Chapter 4

Geography of Density-Dependent Selection in Pitcher-Plant Mosquitoes

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Two questions appear to us to be fundamental to the understanding of life history evolution. First, what sorts of selective forces are impinging on a species over its range or a given environmental gradient? Second, what is the response by this species to this gradient? As has often been pointed out, there is abundant theory relating to general (many assumptions) or special (very specific) circumstances but fewer direct answers to the two questions posed above. Before one can predict the outcome of selection,, one must first know how selection is operating; only then can one test the predictions about what organisms ought to do by comparing these predictions with what one actually finds them doing.

A few investigators have now sought to test theory in the laboratory by imposing artificial selection designed to distinguish between alternative predictions in *Drosophila* (Giesel and Zettler 1980; Taylor and Condra 1980; Mueller and Ayala 1981), bacteria (Luckinbill 1978), protozoa (Luckinbill 1979), and copepods (Bergmans 1984). Although we believe these experiments to be revealing and important tests of theoretical predictions, one must examine the experimental designs carefully to determine what is being selected. For instance, Taylor and Condra (1980) reared *Drosophila* on a small, fixed amount of medium allowing adults continuous opportunity to oviposit (supposed K selection) or on a generous supply of medium, but allowing only the first 100 flies that emerged to oviposit and allowing only 2 days in which to oviposit (supposed r selection). Because of the design of their experiment, they were primarily selecting for rapid development instead of r selection directly; instead of a K-selected strain, they selected for high adult survivorship instead of a higher equilibrium density. It is not, therefore, surprising that their primary result was that "r-selected" strains showed rapid development and "K-selected" strains showed high adult survivorship. Their strains did not differ in either capacity for increase or carrying capacity. Did these latter traits not respond to selection because the selection was not what it superficially appeared to be or because these traits were manifestations of a relatively inflexible genotype?

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examine life history changes along a gradient of selection. One is equally responsible for identifying the factors actually impinging on a population in the field as well in the laboratory. In this chapter, we define a geographical gradient in density-dependent, juvenile mortality in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), and describe what we believe to be some of the potentials and pitfalls of such a basis from which to test theory.

Background on *W. smithii* and Its Host Plant

The mosquito *W. smithii,* is a member of the tribe Sabethini, most of which are tropical mosquitoes that oviposit into and develop in small, circumscribed (container) habitats such as bamboo internodes, bromeliad axils, and pitcher plants. *W. smithii* is no exception as it develops exclusively in the leaves of insectivorous plants belonging to the genus *Sarracenia.* Within *Sarracenia* there are only two species that retain water throughout the year; one, *S. psittacina* Michx., has a cryptic opening, has large internal hairs, and, in occupying the wettest subhabitat of the genus, is submerged much of the time. The other *Sarracenia* with persistent pitchers, and the only one to contain standing water regularly, is *S. purpurea* L., the principal habitat of *W. smithii. S. purpurea* is found from the Gulf of Mexico to Canada, where it ranges from Newfoundland (the provincial flower) to the McKenzie Territory and British Columbia. The range of *W. smithii* follows that of its host from about 30–55°N latitude.

Prey captured by the host leaf constitute the resource base of the community of living insect inhabitants (inquilines). Although prey decompose and disarticulate, arthropod head capsules or cephalothoraces tend to remain intact and identifiable. The number of prey captured is then quantifiable by examining the "gut contents" of a leaf and counting the number of head capsules of prey contained therein. We have shown previously that both mosquito biomass sustained by and pupae produced in a leaf are directly proportional to the number of prey it has captured (Bradshaw 1983a; Bradshaw and Holzapfel 1983). Although a leaf may live for over a year, it is most active in capturing prey only for a short period 2–4 weeks after it has opened. Thereafter, prey capture declines exponentially and becomes highly sporadic. Thus, resource availability is predictable but transient. Ovipositing females of *W. smithii* select only the very youngest leaves and, even though prey capture is proportional to leaf size, females oviposit preferentially into a smaller, younger leaf than into an older, larger one (Bradshaw 1983a). Attractability of a leaf to *W. smithii,* unlike to prey, is immediate on opening and declines exponentially from day zero. *W. smithii* will oviposit into a leaf that has opened the same day, has no standing water, and no prey, and is not yet fully open or hardened. The adaptive significance of this behavior seems straightforward: since mosquitoes hatch into a leaf prior to maximal prey capture, resources will peak as they develop and resource demand increases. Further, where mosquito resources are limiting, early cohorts may have a competitive advantage over later ones (Livdahl 1982). Plant and mosquito behavior combine to create an ideal field experimental system: both mosquito recruitment and resource input are transient and occur early in the life of a leaf. Densities or food levels may be manipulated soon after a leaf opens, populations censused with replacement for the duration of the experiment, and resource level (head capsules) determined by destructive sampling at a later date. Finally, *W. smithii* enter dormancy (diapause) as larvae under the influence of short-day photoperiod (Bradshaw 1976; Bradshaw and Lounibos 1977) and overwinter inside the pitcher plant leaves. Thus, during the winter and early spring, 100% of the population can be censused and resource availability during the previous year may be quantified at the same time.

On a geographical scale, *S. purpurea* along the Gulf Coast and northwards into the Carolina coastal plane live in wet, pine savannahs and the wet areas surrounding cypress domes and titi pocosins. In the mid-Atlantic states, *S. purpurea* may be found in sphagnous marshes, cedar swamps, or bogs. Further north and at higher elevations in the south, they occur in bogs, in sphagnous cattail (*Typha*) marshes, in marl pits, and in moist areas around kettle holes, ponds, and lakes, usually associated with ericaceous shrubbery and tamarack (*Larix larcina* DuRoi). At 54°N, a single meristem of *S. purpurea* may produce two leaves per year; at 30°N, it may produce a dozen leaves or more but seven or eight leaves are about average. It is important to emphasize that, although the macrohabitatof the host plant varies over this range, *W. smithii* is a single species of mosquito whose principal habitat is the water-filled leaves of a single species of plant. Along the Gulf Coast and at low elevations in North Carolina, *W. smithii* diapause as fourth instars although overwintering larvae may be found regularly as second, third, or fourth instars; further north and at high southern elevations, *W. smithii* diapause as third instars and 98–100% of the overwintering larvae are found in that stage.

In the present chapter, we start with a close examination of density-dependent development in a single wet pine savannah at the southern extent of *W. smithii*'s range in north Florida (30°N). We then consider density-dependent development at other localities along the Gulf Coast, at low elevations in North Carolina, and at higher latitudes and altitudes. Finally, we shall argue that the degree of density-dependent selection is measured by the concentration of *W. smithii* per unit resource and that this concentration declines continually with latitude throughout the range of this species.

Phenology of *W. smithii* and Its Host Leaves in North Florida

At Wilma in north Florida, pupation among the 1977–1978 overwintering generation of *W. smithii* (Figure 4.1A) commenced in mid-March, peaked the first week in April, and declined but continued thereafter. Starting in mid-April, small numbers of first instars appeared in the overwintered leaves. The first summer generation remained distinguishable until mid-May when the overlap in their developmental stages made it difficult to tell the two generations apart. We ceased censusing of the last year's leaves and the overwintering generation at this time. New leaves of the host plant first appeared in early to mid-April

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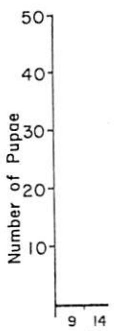

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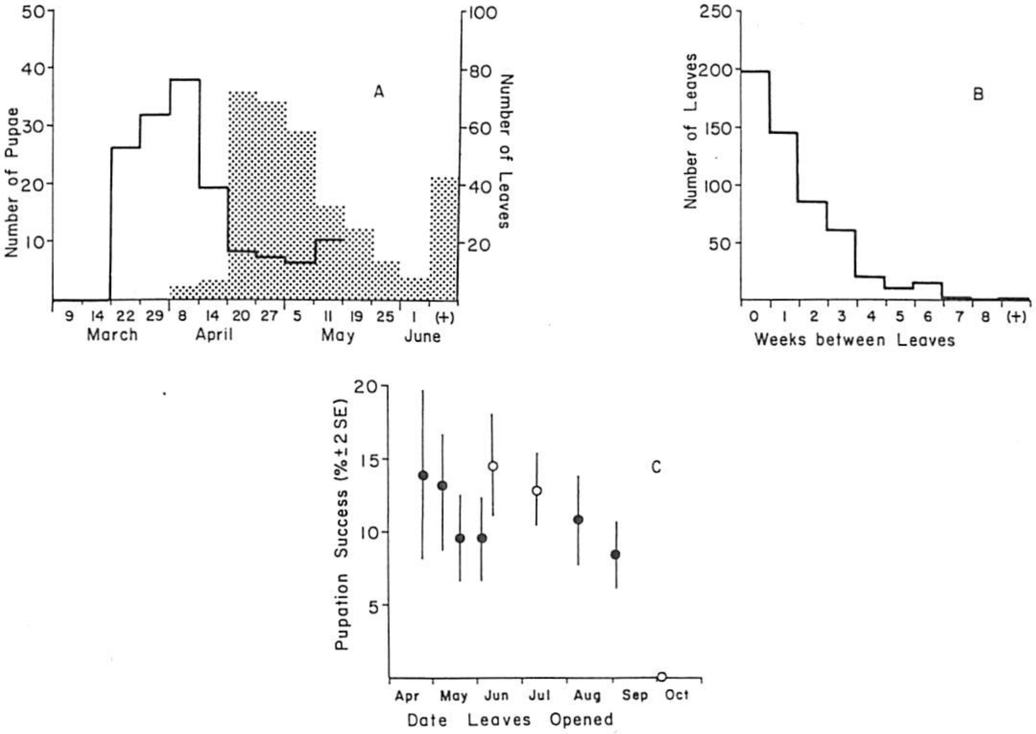

Figure 4.1. Seasonality of *W. smithii* and its host leaves in north Florida. (A) Solid line: number of *W. smithii* pupating in 55 leaves censused weekly from 14 March to 11 May, 1978. Shaded columns: number of plants putting on the first new leaf of the year among 158 plants observed weekly from 29 March until 1 June; (+) represents plants initiating their first leaf after 1 June. (B) Interval (weeks) between opening of 532 leaves on 119 plants after the opening of the first leaf from weekly observations 29 March to 1 August. If two leaves opened on the same plant during a given week, the older leaf was scored as the number of weeks since the previous leaf had opened and the younger leaf as zero weeks. (C) Pupation success among leaves opening from March to October. Pupation success is measured as (the cumulative number of pupae found in weekly censuses from the third to the eighth week after a leaf opened) ÷ (number of *W. smithii* found in the leaf four weeks after it had opened). Heterogeneity among ratios was observed ($\chi^2 = 84.47$; $p < 0.001$; Snedecor 1956, Chap. 9) and significant deviations ($p < 0.01$) from mean expected pupation are indicated by open circles.

(Figure 4.1A) and appearance of the first leaf on plants peaked in late April. The influx of eggs and resultant first instars into the new leaves was immediate and, unlike the previous year's leaves, abundant. After the initial leaves opened, further leaves opened at a median interval of 0.5 weeks (Figure 4.1B) at a rate of about 8 new leaves per 100 plant days (Bradshaw and Holzapfel 1983). *W. smithii* continued to oviposit into and to complete development in these leaves through October. Pupation success averaged 8–15% in leaves opening between April and September but no larvae pupated in leaves that opened after September (Figure 4.1C). Since *W. smithii* diapause as larvae, the decline in pupation late in the season probably resulted from larvae entering diapause as a consequence of declining photoperiod (Bradshaw and Lounibos 1972, 1977; Evans and Brust 1972), food (Istock et al. 1975), or both. These observations suggest (1) that the growing season for *W. smithii* in north Florida is about 7.5 months long, (2) that *W. smithii* emerge about 1–3 weeks before new habitats become available, (3) that new leaves open rapidly and continually once a plant starts producing leaves, and (4) that *W. smithii* readily occupy and develop in these leaves as they appear.

Development During the Summer

Biomass Accumulation

The above observations indicate that in the spring when suitable habitats appear, they are rapidly exploited by an abundance of previously emerged adults. Do these adults saturate the resources and, if so, do their offspring produce sufficient numbers of fertile adults to maintain this saturation throughout the year? To answer this question, we set up an experiment to determine whether leaves could sustain either greater mosquito biomass or pupal production than was already being utilized by the resident mosquito population. On nine separate dates (5 May, 11 May, 19 May, 2 June, 6 June, 10 July, 8 August, 2 September, and 3 October) we selected recently opened leaves on a number of plants and added either (1) 100 first instar larvae "recruited" the same day from local pitcher plant leaves or (2) no mosquitoes but only the pitcher plant water equivalent to that required for transferring 100 first instar larvae. We censused each leaf one at a time in the field, commencing the third week after the experiment was started and continuing weekly through the eighth week. At each census, we counted and removed pupae, counted the number of larvae in each instar, and returned the larvae immediately to the leaf. We determined biomass by ascribing to each individual the mean dry weight for its respective stage of development (Bradshaw 1983b). At the conclusion of each experiment, we harvested the leaves destructively, measured the volume by filling each empty leaf to capacity with water and emptying it into a graduated cylinder, and quantified prey capture by the leaf as the number of head capsules of prey present in the base of the leaf. Among experiments started 5 and 11 May, the number of leaves was not as great as in the other experiments and there was considerable overlap in opening dates of the constituent leaves; consequently, we combined these two treatments to give eight starting dates and two experimental densities totalling, at each density, 76 leaves, which remained intact throughout the duration of their respective treatments.

As a measure of biomass sustained by each leaf, we calculated the biomass of larvae remaining in a leaf on the 8th week and added to it the mass of the cumulative sum of pupae produced up through the 8th week. We found that biomass was positively correlated with prey capture by the host leaf (Figure 4.2A). ANCOVA revealed heterogeneous regression coefficients ($F_{1,148} = 5.50$; $P < 0.05$) between leaves that received 0 or 100 larvae so that it was not realistic

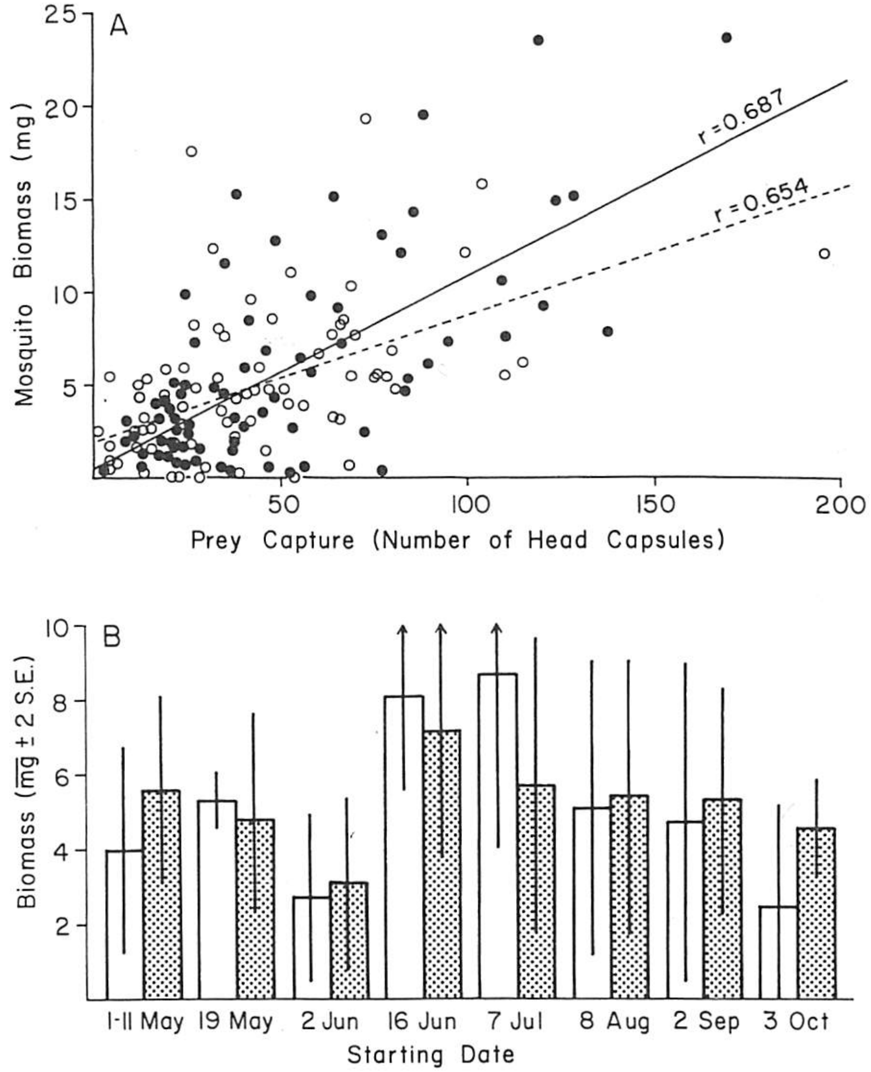

Figure 4.2. Biomass of *W. smithii* supported by leaves of *S. purpurea* in north Florida. (A) Comparison of mean aestival biomass accumulated in leaves to which either zero (——, (●) or 100 (——, (○) supplemental larvae had been added. Both correlations are very highly significant ($p < 0.001$). (B) Comparison of mean aestival biomass accumulated in leaves to which either zero (open columns) or 100 (shaded columns) larvae had been added in experiments starting at eight different times. All leaves had opened during the previous week except 1–11 May when leaves may have been open for up to 2 weeks at the start of the experiment.

to test for differences between adjusted means. If we ignored the correlation between biomass and prey capture, two-way ANOVA (Figure 4.2B) of biomass with number added and starting date as treatments revealed no heterogeneity among cells ($F_{15,136} = 1.28$; $P > 0.05$). When, despite apparent heterogeneity among cells, we examined the least-squares ANOVA, we observed a barely "significant" effect of time of year ($F_{7,136} = 2.17$; $p < 0.05$) but no significant effect of *W. smithii* added ($F_{1,136} = 0.05$; $p >> 0.05$) or interaction between these treatments ($F_{7,136} = 0.53$; $p > 0.05$). Both an examination of the pattern of points about the regression lines in Figure 4.2A and the results of the ANOVA in Figure 4.2B provide no evidence that more biomass is potentially sustainable by leaves than is already being produced. Thus, leaves are, on the average, saturated with mosquito biomass throughout the growing season.

Pupation Success

The accumulation of biomass has little significance if this accumulation does not result in adult emergence. As an index of adult emergence, we counted the number of pupae in our weekly censuses in the convergence experiment above and expressed pupation success as the cumulative sum of pupae through week

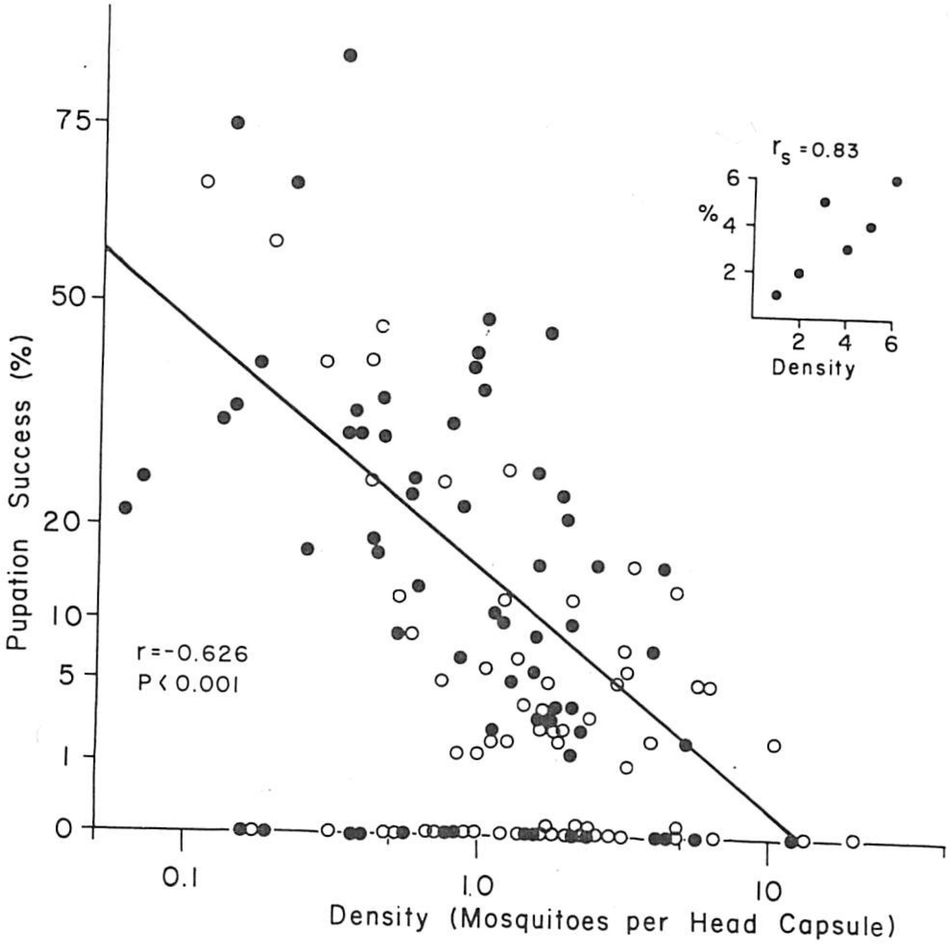

Figure 4.3. Cumulative pupation success for experiments illustrated in Figure 4.2 (omitting those started 3 October). Density is measured as *W. smithii* per head capsule of prey captured by the host leaf. (●), Leaves to which no supplemental larvae were added; (○), leaves to which 100 first instars were added. The solid line plots the regression when zero percent pupation is omitted. Inset: rank correlation between percentage of leaves sustaining no pupation (%) and density.

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8 (after pupation had declined) divided by the number of mosquitoes (larvae +pupae) present on week 4 (by which time few new first instars were appearing in leaves and few larvae had started to die). In our analyses, we omitted consideration of the experiment started in October since we ascribed the absence of pupae to dormancy, not limitations of available resources. Since pupation success is essentially a percentage datum, we used an angular transformation prior to any analysis. Figure 4.3 shows that pupation success was inversely correlated with density. The addition of 100 larvae to leaves as described above resulted in a decline in pupation success (ANCOVA: regression coefficients, $F_{1,127} = 0.04$; $p > 0.05$; adjusted means, $F_{1,128} = 5.55$; $p < 0.05$). If the leaves in which there were no pupae are deleted from the ANCOVA, however, then the treatment effects do not differ (ANCOVA: slopes, $F_{1,86} = 0.16$, $p > 0.05$; adjusted means, $F_{1,87} = 1.05$, $p > 0.05$). Of the 64 leaves receiving 100 larvae, 15 failed to produce pupae and, of the 68 leaves receiving no supplemental larvae, 25 failed to produce pupae but these numbers are not significantly different ($\chi^2 = 3.14$; $p > 0.05$). To see if there was a density-dependent component to the lack of pupation in these leaves, we divided densities into six intervals (larvae per unit prey = 0.25 or less, 0.25–0.50, 0.51–1.00, 1.1–2.0, 2.1–4.0, and 4.1 or greater). Percentage of leaves not producing any pupae within each interval was then positively correlated with density interval ($r_s = 0.829$; $p = 0.029$). Thus, both the probability of producing any pupae as well as the incidence of pupation in productive leaves show a close correlation with density. These results support the previous general conclusion; namely, that there appear to be little or no resources available to convert into additional mosquito biomass or pupae during the aestival growing season.

Overwintering Ecology

The overwintering population of *W. smithii* in north Florida is comprised entirely of larvae in the diapausing (fourth) or in earlier instars (Bradshaw and Lounibos 1977; Bradshaw and Holzapfel 1983; Bradshaw 1983a). It is this population of larvae from which adults are recruited in the spring. In this section, we describe the establishment of the overwintering population and then factors that affect its survivorship and eventual pupation success.

Among the leaves in the convergence experiment to which no supplemental larvae were added, those that opened during the week prior to 3 October produced no pupae and those that opened during the week prior to 2 September produced pupae from 27 September to 24 October (Figure 4.4A) but none thereafter. We attribute the complete lack of pupation during November and December to the combined effects of density and the shortening day length that induce larval diapause. These larvae thus represent members of the population destined to overwinter in the leaves and not pupate until the following spring.

To see to what extent density affects the age structure of larvae at this time of year, we regressed mean age (average instar number) on density (*W. smithii* per prey captured by the host leaf). In this chapter, we shall be concerned with

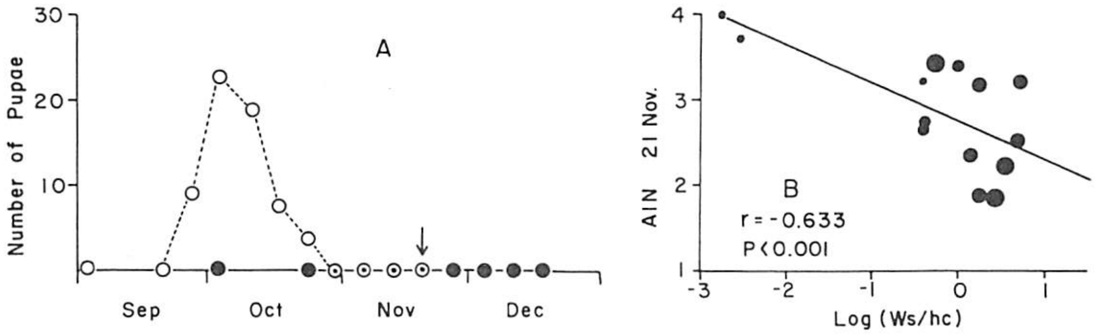

Figure 4.4. Autumnal development of *W. smithii* in north Florida in leaves to which no supplemental larvae had been added. (A) Pupation in leaves that opened on or during the week prior to 2 September (○) or 3 October (●). The latter produced no pupae. (B) Dependence of age structure (AIN, average instar number) on density measured as *W. smithii* per head capsule (*Ws/hc*) on 21 November (arrow in A) in the combined census of leaves shown in A. The number of larvae in each leaf is proportional to dot size: 1–10, 11–25, 26–50; >50 larvae.

what is happening to populations of mosquitoes, not populations of habitats. It is density that is encountered by the average individual that is important. This density is not necessarily that which prevails in the average habitat. Consequently, in calculating regressions and correlations, we summed the sums of squares and cross products over individuals. Because individuals in the same leaf may not be considered independent observations, we made error estimates and tested for significance using the number of leaves to generate degrees of freedom. After summing over individuals, there was a negative correlation between average instar number and density in leaves (Figure 4.4B). Thus, density at the end of the growing season has a depressing effect on the age structure of the overwintering population. This result is especially significant since pupation success in the spring is positively correlated with the average instar number of the overwintering population (Bradshaw andHolzapfel 1983).

To examine the effects of autumnal resource levels and mosquito densities further, we marked leaves that had opened from late October to mid-November and on 21 November added 25, 50, and 100 *W. smithii* recruited on the same day from nearby leaves, 50 or 100 ants (which constitute the main prey captured by *S. purpurea* at Wilma) from a nearby anthill, or nothing (control). We censused these leaves from 30 January through 6 February to determine the overwintering population size and then weekly from 9 March through 27 April. Figure 4.5 shows that pupation success in the spring was adversely affected by increased larval density and greatly enhanced by increased resources the previous fall.

Once established, at least two further factors affected survivorship and pupation success of overwintering larvae: (1) flooding and flushing of leaves by winter and spring rains, and (2) predation of host leaves by the noctuid moth, *Exyra* sp. These effects are illustrated by comparing pupation success in 97

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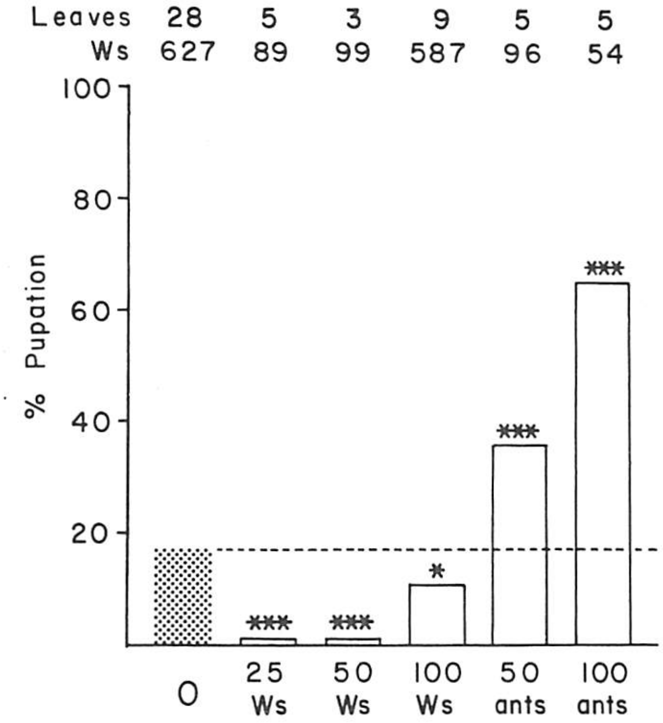

Figure 4.5. Percentage pupation during April and May in leaves that received nothing (0), 25, 50, or 100 first instar *W. smithii* (*Ws*) or 50 or 100 ants (predominant prey of *S. purpurea*) the previous November. χ^2 tests the null hypothesis that percentage pupation is the same in all treatments as in the control (0, screen and dashed line); significance is denoted by $*p < 0.05$; $***p < 0.001$.

leaves marked in November, 1977, and followed through the ensuing winter and spring (Table 4.1). Hereafter, we define the overwintering population as those individuals we found by censusing leaves on 30 January and 6 February, 1978. In the winter, these 97 leaves contained 1974 larvae of which only 8.3% pupated by 27 April. Moths ate three of these leaves; all filled with frass and never produced any pupae. On 6 and 9 March, heavy rains flooded 39 of the leaves while 56 remained above water on these dates. Flooding resulted in a precipitous decline in *W. smithii* populations. Survivorship through the spring was 30.7% in unflooded leaves and only 8.8% in flooded leaves. Among survivors of the flooding, however, pupation success was the same as among individuals in leaves that had not flooded. Thus, flooding and moth predation have a substantial effect on survivorship of the overwintering population but mosquitoes that survive flooding do not appear either to suffer adverse consequences as a result of the flooding or to obtain relief from any density-dependent factors that affect them.

Taken together, these results and observations show that among larvae that survive the hazards of flooding and habitat destruction, overwintering density is a substantial determinant of eventual pupation success. This density de-

Table 4.1 Survivorship and pupation of overwintering *W. smithii* in north Florida

	Number of leaves	Larval population 30 Jan–6 Feb	Percent surviving or pupating by 27 April	Percent total population that pupated	Percent post-flood survivors that pupated
All *W. smithii*	97[a]	1974	18.9	8.3	22.9
W. smithii in nonflooded leaves	56	922	30.7	14.5	23.0
W. smithii in flooded leaves	39	1024	8.8	2.9	22.5
W. smithii in moth-eaten leaves	3	28	0.0	0.0	—

[a] Sum of individual leaves = 98 since 1 leaf was both moth-eaten and flooded.

pendence is influenced by the age structure of the previous fall; these effects may then be reinforced or relaxed by an increase in density or resources, respectively. Density-dependent age structure in the autumnal population is in turn the final manifestation of density-dependent development, which has continued from the first summer generation. Thus, at Wilma in north Florida, *W. smithii* encounter density-dependent constraints year round; not even diapause provides an "escape in time" from the exigencies of resource depletion and density-dependent limitations to development.

Geographical Variation of Density-Dependent Development

The results and observations of the previous section indicate that resources and consequential density dependence at Wilma in north Florida may be measured by some function of prey captured by host leaves. In this section, we address two extensions of this conclusion. First, we ask to what extent prey capture is a reasonable measure of resource availability at other localities and then examine variation in resource-dependent densities encountered by *W. smithii* throughout its range from the Gulf of Mexico to northern Manitoba (30–54°N latitude). To answer these questions, we analyze the interaction between density and age structure of the overwintering population. We chose this population as our basis for comparison because *W. smithii* diapause exclusively as larvae; 100% of the population is thereby confined to leaves during the winter and spring when they may be readily sampled.

From the Gulf of Mexico north through low elevations in the Carolinas, *W. smithii* diapause primarily in the fourth instar. As at Wilma, the average instar number of the overwintering population is somewhere around three, not four (Bradshaw and Lounibos 1977), and is inversely correlated with mosquitoes per head capsule of prey captured by the host leaf (Bradshaw and Holzapfel, 1983).

At northern latitudes or higher altitudes, *W. smithii* diapause as third instars and 98–100% of the population overwinter in this stage (Bradshaw and Lounibos 1977). Istock et al. (1976), running convergence experiments similar to those we ran at Wilma, concluded that *W. smithii* in New York State experience density-dependent development in the spring and fall but escape these constraints during the summer. We have already reported (Bradshaw and Holzapfel 1983) that at the same latitude, average instar number of *W. smithii* during the spring is inversely correlated with mosquitoes per leaf. We now provide data from vernal censuses at six northern and one high elevation, southern locality where *W. smithii* diapause as third instars. As above, since we are interested in the conditions affecting the average individual, not conditions prevailing in the average habitat, we summed regressions over individuals but tested for significance using degrees of freedom based on the number of leaves sampled. We found a significant inverse correlation between average instar number and density (Figure 4.6) at four of the six northern localities but not the high-elevation, southern locality.

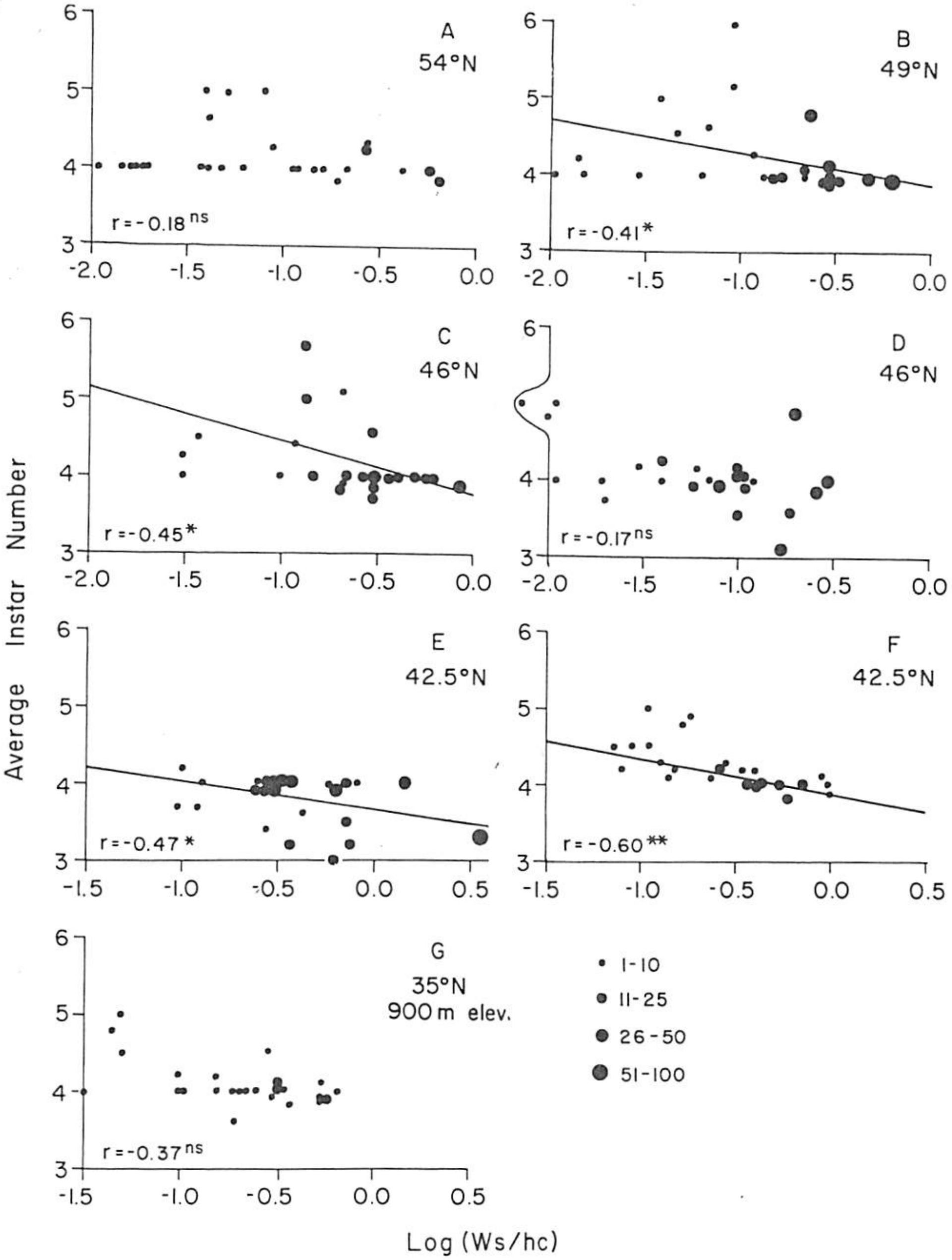

Figure 4.6. Dependence of age structure of vernal populations of *W. smithii* on density at northern (A–F) or high-elevation, southern (G) localities. Pupae are scored as 5 and pupal exuviae (adults) as 6. Density is measured as *W. smithii* per head capsule of prey in the leaf (*Ws/hc*). (A) Northern Manitoba, 2 July, 1979; (B) northern Ontario, 29 June, 1979; (C) northern Wisconsin, 28 June, 1979; (D) northern Maine, 22 June, 1979; (E) southern Michigan, 17 May, 1976; (F) eastern Massachusetts, 12 June, 1976; (G) western North Carolina, 31 May, 1976. Significance of regressions is designated by NS, $p > 0.05$; $^*p < 0.05$; $^{**}p < 0.01$.

A clue to the reason for the lack of correlation between development and density in these three localities emerges from consideration of relative resource availability. We continue to be interested in conditions experienced by the average individual at a locality. Consequently, we calculated the mean intraspecific crowding of *W. smithii* per unit resource (Hurlbert 1978):

$$\frac{\overset{*}{Ws}}{hc} = \frac{\sum \dfrac{Ws_i\,(Ws_i - 1)}{hc_i}}{\sum Ws_i}$$

where Ws_i is the number of *W. smithii* in the *i*th leaf and hc_i is the number of head capsules of prey captured by that leaf. When we plotted census data from four localities along the Gulf Coast, two from a low elevation in North Carolina and nine from more northern localities, we found an inverse correlation between mean crowding per unit resource of the overwintering generation and latitude (Figure 4.7). The average of three censuses at two localities at high elevation in North Carolina produced a mean crowding per unit resource equivalent to about 43°N latitude (Figure 4.7, open circles). The three regressions in Figure 4.6 that did not yield significant correlations between average instar number and density represent the two lowest mean crowdings per unit resource along the latitudinal gradient and the lowest of the three censuses at high elevation in North Carolina. Thus, when resources become sufficiently abundant, there no longer is a significant correlation between development and density.

A more complete manifestation of this trend may be seen by examining the correlation coefficients relating average instar number to density (number of *W. smithii* per head capsule of prey) along this same gradient. We have such data for 13 of the 17 separate localities shown in Figure 4.7. Because the censuses at southern localities were taken in midwinter and at northern and high elevation localities in the spring at different times of different years, we used rank rather than least-squares correlation. As seen in the inset to Figure 4.7, there was a very close correlation ($r_s = 0.84$; $p < 0.01$) between the correlation coefficient relating age structure to density and the mean crowding per unit resource at that locality. This result means that as the number of individuals that must share a given resource increases, their development more clearly becomes dependent on the quantity of those resources.

Conclusions

Many of our analyses have considered what is happening to the average individual in a population. We believe that this type of analysis is, unfortunately, greatly underutilized. It is far easier to sum regressions, ANOVA, and other statistics over samples, be they pitcher-plant leaves or quadrats, particularly now that canned computer programs make for statistics with fewer tears.

There are two main considerations here. Summing over individuals avoids giving undue emphasis to aberrant subhabitats with few larvae in them but it

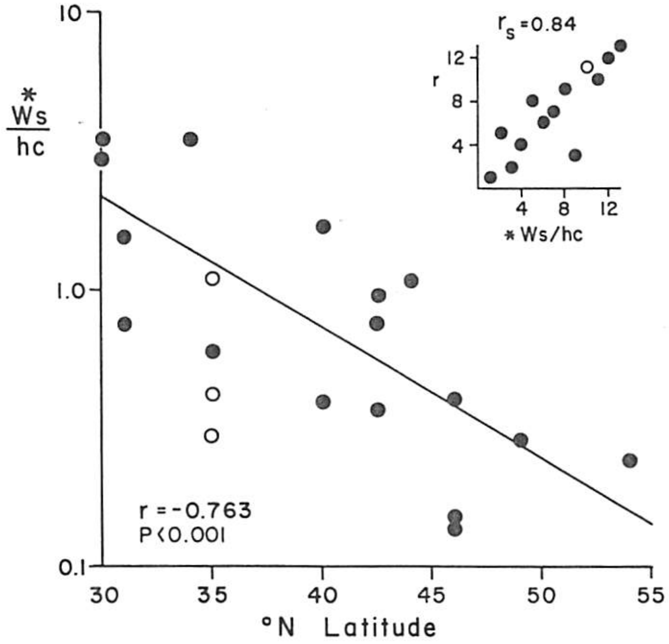

Figure 4.7. Resource availability to the average larva over the range of *W. smithii*. The regression was run on the latitudinal gradient (●); the mean of three censuses at 900 m in North Carolina (○) intercepts the regression line at 43°N. Resource availability is measured as mean crowding of *W. smithii* per head capsule of prey in the leaf during January and February at 30–35°N and during May to July at high elevation at 35°N and all points 40–54°N. Inset: rank correlation between the correlation coefficient (*r*) for the regression of average instar number on density as shown in Figure 4.6 and resource availability (**Ws/hc*), for the 13 localities for which we have such data.

does give much more weight to the subhabitats with many larvae. One's uneasiness here stems from the apprehension that somehow something important is being missed by deemphasizing what is going on in the peripheral or sparsely populated habitats. The underlying assumption in the former analysis is that all habitats and all individuals are equally important. This assumption and uneasiness loses track of two points. First, one is generally interested in what is happening to a population of organisms, not a population of habitats, and second, a leaf with more larvae produces absolutely more pupae than a leaf with fewer larvae. What happens within the more heavily populated leaf will have a greater impact on which mosquitoes will comprise the breeding population than what happens within the more sparsely populated leaf. In Figure 4.3 we presented analyses both including leaves that produced no pupae and excluding these leaves. We believe the latter to be the more important comparison as, in this case, we are dealing with the subset of mosquitoes that will produce the next generation. The proximal causes of developmental failure in leaves not producing pupae has little adaptive significance. The important selective forces are those impinging upon and overcome or exploited by the mosquitoes that eventually reproduce. In the best of all possible worlds, density-dependent effects in the parental generation might then be summed over offspring produced, not the parents themselves, and certainly not over all the subhabitats in which the parental generation might have lived.

Flooding and predation of leaves are severe causes of mortality (Table 4.1). Along with freezing further north, these aspects of seasonal harshness probably constitute the greatest sources of density-independent mortality among the overwintering generation. Yet, unlike the scenario envisioned by Roughgarden (1971), these aspects of seasonal harshness do not relieve the effects of density-dependent constraints in either the overwintering (Table 4.1) or subsequent (Figure 4.2) generation. In *W. smithii,* density dependence exerts its effects in the fall during the establishment of the overwintering population. Once resources have been depleted during the fall and have either been taken up by the plant or incorporated into mosquito bodies, there are no resources remaining for survivors of environmental harshness. Unless density-independent mortality occurs before the density-dependent bottleneck during the life cycle of an organism, it is not necessarily going to relieve that density-dependence. The very important point is that to assess the relative importance of density-dependent versus independent causes of mortality as selective factors molding life history traits of a species, the timing of events must be taken into account.

We are quick to point out that the correlation shown in Figure 4.7 is clearly not perfect, even though measurements were always made on the overwintering generation when 100% of the population was confined to the leaves. Local conditions vary in both space and time; these variations affect measurements of mean crowding as well as measurements of density-dependence using regressions of age, biomass, pupation success, or other life history traits on density. The perception of a gradient depends on the number of observations along the gradient. The perception of geographical variation in density-dependent development among populations of *W. smithii* might have appeared very different had we sampled only two or three localities drawn at random from those shown in Figures 4.6 and 4.7. The literature is full of examples of studies using two points, one insular, one continental; one southern, one northern; one low elevation, one high elevation; one wet, one dry, and so on; but, apart from the problem of controls, two points do not indicate whether the response to the gradient is linear or nonlinear, continuous or discontinuous, or provide an idea of within- versus between-region variation. If the gradient is carefully chosen, one usually wants to know the quantitative relationship between the environmental gradient and the organismic or populational response. Three or even five points are precious few upon which to base a correlation, a regression, or an error estimate; for a two-dimensional gradient (e.g., latitude and altitude) they are simply inadequate.

In the case of *W. smithii*, we concluded that there is a continual, linear decline in density-dependent juvenile mortality from the Gulf of Mexico to northern Canada. To the best of our knowledge, *W. smithii* is the first case for which this statement can be made for the entire latitudinal range of a species. The average mean crowding per unit resource (Figure 4.7) from three censuses of two localities at 900 m in North Carolina intercepts the regression line at 43°N, providing an altitude/latitude conversion factor of 113 m/°N. This value falls between the altitude/latitude conversion factors of 99 m/°N for length of growing season (mean number of frost-free days) over this same range and 122m/°N for Hopkins ''bioclimatic law'' relating phenology to geography (Bradshaw 1976). More studies of the present kind will tell us whether these comparative values are coincidence or represent a general ecogeographical pattern.

Only when one understands the selective forces actually impinging upon populations of a species will one be able to make predictions about the consequesnces of this selection on life history. The quantitative perception of the selection gradient will then provide the basis for quantitative testing of our models. Initially, it makes no difference whether these populations exist in the laboratory or are spread over several continents. Ultimately, however, scholars of life history evolution will have to formulate hypotheses and to identify species and selection gradients that permit us to test theory in the crucible of natural, not artificial, selection.

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