México, Apartado Postal 70-275, Ciudad Universitaria, C. P. 01450, México

ABSTRACT: The large, orb-weaving spider *Nephila clavipes* is found in a diversity of habitats within a narrow latitudinal range in Mexico. This allowed nearly simultaneous study of post-embryonic development of six disjunct populations in dissimilar environments. A common-garden laboratory study utilizing juveniles collected in four sites reinforced the conclusions from the field. The developmental parameters influencing growth in size at ecdysis did not vary within or among populations and may be genetically determined. Although very small juveniles exhibit variation in the growth per ecdysis, larger juveniles exhibit very little variation. These data, compared to data from field and laboratory studies of other tropical populations of *N. clavipes,* indicate that growth per ecdysis is highly constrained. Thus, this developmental parameter establishes a developmental trajectory that may be genetically determined and therefore subject to natural selection.

RESUMEN: La araña *Nephila clavipes,* tejedora de telas orbiculares, se encuentra en habitats diversos en México dentro de un límite angusto de latitúd. Eso permitió estudios casi simultaneos de ontogenia de juveniles en seis poblaciones desunidos en ambientes distintos. Un estudio del laboratorio utilizando juveniles colectados en cuatro sitios fortalece las conclusiones del campo. Los parámetros ontogenéticos determinando el crecimiento por muda no varió dentro ni entre poblaciones, y posiblemente son geneticamente determinados. Aunque juveniles muy pequeños mostraron variación en el crecimiento por muda, juveniles mas grandes no mostraron variación en este parámetro. Estos datos, combinados con datos de estudios de otras poblaciones en el laboratorio y en el campo, indican que el crecimiento por muda esta muy constreñido. Asi, este parámetro establece una trayectoria ontogenética que posiblemente sea geneticamente determinado y sujeto a selección natural.

Determining how environmental factors influence life history requires determination of the developmental parameters at each life-history stage and examination of whether the parameters are phenotypically plastic, responding to the environment, or are genetically determined (Caswell 1983, Via & Lande 1985, Pease & Bull 1988). The development of the large orb-weaving spider *Nephila clavipes* (Linnaeus) (Araneae: Tetragnathidae) can be expressed as a group of interdependent parameters with varying degrees of phenotypic plasticity (Higgins 1992a). Experimental trials showed that weight gain and intermolt interval duration responded to shifts in food availability, but growth per ecdysis did not (Higgins pers. obs.). The duration of the intermolt interval was found to be correlated with the size of the spider and apparently reflected the length of time the individual required to achieve the minimum weight necessary to molt to the next instar. In field studies, whereas the rate of weight gain and the number of juvenile molts varied with habitat within and among populations, the growth per ecdysis did not vary within a population or between two populations in the tropics (Higgins 1992a). The constraints imposed upon development by the constant growth per ecdysis were countered by phenotypic plasticity in other developmental parameters, particularly the number of instars, generating variation in size and age at maturity (Higgins 1992a; Higgins pers. obs.).

The previous studies utilized a range of sites with widely differing physical and biological conditions including photoperiod, making it difficult to distinguish the relative influence of different ecological factors on post-embryonic development. In order to better understand the environmental influence on development, a second field study was undertaken, utilizing the diversity of habitats in which *N. clavipes* is found in Mexico. Choosing sites within a 1° latitude range eliminated variation in photoperiod among sites and allowed nearly simultaneous study of populations experiencing very different environments.

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[1] Current address: Dept. of Zoology, University of Texas at Austin, Austin, Texas 78712 USA

In order to more fully explore the plasticity and constraints of development, juveniles from the four most distinct habitats were brought into the laboratory in a common-garden experiment.

Common-garden experiments, where individuals from different environments are held in a common environment, allow preliminary differentiation between those parameters that are apparently genetically determined and those that are phenotypically plastic (Wise 1987). Parameters that are genetically determined and vary among populations will express similar variation in the laboratory among individuals from those populations. In contrast, parameters that are phenotypically plastic and vary among populations will not vary in the laboratory among individuals from different populations. The combination of laboratory and field observations thus allows distinction between constrained, potentially genetically determined, parameters and phenotypically plastic parameters.

METHODS

Populations studied.—Spiders were observed in six sites spanning Mexico from the Veracruz coast to the Jalisco coast. Three sites were in Veracruz: Playa Escondida, Nanciyaga, and Fortín de las Flores. One site was in the high altitude desert valley of Tehuacán, Puebla. Two sites were west of the central plateau: Arroyo Frio, Michoacan, and Chamela, Jalisco. In 1989 I traveled to Playa Escondida, Nanciyaga, Fortín de las Flores, Arroyo Frio, and Chamela. In 1990, I studied spiders in Playa Escondida, Nanciyaga, Fortín de las Flores, Tehuacán, and Chamela. Because these sites varied only 1° latitude, there was no significant difference in photoperiod among them. However, the sites varied in many environmental parameters including type and degree of seasonality, and prey capture rates (Table 1).

Playa Escondida and Nanciyaga are privately owned forest preserves about 13.5 km apart on the Veracruz coast, separated by cattle ranches. Both have wet climates with relatively cool winters. Playa Escondida is approximately 1 km from a previous study site, the biological station "Los Tuxtlas" (Higgins 1992a, b). The *N. clavipes* population at the latter site disappeared shortly after dispersal of juveniles early in 1989, and the study was continued at Playa Escondida. The third Veracruz site, Fortín de las Flores, is a mid-altitude area of coffee plantations (Benton & Uetz 1986). This site is cooler and experiences stronger winters with minimum temperatures as low as 0 °C. On the western, dry side of the Sierra Madre Oriental in the valley of Tehuacán, I studied the spiders at the Secretaria de Ecología y Desarrollo Urbano cactus garden near Zapotitlán Salinas, Puebla. Annual rainfall in Tehuacán is very low and there is a relatively cold winter. Climatological data are not available for Arroyo Frio, located near Perdenales in southwestern Michoacan. The area is seasonally dry and, due to the altitude, seasonally cool. However, there is a permanent stream through the site that maintains high relative humidity within the arroyo where the spiders are found. On the Pacific coast of Jalisco, the spiders were studied at Chamela field station, owned and run by the Instituto de Biología, Universidad Nacional Autonoma de Mexico (UNAM). This site is seasonally dry but never cool. Spiders were found during the rainy season at all sites, but are facultatively bivoltine at both coastal Veracruz sites (Higgins in press). There was no second generation at these sites in 1989 and 1990, so data are presented from only the rainy season.

Rates of prey capture were estimated through trap-line surveys (Turnbull 1960). The spiders in Playa Escondida captured fewer prey than the spiders at other sites (Table 1) (Higgins pers. obs.). Predation load, estimated as the proportion of juveniles less than 0.5 cm leg I tibia + patella length that abandoned intact orb webs following predator attack (as in Higgins 1992b), was higher in Tehuacán, Arroyo Frio, and Chamela, but the differences were not significant (Higgins pers. obs.).

I traveled alternately east and west from Mexico City, visiting inland sites both on my way to and returning from the coastal sites. The visits at the coastal sites were slightly longer than visits to the inland sites. The combination of longer visits at the end points and repeated visits at intermediate sites enhanced the probability of observing molts by marked individuals.

Field observations.—Field observations of individuals utilized the methodology previously described in detail (Higgins 1992a). At each site, censuses of spider abundance and size were made and web sites were flagged. Measurements, made with Helios needle-tipped calipers, included spider leg I tibia + patella length (TPL, cm), abdomen length, and abdomen width. Individuals larger than 0.5 cm TPL were marked with enamel paint on their legs. From abdomen length and width, the abdominal volume was estimated as

Table 1.—Location, climate and relative prey capture rates at each site. Annual rainfall and mean temperature were taken from the nearest weather station reported by Garcia (1973) for all sites except Chamela. Data from Chamela come from Bullock (1986 and pers. commun.). Temperatures are for the growing season. No weather data are available for Arroyo Frio. (§ Mean prey capture per 12 diurnal hours per spider (Higgins, pers. obs.), determined as in Higgins & Buskirk 1992.)

Site	Coordinates	Altitude (m)	Annual rainfall (m)	Mean temp. °C	Prey capture§
Playa Escondida	18°35′N, 95°W	0	4.5	26	low
Nanciyaga	18°35′N, 95°W	100	4.5	26	high
Fortín de las Flores	18°50′N, 97°W	1000	2.5	22	high
Tehuacán	18°20′N, 97°30′W	1200	0.3	20	high
Arroyo Frio	19°10′N, 101°30′W	1200	—	—	—
Chamela	19°30′N, 105°W	50	0.7	—	high

a cylinder. For a given TPL, abdomen volume is highly correlated with spider weight (Higgins 1992a). Each individual found on a non-viscid silk platform was assumed to be pre- or post-ecdysis (Higgins 1990). The size of the abdomen relative to the legs distinguished between these conditions: pre-ecdysis individuals have large, distended abdomens whereas post-ecdysis individuals have much smaller abdomens relative to leg length and carapace width. Collection of exuviae provided additional data for the analysis of growth per ecdysis. Post-ecdysis spiders often hang the exuvium in the barrier webs near the hub connection, and TPL of an exuvium is not significantly different from the spider size in the previous instar (Higgins 1992a). When several exuviae were present, I only measured the largest, from the most recent molt.

These data are used to compare the relationships among abdomen volume, premolt TPL, and postmolt TPL within and among the populations over the entire life-cycle of the spiders. These parameters all describe the growth per molt, and as such are not strictly independent. However, because there was a chance that spiders gain weight beyond that required to successfully complete ecdysis (particularly relevant for penultimate-instar individuals), premolt abdomen volume was compared as well as premolt and postmolt TPL. To describe growth patterns of the species in North America, data from previous studies in Texas, USA, "Los Tuxtlas", Veracruz Mexico, and Panama (Higgins 1992a) were compared to those presented here.

Common garden experiment.—In 1990, juvenile spiders from two wet and two dry sites were brought into the laboratory. The populations selected for laboratory study were Nanciyaga, Fortín de las Flores, Tehuacán and Chamela. The spiders were maintained on three dimensional frames made of two intersecting 30 cm circles made of fiberglass strips, and were free to move about the laboratory. From each site, spiders of 0.2–0.4 cm TPL were collected. Twenty-three spiders were used in the experiment, as follows: Nanciyaga (3 females, 1 male), Fortín (3 females, 3 males), Tehuacán (2 females, 4 males), Chamela (2 females, 5 males). The ratio of juvenile males to females depended upon the exact dates of collection. Later in the season, small spiders are more likely to be males (pers. obs.). In addition, three spiders escaped prior to being marked during a trip to Fortín and Tehuacán. These animals were included in the description of weight gain during the intermolt interval.

The spiders were maintained 3–4 days with only water to increase the probability of spinning when released, and were offered food immediately after a web was spun. Any spider that did not spin an orb within three days of release in the laboratory was not included in the experiment. Throughout the experiment, each spider was offered three *Drosophila virens* each day. For a period of one week, only *D. melanogaster* was available; two of these were substituted for each of the larger *D. virens* (for a total of six flies). At the initiation of the experiment, the spiders were measured (TPL, abdomen length, abdomen width), and these measures were repeated with each molting. Abdomen volume was measured every other day during the intermolt interval to monitor weight gain. The majority of the spiders were held for two molts; one individual from Fortín failed to molt a second time in the lab-

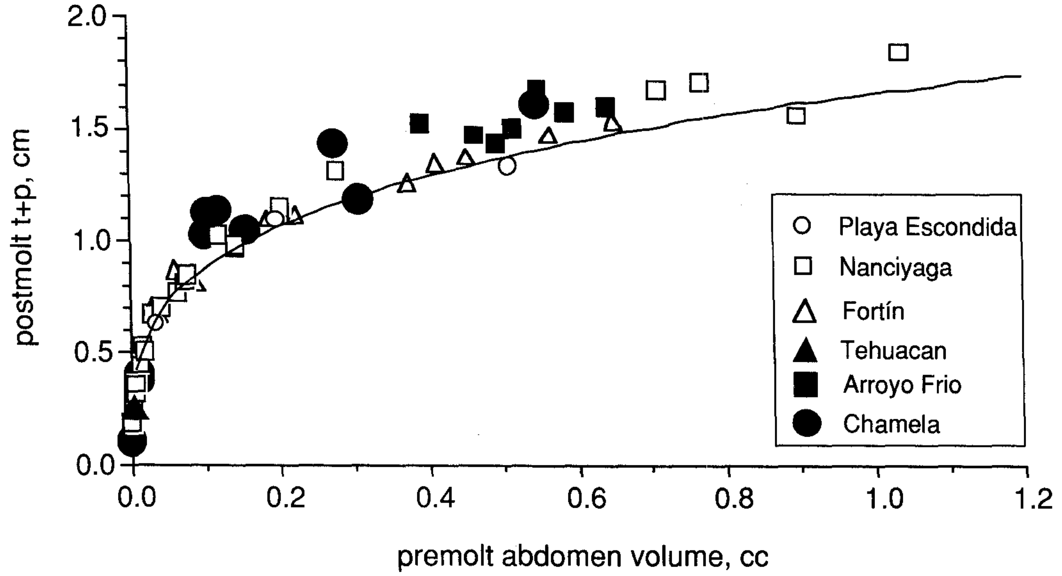

Figure 1.—Postmolt size (leg I tibia + patella length, TPL) as a function of premolt abdomen volume at all sites. Only data from juveniles and females molting to maturity are included in the graph. The curve represents a fit to the entire data set, $y = 1.66 \times {}^{0.28}$ ($R^2 = 0.99$).

oratory. Observations also distinguished those days when the spiders were foraging and had partially or wholly renewed orbs from the days immediately pre- or post-molt, when the spiders were not actively foraging. Data presented here concern the growth per ecdysis, length of the intermolt, days spent foraging during the intermolt and pattern of abdomen volume gain during the intermolt period.

Statistical analyses.—The developmental parameters examined in this study are dependent upon the size, TPL, of the individual. Therefore, all analyses tested for a significant regression between TPL and the measurement in question. If the regression analysis was significant for each population, further analysis tested for significant variation in the slope of the regression lines among populations. If there was a significant interaction of population and TPL, indicating difference in slope, then the analysis was halted (Sokal & Rohlf 1981). If the interaction terms were not significant, a final analysis of covariance (ANCOVA) with TPL as covariate tested for variation in the altitude of the line (y intercept). Lastly, if no difference was found due to population or treatment in a biologically important variable, *a posteriori* power tests were calculated to determine the minimum percent difference in the slope or intercept that could have been detected with these data.

RESULTS

Field observations.—*Abdomen volume and molting:* The relationships among premolt abdomen volume, premolt TPL and postmolt TPL vary little within or among the six populations studied. Postmolt TPL is related to the abdomen volume by a concave function, approximately a function of the cube root of abdomen volume (Fig. 1). The data are insufficient to allow comparison among the sites because recording premolt abdomen volume and postmolt TPL for the same individual was unlikely in the field.

The premolt abdomen volume is a function of premolt TPL, and males and females molting to sexual maturity do so at a lower abdomen volume compared to juveniles molting to juvenile instars. The data from Fortín included the greatest number of observations of molts to sexual maturity (Fig. 2). Penultimate instar males and females were identified as follows: almost all females of TPL greater than 1.0 cm are molting to sexual maturity (pers. obs.) and penultimate males have swollen palpi. After correcting for heteroscedasticity by taking the square-root of the dependent variable (cube root of abdomen volume), ANCOVA of the data from Fortín revealed that the differences among juveniles, penultimate instar males, and penultimate instar females were significant (TPL: $F_{(1,52)} = 1475.6$, $P < 0.001$; sex/

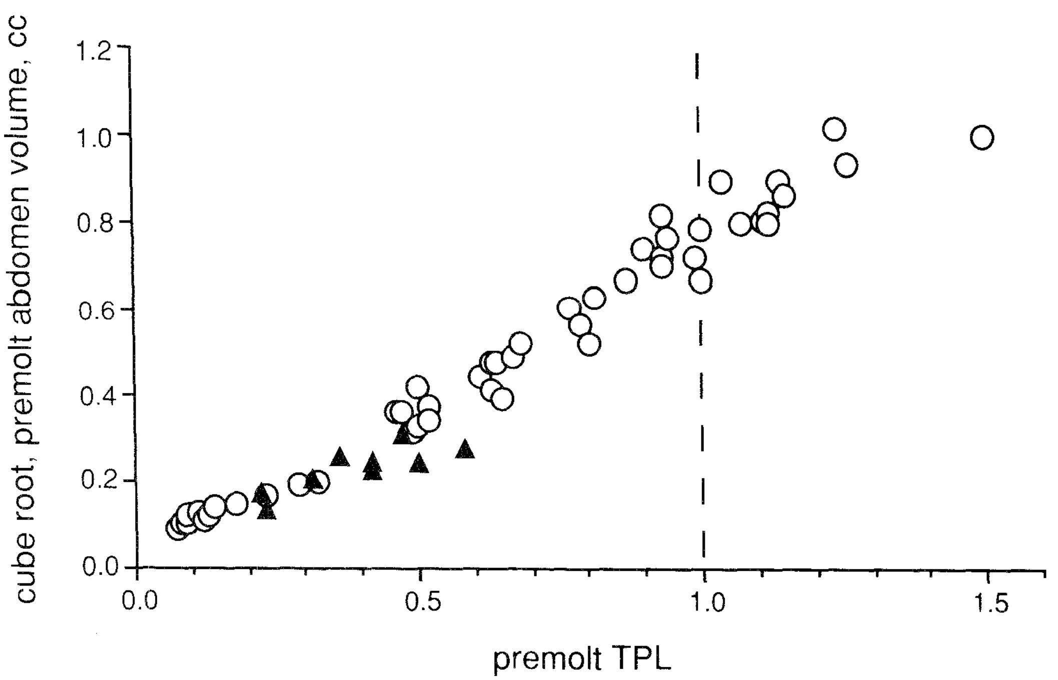

Figure 2.—The cube root of premolt abdomen volume as a function of premolt TPL (leg I tibia + patella length) for spiders in Fortín. These data include males molting to sexual maturity (= ▲) and juveniles and females (= ○). The points above premolt TPL = 1.0 cm are females molting to sexual maturity.

age: $F_{(2, 52)} = 8.85$, $P < 0.001$; interaction: $F_{(2, 52)} = 13.75$, $P < 0.001$). Separate comparison of males and females molting to sexual maturity indicated that the function of premolt abdomen volume on premolt TPL have the same slope ($F_{(1, 15)} = 2.28$, *ns*, power test: 4.1% detectable difference in slope).

Data from the remaining populations included few observations of spiders molting to maturity, so the comparison of the function of premolt abdomen volume on premolt TPL among sites utilized only data from juvenile molts. Arroyo Frio data were excluded from the final analysis because few spiders were observed in the size range of 0.5 cm–1.0 cm TPL. ANCOVA of premolt abdomen volume (to the 0.15 power) with TPL as covariate revealed that there was no difference in slope among all five sites ($F_{(4, 107)} = 1.75$, *ns*, power test: 1.8% detectable difference). The interaction term was dropped from the final ANCOVA, and this test showed that spiders in Chamela molted at a slightly but significantly lower premolt abdomen volume for their size [ANCOVA. TPL: $F_{(1, 111)} = 4283.4$, $P < 0.001$; site: $F_{(4, 111)} = 7.63$, $P < 0.001$; regressions: Chamela: $y = 0.30 + 0.54$ (TPL); remaining sites: $y = 0.32 + 0.55$ (TPL)].

Growth per ecdysis: Growth per ecdysis was compared within and among populations using regression analysis of postmolt TPL on premolt TPL. The slope of the regression line is an indication of the rate of size-specific growth. Molting to sexual maturity was presumed to affect the rate of growth at ecdysis because postmolt TPL is correlated with premolt abdomen volume, and whether the spiders were molting to maturity influenced the premolt abdomen volume. Therefore, the observations of molting juveniles, males molting to maturity, and molting females larger than 1.0 cm TPL were considered separately. Within sites, preliminary regression analyses showed that premolt and postmolt TPL were significantly correlated for juvenile molts at all sites (all $P < 0.003$) (Fig. 3). Individual ANCOVA were run to check for differences in growth during juvenile molts between years at the sites studied in both years: Nanciyaga, Playa Escondida, Fortín de las Flores, and Chamela. No significant differences were found (all $P \geq 0.12$); therefore, data from 1989 and 1990 were combined for the remaining tests. Due to small numbers of observations, the data from both years were pooled for the analyses of growth during molts to sexual maturity. Preliminary regression analyses of

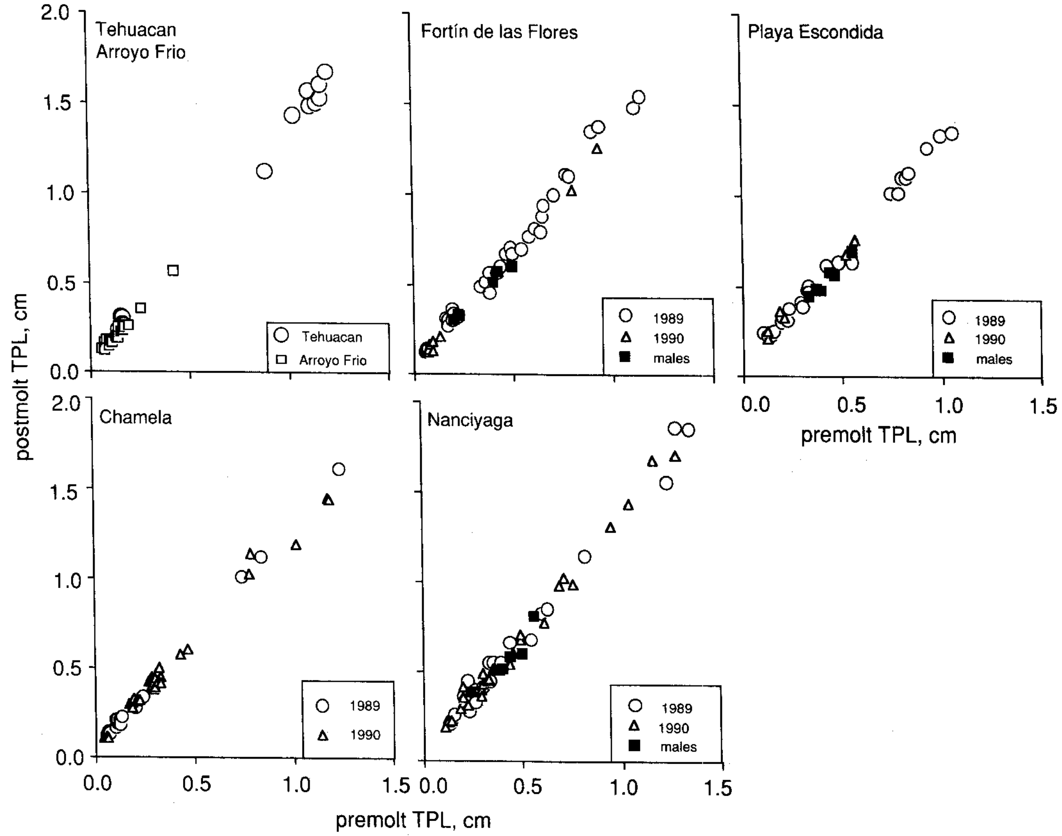

Figure 3.—Growth per ecdysis, determined as the relationship between premolt TPL (leg I tibia + patella length) and postmolt TPL for all populations observed. Arroyo Frio (1989) and Tehuacán (1990) are plotted together, all other plots contain data from two years. Males molting to maturity are indicated by solid squares (■).

postmolt TPL on premolt TPL were significant for males from Playa Escondida, Nanciyaga, and Fortín, and for females from Nanciyaga ($P <$ 0.02).

Travel precluded collecting complete data sets for all populations, therefore comparison between age and size classes (juvenile, male or female) were restricted to a few sites. Data from Nanciyaga indicated that there was no difference in growth per ecdysis between juveniles and females molting to maturity (no interaction effect $F_{(1,57)} = 0.01$, *ns*; ANCOVA. TPL: $F_{(1,58)} = 1567.1$, $P < 0.001$; maturity: $F_{(1,58)} = 2.01$, *ns*). In all three Veracruz sites, males were observed molting to maturity. ANCOVA showed significantly lower growth per ecdysis (slope of the line) in males molting to maturity than in molting juveniles less than 1.0 cm premolt TPL (Table 2).

Table 2.—ANCOVA of growth per molt of juveniles and males molting to maturity in Playa Escondida, Nanciyaga, and Fortín de las Flores. (* $P \leq 0.01$, ** $P \leq 0.001$)

Factor	*df*	*F* ratio
Premolt TPL	1	419.3**
Site	2	1.26
Maturity	1	3.28
Site × premolt TPL	2	2.98
Maturity × premolt TPL	1	7.81*
Site × maturity	2	0.26
Error	133	

Comparisons made among sites for juvenile molts revealed no difference among sites in growth per ecdysis either in slope or in intercept (slope: $F_{(5,208)} = 1.98$, *ns*, power test 0.9% detectable difference; intercept: $F_{(5,213)} = 1.48$, *ns*, power test 1.9% detectable difference) (Table 3). However, these regression analyses obscure a slight non-linearity of the data. Closer exami-

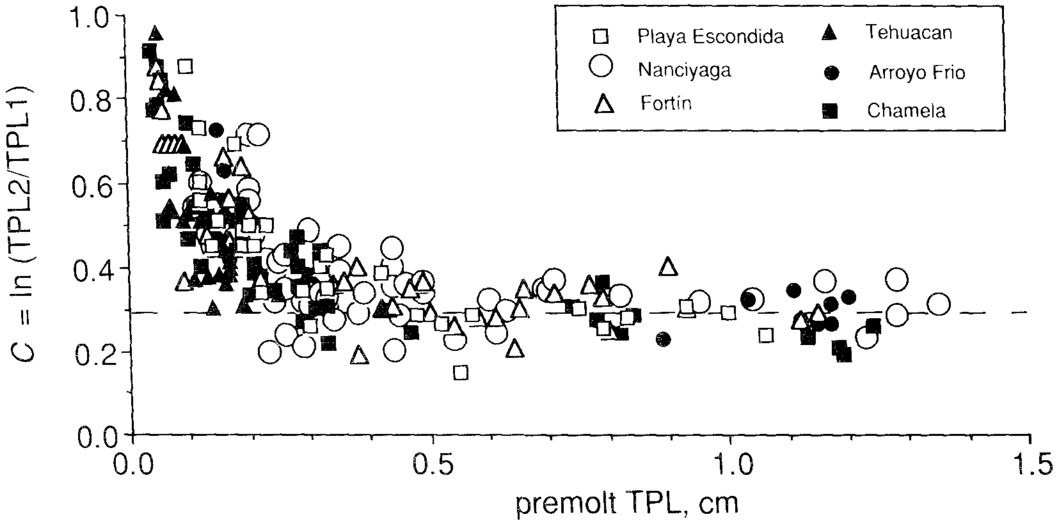

Figure 4.—The growth statistic C as a function of premolt TPL (leg I tibia + patella length). The value of C equal to the average slope of the equations of growth per ecdysis (Table 3) is indicated by the dotted line.

nation of variation in the growth per ecdysis was permitted by calculation of a growth statistic, $C = \ln(\mathrm{TPL2}/\mathrm{TPL1})$. This growth statistic is related to Huxley's growth equation (1972 p. 6) as $C = \ln(\alpha G)$ when $dt = 1$ instar, but it is not the same as k. Mean C is a function of the regression equation slope (ln(slope) = mean C). This transformation revealed that although C was independent of premolt TPL for larger spiders, for individuals of the first and second instars (TPL < 0.3) C was strongly dependent upon premolt TPL (Fig. 4). The data from all populations appear to fall on the same curve. The high values of C for small spiders reflect the large changes in TPL at ecdysis. Spiderlings with premolt TPL of 0.05 cm often molt to TPL of 0.11 cm, an increase of 100% or $C = 0.8$.m.

The data concerning juvenile growth per ecdysis can be compared to data collected in earlier studies of populations at Barro Colorado Island, Panama, at Los Tuxtlas, Veracruz, Mexico, and in southeastern Texas, USA (Higgins 1992a). The older data set did not distinguish males molting to sexual maturity. Therefore, the ANCOVA was run with juveniles of less than 1.0 cm premolt TPL and males. There was no difference among any of the tropical populations (no interaction effect: $F_{(7,\ 365)} = 1.67$, *ns*, power test: 2.2% detectable difference; ANCOVA. TPL: $F_{(1,\ 372)} = 19{,}370.4$, $P < 0.001$; site: $F_{(7,372)} = 0.99$, *ns*, power test: 4.7% detectable difference). Inclusion of observations from the University of Houston Coastal Center in Galveston County, Texas, resulted in a significant site effect on slope, reflecting the significantly lower slope of the growth per ecdysis for the Texas population (ANCOVA. TPL: $F_{(1,\ 425)} = 5776.6$, $P < 0.001$; site: $F_{(8,\ 425)} = 1.14$, *ns*; site × TPL: $F_{(8,\ 425)} = 4.91$, $P < 0.001$).

Table 3.—Regression equations for juvenile growth per ecdysis, where initial TPL is less than 1.0 cm. (**$P < 0.001$)

Site	n	Regression intercept	Regression slope	R^2	F (regression)
Playa Escondida	31	0.068	1.24	0.99	2671.1**
Nanciyaga	55	0.051	1.29	0.97	1959.7**
Fortín de las Flores	41	0.043	1.32	0.99	3150.0**
Tehuacán	24	0.053	1.19	0.96	591.0**
Arroyo Frio	17	0.078	1.25	0.99	1423.0**
Chamela	52	0.047	1.29	0.99	4743.5**

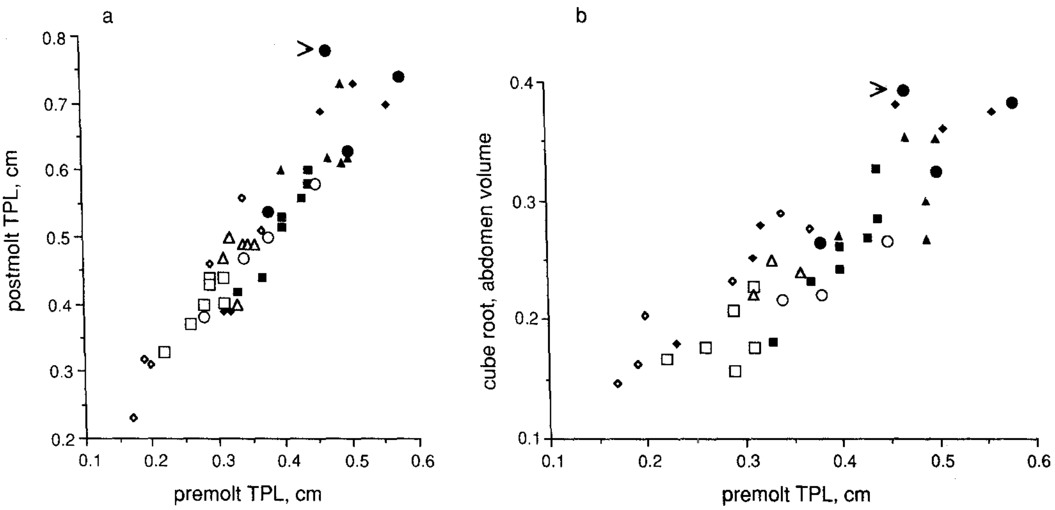

Figure 5.—Growth in the laboratory by spiders from four populations. Data from two molts are plotted, with the first molt indicated by open symbols and the second indicated by closed symbols. Squares = Chamela; diamonds = Tehuacán; triangles = Fortín; circles = Nanciyaga. The arrows indicate values for the male from Nanciyaga that delayed molting. Graph *a* = Growth per ecdysis in the laboratory, plotted as postmolt TPL (leg I tibia + patella length) *vs.* premolt TPL. Graph *b* = Premolt abdomen volume as a function of premolt TPL.

Common garden experiment.—Twenty-three spiders with TPL of 0.2–0.4 cm were brought to the laboratory from Nanciyaga, Fortín, Tehuacán, and Chamela and held for one complete intermolt cycle (two molts). The entire study lasted from June to October, and while all changes in TPL were verified by myself, variation caused by different persons making measurements produced increased error in the estimations of abdomen volume. In particular, data from two dates had to be excluded from the analysis of increasing abdomen volume, resulting in removal of five observations.

A total of 13 males were included in the study and five of them molted to maturity in the second molt in the laboratory: one from Nanciyaga, two from Fortín and two from Tehuacán. Therefore, analyses included the parameter of juvenile *vs.* maturation molt where appropriate.

Utilizing both observed molts for each individual, regression analysis of growth per ecdysis was significant for each population (all $P < 0.003$) (Fig. 5a). ANCOVA of growth per ecdysis showed no significant difference among these populations or between juvenile and maturation molts (no interaction effects, $P > 0.1$; ANCOVA. TPL: $F_{(1,\,38)} = 230.9$, $P < 0.001$; population: $F_{(3,\,38)} = 0.55$, *ns*; maturation: $F_{(1,\,38)} = 0.004$, *ns*). These data were compared to 37 observations of molts in the field from the same sites and the same premolt TPL (0.2–0.5 cm). Preliminary analysis revealed no significant difference in slope among sites or between conditions (field or laboratory), nor a significant interaction of site and condition (ANCOVA: site × TPL: $F_{(3,\,68)} = 0.43$, *ns*; condition × TPL: $F_{(1,68)} = 0.10$, *ns*; site × condition: $F_{(3,\,68)} = 0.14$, *ns*). Final ANCOVA testing for primary effects showed no significant variation due to site or condition (TPL: $F_{(1,\,75)} = 450.9$, $P < 0.001$; site: $F_{(3,\,75)} = 1.52$, *ns*; condition: $F_{(1,\,75)} = 0.65$, *ns*).

Several parameters describing the intermolt interval were collected from the laboratory animals (Table 4). ANOVA showed no significant difference among populations in mean TPL following the first molt in the laboratory, although Nanciyaga and Fortín individuals were slightly larger. The total intermolt interval and the number of days foraging between molts were not affected by these slight differences in size (regression of total intermolt duration: TPL: $F_{(1,\,15)} = 1.35$, *ns*; regression of days foraging: TPL: $F_{(1,\,14)} = 3.43$, *ns*). The total intermolt interval and days foraging in the laboratory varied among sites but was not affected by whether the individual molted to sexual maturity (ANOVA of intermolt interval. site: $F_{(3,\,16)} = 3.69$, $P = 0.03$; maturity: $F_{(1,\,16)} = 1.64$, *ns*; ANOVA of days foraging. site: $F_{(3,\,15)} = 4.69$, $P = 0.02$; maturity: $F_{(1,\,15)} = 0.002$, *ns*). Differences between Nanciyaga and the re-

Table 4.—Intermolt duration in the laboratory for spiders from four populations. The TPL (leg 1 tibia + patella length) reported is the measurement following the first molt in the laboratory. Letters refer to statistically similar values among sites.

Site	*n*	TPL ± 1 SD	Total days ± 1 SD	Days foraging ± 1 SD
Nanciyaga	4	0.48 ± 0.08	26.8 ± 8.0 (a)	24.3 ± 9.0 (c)
Fortín de las Flores	6	0.47 ± 0.04	18.8 ± 4.0 (b)	15.4 ± 2.5 (d)
Tehuacán	6	0.40 ± 0.13	17.3 ± 4.3 (b)	14.8 ± 3.9 (d)
Chamela	7	0.40 ± 0.04	18.7 ± 3.1 (b)	13.2 ± 1.7 (d)

maining sites appear due to one male from Nanciyaga that took over 30 days to complete the intermolt interval and molt to maturity, twice the usual intermolt duration for spiders of this size.

The abdomen volume gain in the laboratory was independent of site. In the first molt, spiders from Tehuacán molted at a significantly higher premolt abdomen volume (no interaction affects; ANCOVA. site: $F_{(3, 13)} = 5.42$, $P = 0.01$). All spiders molted at the same relative abdomen volume in the second molt (no interaction affects; ANCOVA. TPL: $F_{(1, 16)} = 43.4$, $P < 0.001$; site: $F_{(3, 16)} = 2.61$, *ns*; maturity: $F_{(1, 16)} = 1.3$; *ns*) (Fig. 5b). Because there was no difference in the second molt in premolt abdomen volume among sites or between molts to maturity and juvenile molts, data from all individuals held for a complete intermolt cycle (including three of unknown origin) were combined to describe the pattern of abdomen volume increase over the intermolt. The relative change in abdomen volume [ln(av(d)/av (0)], where d = day and 0 = day of molt, was plotted against time for spiders molting within 20 days, for spiders molting in 20–26 days, and for the individual from Nanciyaga requiring 36 days (Fig. 6). The general trend was for the rate of abdomen volume increase to slow as the spiders approached the next molt. The individual from Nanciyaga that took longer between molts did achieve a greater premolt abdomen volume and grew slightly more at ecdysis than the other spiders, as indicated in Figs. 5a and 5b by arrows.

DISCUSSION

In order to interpret variation in phenology and size at maturity, the proximal developmental causes of the variation must be identified. In arthropods, variation in two developmental parameters can lead to differences in size at maturity: there may be variation in the change in size at each molt, or there may be variation in the number of juvenile molts. Variation in either parameter can result in the same adult size, but the conditions under which each varies may be distinct. Genetic variation or phenotypic plasticity can lead to differences in development within and among populations, but the evolutionary consequences of each source of variation are distinct (Pease & Bull 1988). Longitudinal observations of juvenile growth are a first step towards determining how environmental factors generate differences in adult size, and whether these differences are the result of phenotypic response to the environment or genetic variation among individuals within or among habitats. The results of the studies of *N. clavipes* imply that some developmental parameters are highly plastic while growth per ecdysis is constrained and may be genetically determined (Higgins 1992a, present study). Such information is not available from the census data presented in past arachnological studies without making basic assumptions concerning developmental processes.

Traditionally, field measures of growth utilized either frequency distributions of a single measure, such as carapace width, or the regression of two allometric body parts of individuals. The difficulties of determining growth and instar number from the former measure have been recognized (Polis & Sisson 1990); however, the latter analysis also presents incomplete information (Teissier 1960). In his formulation $dx/dt = \alpha Gx$, Huxley assumes constant growth per unit time if the environmental factors represented by G are constant (1972, p. 6). In order to determine the rate of growth or the growth per ecdysis (t = 1 instar), one must assume that the individuals are moving along the trajectory described by the allometric relationships at a constant rate o growth per ecdysis, an assumption that may b invalid if the growth per ecdysis responds to en vironmental factors (G). This assumption is in valid for the linyphiid *Linyphia triangular* Clerck and may be invalid for the lycosid *Lycos helluo* (Turnbull 1962, Uetz et al. 1992). Turn

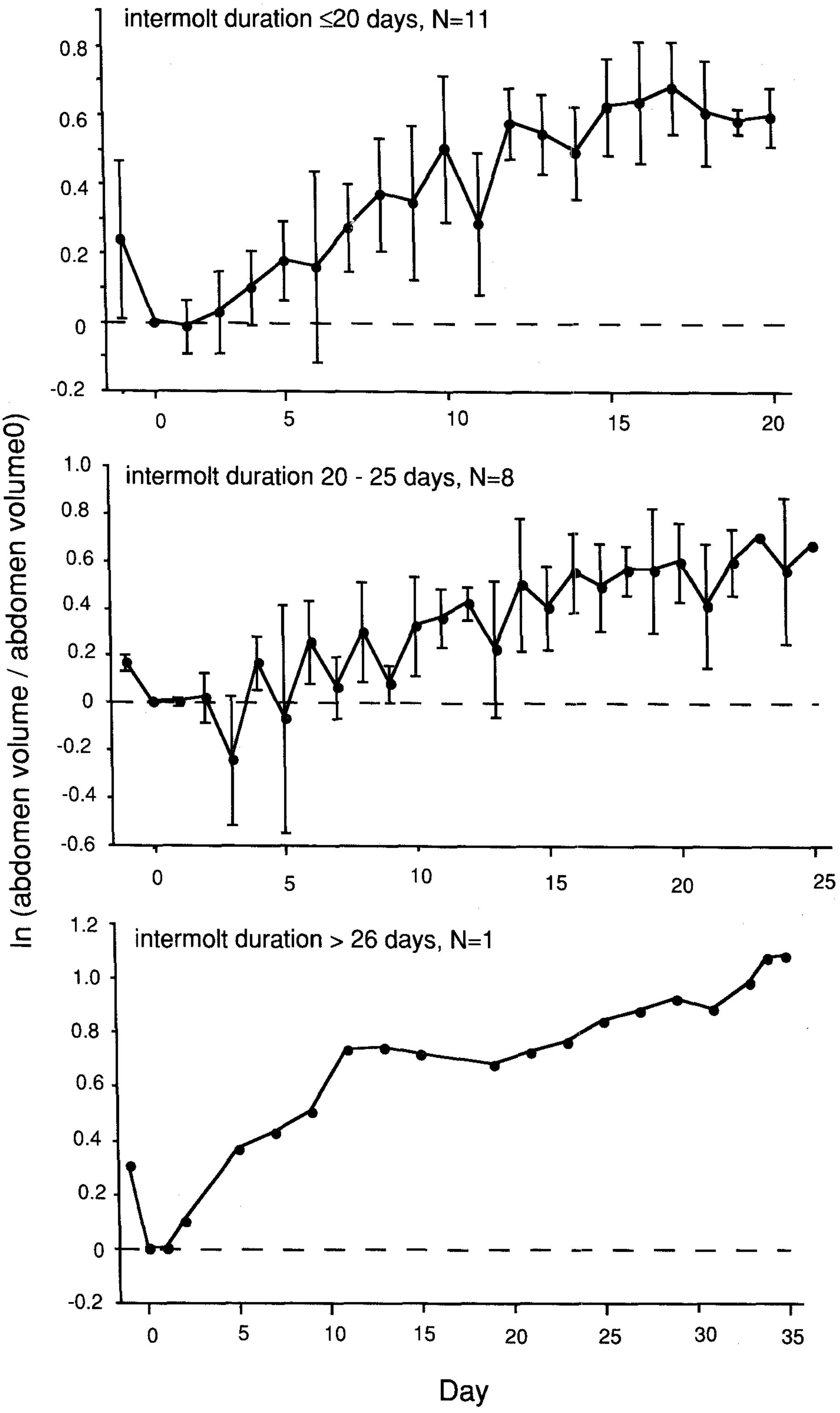

Figure 6.—Mean and SD of abdomen volume gain in the laboratory over time. Spiders from all sites were grouped according to the duration of the intermolt.

bull (1962) found that juvenile *L. triangularis* raised on quantitatively different diets molted at distinct, diet-dependent premolt weights. Implicit in his data is that the spiders grew different amounts in ecdysis, and due to this variation achieved different sizes at sexual maturity. He apparently did not observe variation in the number of juvenile instars. Uetz et al. (1992) found that sibling groups of *L. helluo* reared to maturity on qualitatively different diets varied in age and size at maturity as well as in juvenile mortality. It is unknown what developmental parameter varied with diet. Variation in the number of juvenile instars has been found for spiders in diverse families (Levi 1970), but the relative importance of variation in growth per ecdysis and variation in the number of juvenile instars in determining final adult size is unclear in previous studies of spiders (Levi 1970, Edgar 1971, Miyashita 1986, Wise 1987). Strikingly, despite the temporal variation in food levels experienced by many spiders (Riechert & Luczak 1982, Higgins & Buskirk 1992), there is as yet no evidence in spiders of the retrogressive or supernumary molts characteristic of many holometabolous insects subjected to poor diets or starvation (e. g., Beck 1972, Nijhout & Williams 1974). However, allometric data collected without longitudinal studies would not detect such molts if they did occur.

In *N. clavipes,* there is surprisingly little variation in the developmental trajectory described by growth per ecdysis. The data from field and laboratory reinforce the previous data, revealing stronger constraints in growth per ecdysis than previously reported (Higgins 1992a). Only the smallest individuals showed variation in growth per ecdysis, whereas larger juveniles and penultimate-instar individuals molt at the minimum premolt weight. I postulate that this shift from variable to constant growth per ecdysis may reflect either the reduction in predation pressure with increased size of the spiders or changes in the benefit of delayed ecdysis.

Growth per ecdysis in this spider is highly correlated with the premolt abdomen volume, and the premolt abdomen volume is size-specific and does not vary among the Mexican populations. Spiders from the isolated, desert population at Tehuacán might be expected to differ in their development, as they experience a short growing season and low rainfall (Higgins pers. obs.). In fact, the slope of growth per ecdysis appears to be lower in Tehuacán (Table 3) and is the same as that reported for the Texas, USA, population (Higgins 1992a). However, perhaps due to the small sample size from Tehuacán, the slope of growth per ecdysis did not differ statistically from the other five Mexican sites. Among these five sites, there is no apparent or statistical difference in growth per ecdysis, and these developmental trajectories are equal to those observed in Panama and in another coastal Veracruz site, Los Tuxtlas. Although I previously predicted that the spiders under good conditions (such as warm, moist coastal Veracruz) might accelerate development by delaying each molt, surpassing the minimum premolt abdomen volume and growing more at the next ecdysis (Higgins 1992a), this was observed only for spiders in the earliest instars. These small spiders are in the most heavily predated size class (Higgins 1992b) and may be seeking to escape predation by rapidly increasing their size (Wilbur & Collins 1973). Growth per ecdysis declines and is less variable after the spiders reach 0.3 cm TPL.

The shifts from variable to constant growth per ecdysis could also reflect the allometric relationship of postmolt TPL and premolt abdomen volume, described by a concave curve. For very small spiders, slight changes in premolt abdomen volume greatly alter postmolt TPL, so small delays in molting accompanied by weight gain will have a large effect on growth. For larger juveniles, small changes in abdomen volume have little effect on postmolt TPL, so much longer delays in molting are required for a significant change in growth in ecdysis. This is seen in the very slight increase in growth per molt for the individual from Nanciyaga that delayed molting during the common-garden experiment. This reduced benefit of delayed ecdysis could also explain the patterns seen in animals molting to maturity, which are of sizes found on the asymptote of the curve.

Spiders in the penultimate instar have partially developed external genitalia and are committed to becoming sexually mature in the next instar. Therefore, delaying maturity through additional juvenile molts is not an option. Males and females molting to sexual maturity molt at lower relative premolt abdomen volumes than juveniles molting to a juvenile instar. This is presumably due to the high benefit of reaching sexual maturity early compared to the slight increase in size that may be achieved by delayed ecdysis in the last instar. Both male and female reproductive success increase with increased adult size

(Christenson & Goist 1979, Vollrath 1980, Higgins 1992a), but the importance of early maturation is known only for females. Females maturing earlier in seasonal environments have greater likelihood of reproducing and greater likelihood of producing several egg sacs than do females maturing later (Higgins pers. obs.). There is an increase in fecundity per egg sac with increased TPL (Higgins 1992a), but increasing the number of egg sacs is proportionally more important as each egg sac may contain over 1000 eggs.

Although *N. clavipes* exhibits wide variation in size at sexual maturity, the developmental trajectory of this species described by growth per ecdysis is apparently highly constrained within a population and may be genetically determined. The spiders must achieve a given minimum premolt weight (here presented as premolt abdomen volume) prior to molting, and most spiders molt soon after reaching this weight. Premolt weight is size-dependent and independent of diet either in the field (current study) or in the laboratory (Higgins pers. obs.). That it is possible for the spiders to surpass the minimum premolt weight is apparent from the growth patterns of the smallest juveniles, and from occasional observations of spiders in the laboratory that surpass the minimum weight and grow more in the subsequent molt (present study; Higgins pers. obs.). This may reflect either phenotypic plasticity or genetic variability in minimum premolt weight. If phenotypic plasticity exists for premolt weight, it is rarely expressed after the fourth instar. If we assume that the selection has operated to optimize the developmental trajectory, these data indicate that, during the later juvenile instars, the costs of delayed molting outweigh the benefits of increased growth per ecdysis. This may be particularly true if, as implied by the concave relationship between premolt weight and postmolt size, increasingly long delays are required for significant differences in growth. However, the physiological and ecological costs of molting are not well enough understood to describe this optimization function, and the presence of specific constraints in premolt conditions must be determined first. Only future studies of the physiological and genetic controls of ecdysis in spiders will clarify this optimization function.

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