Temperature-Dependent Ovarian Development in *Coccinella septempunctata* (Coleoptera: Coccinellidae)

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ABSTRACT A linear relationship was observed between ovarian developmental rate in *Coccinella septempunctata* L. and five constant temperatures (14–30°C). The estimated lower temperature threshold for follicle development was 13°C. Development of follicles to maturity (egg chorionation) required 833 degree-hours (°H > 13°C). A five-stage rating system to describe ovarian development in *C. septempunctata* was based on the length of the terminal follicle, number and shape of developing follicles in each ovariole, and presence of yellow color in the terminal oöcyte. In 60–70% of females at each temperature, follicle development in relation to female age (time) followed a sigmoidal pattern. This sigmoidal curve had three phases, a previtellogenic or lag phase, a vitellogenic development phase characterized by rapid increase in follicle length, and a postvitellogenic or chorionization phase characterized by a constant follicle length. An equation to predict either the proportion of gravid females on the basis of degree-hour accumulations, or the degree-hour accumulations on the basis of proportion of gravid females, was developed.

KEY WORDS *Coccinella septempunctata,* ovarian development, age-grading

REPRODUCTIVE ORGANS OF female insects may undergo cyclic physiological and morphological changes through time. These changes, particularly morphological changes in ovaries, have been used to determine population age structure and phenology for several insect species, including the cyclorrhaphous Diptera (Detinova 1962, Krafsur 1985, Spradbery et al. 1991, Wall et al. 1991) and selected cocrophagous scarab beetles (Tyndale-Biscoe 1978, Tyndale-Biscoe et al. 1981, Lumbreras et al. 1991).

Ovarian methods of determining population age structure are based on the degree of egg development and the number of completed ovarian cycles. The remnants of previous ovipositions in Diptera (i.e., disintegrated nurse cells and follicular epithelium in the ovarioles) are used as criteria to determine the number of completed ovarian cycles and to distinguish nulliparous (females that have not oviposited) from parous females (females that have laid at least one batch of eggs) (Tyndale-Biscoe 1984). These characteristics provide a measure of reproductive age (i.e., number of gonotrophic cycles completed and stage of oöcyte development) of the adult female. The reliability of using ovarian stages to age-grade females depends on an understanding of the relationship between environmental factors, particularly temperature and food supply, and ovarian developmental rates.

Ovarian development rates may be useful in understanding predator–prey population dynamics, e.g., predicting changes in the age structure and phenology of insect predators through time and assessing both their efficiency and synchronization with prey populations. Despite their potential for forecasting temporal events in insect populations (pests and beneficials), ovarian methods have not yet been used to determine age structures of predators.

Changes in ovarian development have been used to determine diapause incidence (i.e., diapause is characterized by undifferentiated ovaries immersed in hypertrophied fat body) in a number of coccinellid species. Also, histological studies of ovulation and corpus luteum formation during ovarian development of various coccinellid species, namely in *Coccinella septempunctata* L. (Singh & Nayar 1961), in *Chilocorus bipustulatus* L. (Vaghina 1974), in *Cryptolaemus montrouzieri* Mulsant (Yermolenko 1963), and in *Epilachna vigintioctopunctata* F. (Kulshrestha 1968) showed that, following ovulation of the terminal oöcyte, the follicular epithelium layer disintegrates to form a tubular and colorless corpus luteum. This structure gradually undergoes changes, including contraction in length and accumulation of yellow pigmentation, which are accompanied by thickening of the tunica propria (an elastic membrane inside the outer ovariole sheath). If the rate of these physiological changes is known, in conjunction with rates of oogenesis, the reproductive age structure of coccinellid populations can be determined and converted to calendar dates.

As a first step toward developing methods to determine age structures of predacious coccinellid

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Table 1. Experimental design for determining rate of ovarian development of *C. septempunctata*; photoperiod of 18:6 (L:D) h; prey, *A. pisum*

Temp, °C	No. mating pairs	Max age, h[a]	Age interval at each sample, h[b]
14	50	720	72
18	30	288	48
22	25	120	24
26	35	84	12
30	25	60	12

[a] Maximum age reached by the last five pairs sampled.
[b] Five females were sampled at each interval, frozen, and later dissected.

species, we described and quantified the ovarian development stages in *C. septempunctata* and determined the relationship between temperature and rates of ovarian development of *C. septempunctata*.

Materials and Methods

Adult *C. septempunctata* were obtained from the Beneficial Insects Introduction Research Laboratory (BIIRL), USDA–ARS, at Newark, Del., in 1990 and 1991. These adults were collected in New Castle County, Delaware, by BIIRL personnel and shipped to Ames, Iowa. Adult *C. septempunctata* used in the first replication were collected from alfalfa and clover fields during summer of 1990. The offspring (F_1 adults) of field-collected beetles were used in replications two and three. Rearing of one laboratory generation for replications two and three was required because of heavy parasitization of field-collected adults by the parasitoid, *Dinocampus coccinellae* Shrank, and adults obtained for the second replication had been collected during winter (December 1990) from hibernation sites and were in diapause.

For the first replication, adults were set up into 165 pairs on the day received and assigned to five constant temperatures, at a photoperiod of 18:6 (L:D) h (Table 1) in tabletop environmental chambers (Percival, Boone, Iowa). Each pair was held in a paper can (8.6-cm diameter, 4.8-cm height) (Fonda, Union, N.J.), covered with white organdy. The pairs were provided with a standard diet consisting of a 1:1 mixture of honey and Wheast (Qualcepts, Minneapolis, Minn.), water, and an excess daily supply of pea aphids, *Acyrthosiphon pisum* (Harris). Dead aphids were removed, and oviposition was recorded daily. Pairs were sampled at specified time intervals at each temperature (Table 1); females from sampled pairs were frozen for later dissection.

To obtain F_1 adults for the second and third replications, pairs of field-collected adults were maintained at 26 ± 1°C, at a photoperiod of 18:6 (L:D) h. Beetles were provided with food and water as in the first replication. Eggs from each pair were incubated at 26 ± 1°C, 18:6 (L:D) h; larvae were reared on *A. pisum*. First-generation adults were sexed and paired on the day of eclosion and placed at five temperatures as was done for the first replication (Table 1). Each member of a pair came from a different female parent. The same sampling interval and maximum sampling age was used (Table 1).

Dissections of sampled females were done in saline solution using an Olympus SZH stereomicroscope at 37.5× magnification. The saline solution was prepared by dissolving 6.8 g NaCl, 1.0 g KCl, 0.2 g $CaCl_2$, 0.1 g $MgCl_2$, 0.2 g NaH_2PO_4, and 12.87 g glucose in 1 liter of distilled water (Jones 1977). The stage of ovarian development was determined by measuring the length and width of six terminal (primary) follicles, three from each ovary. In addition, the shapes of the follicles (spherical, slightly oval, distinctly ellipsoidal, and elongate), the level of yolk deposition (none, initial accumulation indicated by appearance of yellow color, and full yellow coloration of the oöcyte), and the length of the germarium and total length of each of the six ovarioles were recorded. If present, subterminal or secondary follicles were also measured. Accumulated fat body was also examined and qualitatively rated as absent, low, or high.

To describe the relationship between temperature and rate of *C. septempunctata* ovarian development, ages of dissected females were converted to follicle developmental rates (h^{-1}), which were then plotted against temperature. Females that were parasitized or did not develop follicles were not included in the analyses.

The proportion of females that became gravid (females with mature eggs in their ovaries), P, in response to temperature, T, and time, t, was determined, i.e., $P = f(T, t)$. The effects of temperature (T) and time (t) were combined into degree-hours ($°H_b$), a physiological time scale that expresses accumulation of heat above a hypothetical minimal ovarian/follicle developmental threshold (b); therefore, $P = f(°H_b)$.

Data were analyzed by using analysis of variance (ANOVA) procedures of general linear models (PROC GLM) and regressions (PROC REG) in SAS (SAS Institute 1985). To quantify the relationship between proportion of gravid females and degree-hour accumulations, probit analysis using the SAS PROC PROBIT (SAS Institute 1985) was used. For the proportion gravid females, degree-hour accumulation relationship, becoming gravid is assumed to be an all-or-nothing response to differing degree-hour accumulations (stimulus for follicle growth). The significance level of $\alpha = 0.10$ was used for goodness-of-fit chi-square test between observed and predicted proportions of gravid females.

Results

Ovarian Development Rating System. Ovarian developmental stages in *C. septempunctata* were

Table 2. Stages of ovarian development for *C. septempunctata* based on follicle size, shape, and intensity of yellow coloration

Ovarian stage	Size of primary follicle			Shape of primary follicle	Yellow color intensity in primary follicle[a]	No. follicles in an ovariole	Vitellogenesis stage	n^b
	Length[c]	Width[d]	Volume[e]					
0	<1.2	<0.02	<0.01	Undifferentiated	None	0	Previtellognic	47
1	1.6– 2.8	0.8–1.4	0.01– 0.29	Spherical	None	1	Previtellogenic	201
2	3.2– 5.6	1.6–2.7	0.45– 2.1	Slightly oval	Hint of yellow	2	Vitellogenic	101
3	6.0–10	2.8–5.2	2.4 –14.1	Distinctly ellipsoidal	Completely yellow	3	Vitellogenic	24
4	>10.0	>5.2	>14.1	Ellipsoidal and elongate	Yellow and chorionated	3	Postvitellogenic	103

[a] Coloration was used as an index of yolk deposition in the primary oöcyte. Color copies of photographs depicting stages of ovarian development are available on request from M.W.P.
[b] Number of females examined.
[c] Follicle length, length × 10^{-1} mm.
[d] Follicle width, width × 10^{-1} mm.
[e] Follicle volume, volume × 10^{-2} mm^3.

determined from five characteristics of the ovarioles (Table 2). These included size (length, width, and volume) of primary follicles, particularly follicle length, different shapes of primary follicles, and presence of yellow color in the primary oöcyte, which characterized different ovarian stages by distinguishing between previtellogenic and vitellogenic follicles, i.e., the intensity of yellow coloration (estimated visually) distinguished newly vitellogenic follicles from those at an advanced vitellogenic stage. The fourth characteristic was the presence of a chorion, which distinguished postvitellogenic (with mature oöcytes) from vitellogenic follicles; and finally, the number of developing follicles in each ovariole was also used to describe different stages of ovarian development. These characteristics were used to develop a five-stage rating system to describe *C. septempunctata* ovarian development. Because *C. septempunctata* females have asynchronous follicular development (i.e., gonotrophic discordance) (Phoofolo 1993), the most developmentally advanced cohorts of ovarioles were used to determine stages.

Ovaries at stage 0 consisted of undifferentiated ovarioles, 0.5–0.6 mm long. This stage was found mostly in newly eclosed adult beetles, but also in diapausing females. The ovaries at this stage were surrounded and entwined by skeins of trachea. Stage 1 was characterized by ovarioles whose primary follicles had begun to differentiate, becoming roughly spherical. The length and width of primary follicles in stage 1 ranged from 0.12 to 0.28 mm and 0.04 to 0.14 mm, respectively. The volume of stage 1 primary follicles ranged from 0.0001 to 0.0029 mm^3. Follicle volume was approximated from the formula for prolate spheroid volume, $(4/3)\pi ab^2$, where a, the major semiaxis, was equal to half the follicle length, and b, the minor semiaxis, was equal to half the follicle width. Both stage 0 and stage 1 ovarioles were translucent and whitish.

Females at stage 2 of ovarian development had ovarioles with two follicles in the vitellarium; the primary follicles ranged from 0.32 to 0.56 mm long and 0.16 to 0.27 mm wide (volume ranges from 0.0045 to 0.021 mm^3), whereas the secondary follicles varied from 0.12 to 0.24 mm long. The primary follicle became slightly oval and the secondary follicle was spherical. Vitellogenesis in the primary oöcyte began at this stage (indicated by yellow coloration), and the oöcyte contents assumed a gel-like consistency.

Stage 3 of ovarian development was characterized by three follicles in the vitellarium; the primary follicles ranged from 0.60 to 1.00 mm long and 0.28 to 0.52 mm wide (volume ranges from 0.024 to 0.14 mm^3). Secondary follicle lengths were 0.26–0.36 mm long, and tertiary follicle lengths were 0.12–0.24 mm. In addition, primary oöcytes became completely yellow (indicating the near completion of vitellogenesis) and ellipsoidal. Ovarioles at stage 4 had mature primary follicles that appeared elongated and were >1.00 mm long (mean ± SEM, 1.28 ± 0.0009 mm) and >0.52 mm wide (0.58 ± 0.0003 mm). Follicle volume was estimated to be >0.14 mm^3. Egg chorionization, a process signifying completion of vitellogenesis, occurred during this stage. This stage may have been followed by ovulation/oviposition during which time the secondary follicle became primary, ranging from 0.60 to 1.00 mm long, and tertiary follicle became secondary.

Temperature Effect on Rate of Ovarian Development. Because adult females used in the first replication were collected from the field, whereas F_1 adults were used for the second and third replications, we tested for statistical differences in developmental times of primary follicle among the three replications at all temperatures. No statistical differences among replications were observed in the ovarian growth stages when one-way ANOVA were applied (stage 1: $F = 1.61$; df = 2, 164; $P = 0.20$; stage 2: $F = 0.39$; df = 2, 58; $P = 0.39$; stage 3: $F = 1.94$; df = 2, 20; $P = 0.16$; stage 4: $F = 0.31$; df = 2, 98; $P = 0.73$).

At each temperature, the development of follicles in relation to female age followed a sigmoidal

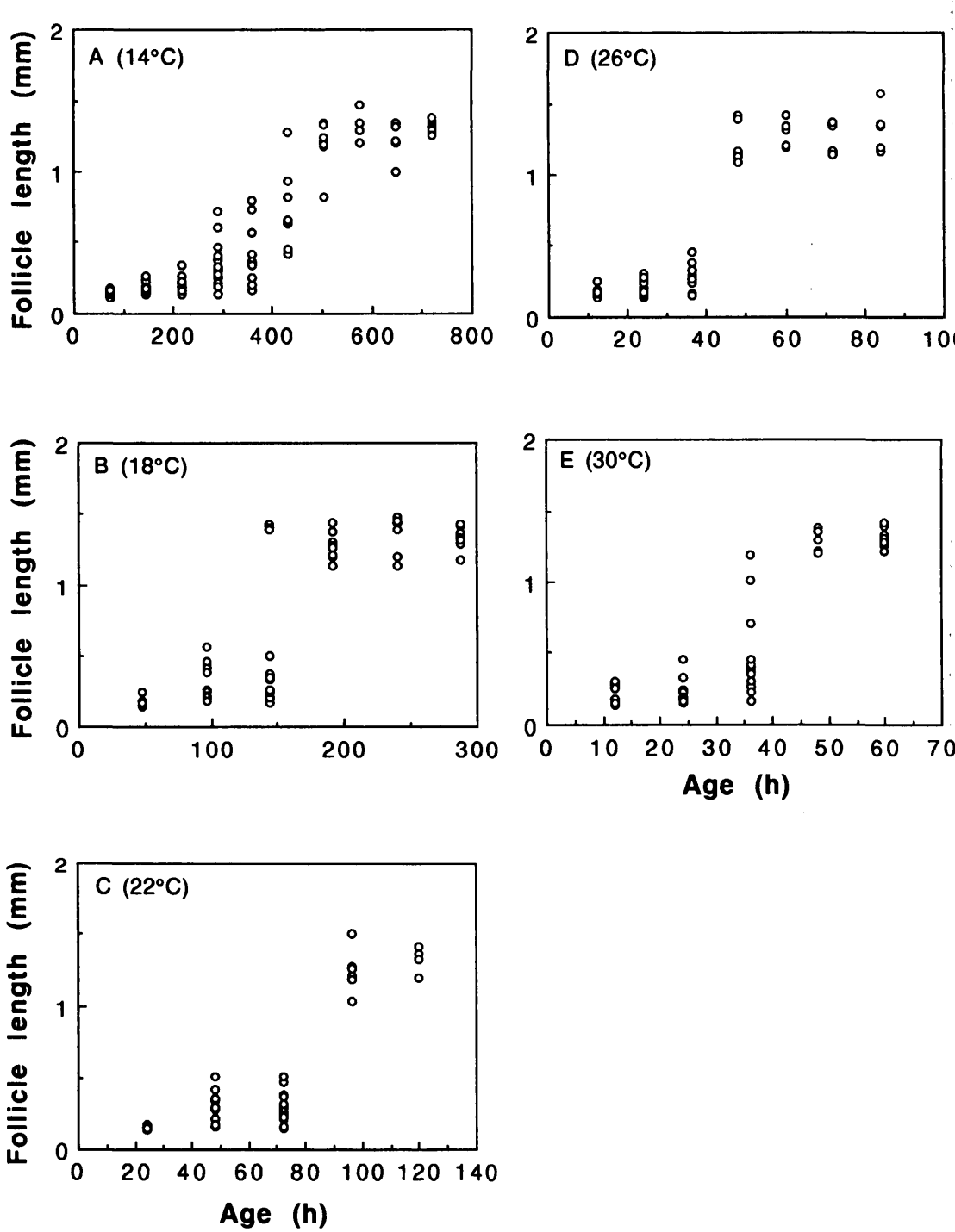

Fig. 1. Development of follicles in relation to female age at (A) 14°C, (B) 18°C, (C) 22°C, (D) 26°C, and (E) 30°C.

pattern (Fig. 1). During the previtellogenic oöcyte growth (stages 0 and 1), the length of the terminal follicle increased gradually. Once vitellogenesis started (stage 2), primary follicle length increased quite rapidly until the oocyte became mature and chorionated (stage 4). However, this sigmoidal pattern of follicle growth was not found in all females. Sixty to seventy percent of females became gravid; ovaries in the remaining females were either undifferentiated or were previtellogenic (stages 1 and

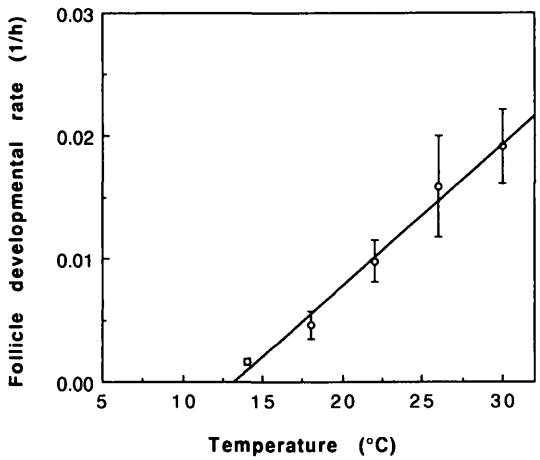

Fig. 2. Rate of stage 4 follicle development against temperature. Bars represent standard deviation.

2), indicating that they were in diapause. These diapausing females were not included in the analysis of the relationship between temperature and rate of ovarian development.

Seventy two-hour-old females at 14°C had individual ovarioles with follicles 0.150 ± 0.004 mm long. Yolk first became visible in 288-h-old females, when follicles reached ≈0.40 mm (Table 2). The youngest female age, at 14°C, at which ovarioles reached stage 4 (egg maturity) was 432 h (Fig. 1A). At 18°C, stage 1 ovarioles were found in 48-h-old females, stage 2 ovarioles in 96-h-old females, and stage 3 and stage 4 ovarioles were in 144-h-old females (Fig. 1B). Follicle growth at 22°C was faster; oöcytes matured in 96-h-old females (Fig. 1C). At 26 and 30°C, follicle growth was more rapid, oöcytes matured after 48 and 36 h, respectively (Fig. 1D and 1E).

The rate of primary follicle development (for most advanced cohorts of ovarioles) increased in direct proportion to increasing temperature (ANOVA, $F = 742.34$; df = 1, 102; $P = 0.0001$). This relationship was described by linear regression as:

$$Y = -0.01521\,(\pm 0.00094) + 0.00116\,(\pm 0.00004)x,$$
$$R^2 = 0.88$$

where Y is the rate of follicle development (h^{-1}) and x is the temperature at which the females were maintained (Fig. 2). This regression equation was used to estimate the lower temperature threshold for follicle development as 13°C. The reciprocal of the regression coefficient (slope) gave a measure of the degree-hours (the thermal constant) required to complete follicle development. Thus, 833 degree-hours were estimated for complete development of *C. septempunctata* follicles.

On the basis of the proportion of gravid females sampled at different degree-hour accumulations (Table 3), probit analysis was performed to calculate expected proportions of gravid females at various degree-hour accumulations from the linear regression equation:

$$E_g = \alpha + \beta \log_{10} {}^{\circ}H_b$$

where E_g is the expected proportion of gravid females expressed in probits, ${}^{\circ}H_b$ is accumulated degree-hours >13°C, α is the intercept, and β is the slope of regression line, both of which are estimated by the linear regression after probit transformation of the proportions of gravid females. The estimate of α ± SEM was −5.96 ± 1.2 ($\chi^2 = 79$, df = 1, $P = 0.0001$); the estimate of β ± SEM was 3.63 ± 0.43 ($\chi^2 = 71$, df = 1, $P = 0.0001$). The regression equation is:

$$E_g = -5.96 + 3.63 \log_{10} {}^{\circ}H_b.$$

This equation can be used either to predict the proportion of gravid females, when the degree-hour accumulations are known, or to predict the degree-hour accumulation, when the proportion of gravid females is known. However, from Table 3 it can be seen that, between 816 and 1,440 degree-hour accumulations, the percentage of gravid females does not increase. This suggests that the predictive use of this equation is limited to estimating percentages <60 (solid part of the curve in Fig. 3) and that percentages of gravid females >60 (if they

Table 3. Proportions of females that become gravid (stage 4) as a function of degree-hour accumulations >13°C

Degree-hours[a]	n[b]	Observed % gravid females	Expected % gravid females[c]
72	15	0	0
144	15	0	0.10
156	15	0	0.11
204	15	0	0.49
216	30	0	0.50
240	15	0	1.00
288	15	0	2.10
312	15	0	2.80
360	15	0	4.60
408	15	0	6.80
432	30	3.33	8.20
468	15	6.67	10.20
480	15	0	10.90
504	15	40.00	12.50
576	15	33.33	17.35
612	15	13.33	20.05
624	15	26.67	20.90
648	30	20.00	22.70
720	30	40.00	27.80
780	15	40.00	32.30
816	15	53.33	34.80
864	15	33.33	38.20
936	15	33.33	43.30
960	15	53.33	44.80
1,020	15	60.00	48.40
1,080	15	53.33	52.00
1,090	15	46.67	52.80
1,200	15	46.67	58.70
1,440	15	53.33	69.10

[a] Degree-hours accumulated >13°C.
[b] n, All females at five temperatures (14–30°C).
[c] Determined from the regression equation (through probit transformations to percentages).

occur) can not be predicted by the equation (as represented by the broken part of the curve).

The SAS PROBIT procedure performs a goodness-of-fit test based on the log-likelihood ratio chi-square to examine how the data agree with the predicted values. The chi-square value obtained was 32.63 (df = 27, P = 0.21). This relatively small chi-square statistic indicates that the data fit the lower (solid) part of the probit regression curve well.

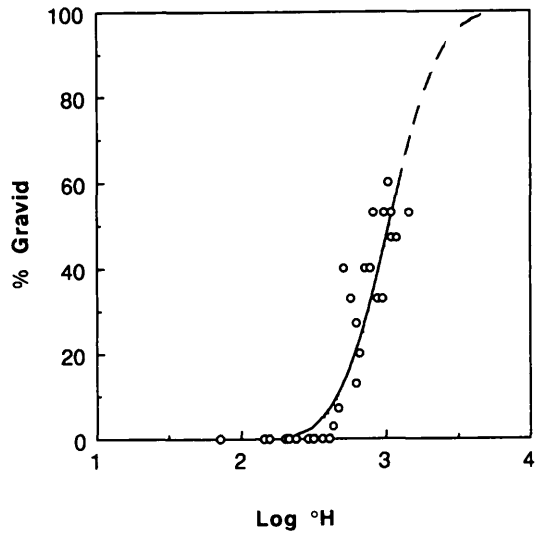

Fig. 3. Predicted relationship (from probit analysis) between proportion of females that become gravid and heat units (degree-hours) accumulated. (Circles represent observed proportions; curve represents expected proportions.)

Discussion

In *C. septempunctata,* as in all coccinellid species reported in the literature, each ovary consists of meroistic telotrophic ovarioles (Singh & Nayar 1961, Yermolenko 1963, Vaghina 1974). The ovarian development rating system developed in this study for *C. septempunctata* is based on five quantitative and qualitative ovarian characteristics. The range of size measurements for follicles in stages 1, 2, and 3 is the result of continuous growth, as well as variation in size among different follicles. The follicles in stage 4 are mature, and the range in their lengths reflects only variation in size.

Size measurements alone do not provide an adequate rating of ovarian stages because of variation, which results in overlap between stages. Other characteristics that further define ovarian stages include number of follicles, which ranges from 0 to 3, shape of developing follicles, and presence of yellow in the primary oöcytes. These characteristics also distinguish previtellogenic from vitellogenic ovarioles. The intensity of yellow color serves as an index of level of vitellogenesis. A combination of these quantitative and qualitative characteristics provides a reliable method of distinguishing between different stages of ovarian development in *C. septempunctata* (and possibly other predacious coccinellid species). Previous studies of *C. septempunctata bruckii* used only qualitative characteristics to assess stages of ovarian development (Sakurai et al. 1986).

In polytrophic ovarioles observed in Diptera, Lepidoptera, and Hymenoptera, yolk is deposited progressively, filling the growing oöcyte and replacing disintegrating nurse cells, making it possible to measure yolk deposition levels (Tyndale-Biscoe & Hughes 1969, King 1970, Telfer 1975, King & Buning 1985). In contrast to polytrophic ovarioles, yolk is deposited evenly throughout the entire oöcyte in telotrophic ovarioles (King & Buning 1985). This makes it difficult to use the quantity of yolk deposition quantitatively to characterize different ovarian growth stages in telotrophic ovarioles of *C. septempunctata.* However, yolk deposition, as indicated by intensity of yellow color in the oöcytes, in *C. septempunctata* provided a qualitative basis to distinguish previtellogenic from vitellogenic ovarioles.

The sigmoidal growth pattern in *C. septempunctata* at the five temperatures can be divided into three phases. The first phase is the previtellogenic development, the slow growth or lag phase (Fig. 1). The second phase is vitellogenic development, characterized by rapid increase in follicle length caused by yolk accumulation. The third phase is postvitellogenic development, characterized by a plateau caused by cessation of increase in follicle length (Fig. 1). This phase is accompanied by chorionization of mature oöcytes.

During vitellogenesis, the follicular epithelium that surrounds a developing oöcyte becomes highly permeable to the yolk protein precursors, produced by the fat body, which accumulate in the oöcyte (Zhai et al. 1984, Raikhel & Dhadialla 1992). During the previtellogenic phase of follicle development, all the materials (RNA, proteins, lipids, carbohydrates, and cytoplasmic organelles such as ribosomes and mitochondria) found in the follicles come from the trophic region in the germarium that are transported by way of the trophic cords connecting follicles to nurse cells (Telfer 1975; Buning 1979a,b; King & Buning 1985; Raabe 1986). The differences in sources and types of materials found in the developing oöcyte during previtellogenic and vitellogenic phases may account for differences in the rates of follicle development during the two phases.

The predictive curve relating the proportion of gravid females to degree-hour accumulations fit the data, even though the upper end of the predictive curve did not have data with which to be compared (Fig. 3). However, in a related study (Phoofolo & Obrycki 1994), we found that no more than 60% of *C. septempunctata* females from four geographic locations would oviposit during a 74-d

period at 26°C, a photoperiod of 18:6 (L:D) h, and unlimited aphid prey. Therefore, we do not expect the percentage of gravid females to increase as degree-hours increase >900 °*H*, but instead, we predict that the highest percentage of gravid females will remain at ≈60%. It is therefore, important to emphasize that the predictive equation is useful only until 800–900 degree-hours have been accumulated.

Although these findings were determined in the laboratory (where insects were supplied with abundant prey at optimal environmental conditions of long day lengths and moderate temperatures), they can serve as a basis for studying such relationships in field populations. Furthermore, the information from this experiment can be used to assess reproductive status of *C. septempunctata* females in field populations through determination of ovarian stages. Previously, Boiteau et al. (1979) used the degree of ovarian development to group field populations of the bean leaf beetle, *Cerotoma trifurcata* (Forster). However, for accurate determinations of *C. septempunctata* population age structure, further studies are needed to distinguish between parous and nulliparous females and to determine the number of previous ovipositions (ovarian cycles) in parous females. Also, further studies are needed to address the question of why only 50–60% of all the females become gravid or oviposit. At this point, we can only speculate that, similar to other life-history traits that are highly variable within a *C. septempunctata* population, reproductive status is evidence of phenotypic plasticity that results in occurrence of contrasting phenotypic characters (e.g., reproducing versus diapausing or nonreproducing females) in the same genotype. Phenotypic plasticity is considered an important adaptive strategy (West-Eberhard 1986, 1989; Scheiner 1993), which in *C. septempunctata,* could potentially be one of the factors contributing to its widespread distribution both in its indigenous (Palearctic) and invaded (Nearctic) regions.

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