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MORPHOLOGICAL AND CHROMATIC CHANGES IN THE LAST INSTARS OF ERYTHEMIS SIMPLICICOLLIS (ODONATA)

by

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INTRODUCTION

Many aspects of the adult life history of the dragonfly Erythemis simplicicollis are well-known resulting from an excellent paper by Williamson (1923). This species (Family Libellulinae) is the "green jacket" dragonfly one finds almost everywhere around ponds and lakes. Very little information, however, has been recorded about its aquatic larval stage. The best of the papers in this area is that of Bick (1941) in which he discusses the developmental stages of this species. Using only a small number of eggs, he reared the insects in laboratory conditions and studied morphological changes beginning with the first instar. All but one died before maturing; the surviving one reached adulthood in a larval period of 113 days which included thirteen instars. Other studies carried out on this species by Wilson (1917) and Needham (1901) focused either on the early or on the final instar larvae. Thus, one is struck by the void of information on this abundant species for most of its developmental history from egg to larva through successive instars to the adult.

The present investigation was carried out to determine the sequence of morphological changes in the larval stage, especially in the later instars. Emphasis was placed on the sequence of changes that take place in the final instar and just before emergence. The detection of these significant occurrences would serve to pinpoint the "day" of emergence in advance.

In addition, very little seems to have been written about color changes in larval dragonflies. Corbet (1962) states that color changes are associated with differences in illumination in species such as Calopteryx, while in other species such as Anax, color changes are made in response to a change in the background color. In E. simplicicollis there are no published studies on any aspects of color changes. Even in the description of the larva by Needham and Westfall (1955), no mention is made of the obvious fact that the eyes of the larva are differentiated into dorsal and ventral parts on the basis of different colors, the ventral part usually being a lighter yellow or orange. Thus, in the present investigation, color changes were studied to determine the extent of color change in the body and in the eyes as a response to different color immediate backgrounds.

MATERIALS AND METHODS

A. General

Individuals of the dragonfly Erythemis simplicicollis were collected at varying intervals over a period of seven months (June-December, 1965). Collections were made in a small impoundment located northwest of Greensboro, North Carolina immediately behind Claxton Elementary School. Organisms were brought to the laboratory and placed in individual finger bowls four inches in diameter. All were maintained under a fourteen hour photoperiod and at a temperature of 20°C ± 1°C. On alternate days they were fed approximately the same numbers of the crustacean Daphnia magna so that they were rarely without food. De-chlorinated tap water was used as the environmental medium, and its pH values, determined intermittently, remained essentially the same (5.7-6.8 with a median and mode for the organisms of 6.4) throughout the experimental period.

B. Morphological

A total of 82 insects were measured at weekly intervals using a binocular microscope with a calibrated ocular micrometer. The insects were removed from their bowls with rubber-tipped forceps and placed on a flat glass plate on the microscope stage. The dorsal surface of their eyes was brought into sharp focus, and measurements were taken of total body length from the caudal cerci to the tip of the head. This is distinct from the method used by Lutz (1962) where measurement was made from the labium to end of ninth abdominal segment lateral spines in Tetragoneuria cynosura.

Measurements were also made on wing-sheath separation (the widest separation at the most distal part of the mesothoracic wings) and on head and abdominal widths at their broadest points. A measurement was then taken of the distance between the most lateral extensions of the brown pigmented tissue on the dorsal surfaces of the eyes; later, measurements of the distance between the most mesial extensions of this pigment were made (Fig.1). The insect was then elevated to an angle approximately 30°-45° to the microscope stage with the eyes focused clearly. Measurements were taken at the level of antennal attachment of (1) the distance between the most lateral extensions of pigmented eye tissue and (2) the distance between the most mesial extensions of the pigmented eye tissue (Fig. 2).

To supplement these eye measurements, animals within three-four days of emergence as indicated by retracted imaginal labiums, were dissected to ascertain more clearly underlying eye structures. Before dissection, a preliminary sketch was made of the head region using a grid scale as a guide. The cuticle on the dorsal surface of the head was then split, peeled, and broken off using forceps and scapel. After removal of the chitinous covering, a sketch was made again.

Detection of the retracted imaginal labium was visual. When it was seen through the chitinous covering of the larval labium, the latter was pulled out so that the degree of retraction of the adult labium could be ascertained. When it was seen to be fully retracted, it was recorded as such.

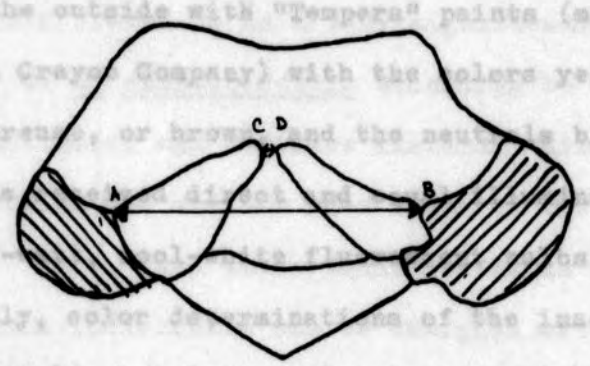

Fig. 1. Dorsal view of head showing eye pigment extensions. A-B, distance between lateral extensions. C-D, distance between mesial extensions. (X20)

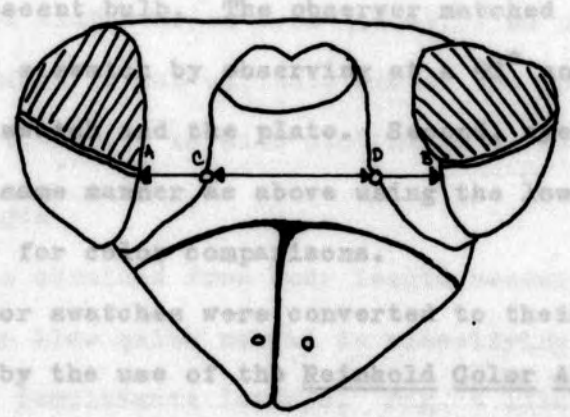

Fig. 2. Frontal view of head showing eye pigment extensions. A-B, distance between lateral extensions. C-D, distance between mesial extensions. (X20)

C. Color

All finger bowls in which the animals were maintained were painted on the outside with "Tempera" paints (manufactured by the American Crayon Company) with the colors yellow, orange, green, chartreuse, or brown, and the neutrals black or white. All organisms received direct and equal illumination from two overhead, 15-watt, cool-white fluorescent bulbs.

Initially, color determinations of the insects were made at two-day intervals, but later only at seven-eight intervals. Their colors were matched to standard color swatches mounted on white paper. Two color matches were made. First, the insect's color along the lateral edge of the thorax and upper abdomen served as that area where body color was noted. The color swatches and insect were simultaneously held at an angle of 45° to the cool-white fluorescent bulb. The observer matched the insect's color with that of a swatch by observing at a 90° angle from the surface of the swatch and the plate. Second, eye color matches were made in the same manner as above using the lower part of the eye as the basis for color comparisons.

The color swatches were converted to their wavelength (lambda) equivalents by the use of the Reinhold Color Atlas (1962). The neutrals which have no wavelength equivalents, were converted to their value equivalents as designated in the Munsell system (1929). These values corresponded very nearly to luminosity, i.e., the amount of reflected light. Munsell's scale has ten units for the neutrals, running from black with a value of one to white with a value of ten. What we perceive as white, however, usually has a value of nine rather than ten.

RESULTS

A. Head Width

Head width proved to be a very significant means of classifying larvae of E. simplicicollis according to antepenultimate, penultimate, and ultimate instars. Almost immediately following molting the maximum head width of the succeeding instar is attained. Thus, this measurement serves as an excellent criterion by which individuals can be assigned with confidence to instars immediately after a molt. These data are shown (Fig.3) in the form of head width frequency histograms for the three last instars. The non-overlapping nature of the histograms enables the experimenter to accurately classify the individuals into one of the last three instars. The head width of the antepenultimate instar ranges from 3.24-3.40 mm with a mean of 3.29 mm. The penultimate head width varies from 3.98-4.31 mm with a mean of 4.09 mm. Individuals in the ultimate instar had head widths which ranged from 4.44-5.34 mm with 5.04 mm as an average width.

B. Body Length

The data obtained from body length measurements indicated this parameter was also quite useful in classifying the insects into ultimate and penultimate instars. Fig. 4 illustrates body length data obtained at the beginning and end of the last two instars. This graph illustrates the non-overlapping nature of the body lengths. Body length increased in the penultimate instar on an average of 11.8% and in the ultimate instar, 12.3%. At the molt into the ultimate instar, there was an average increase in body length of 14%.

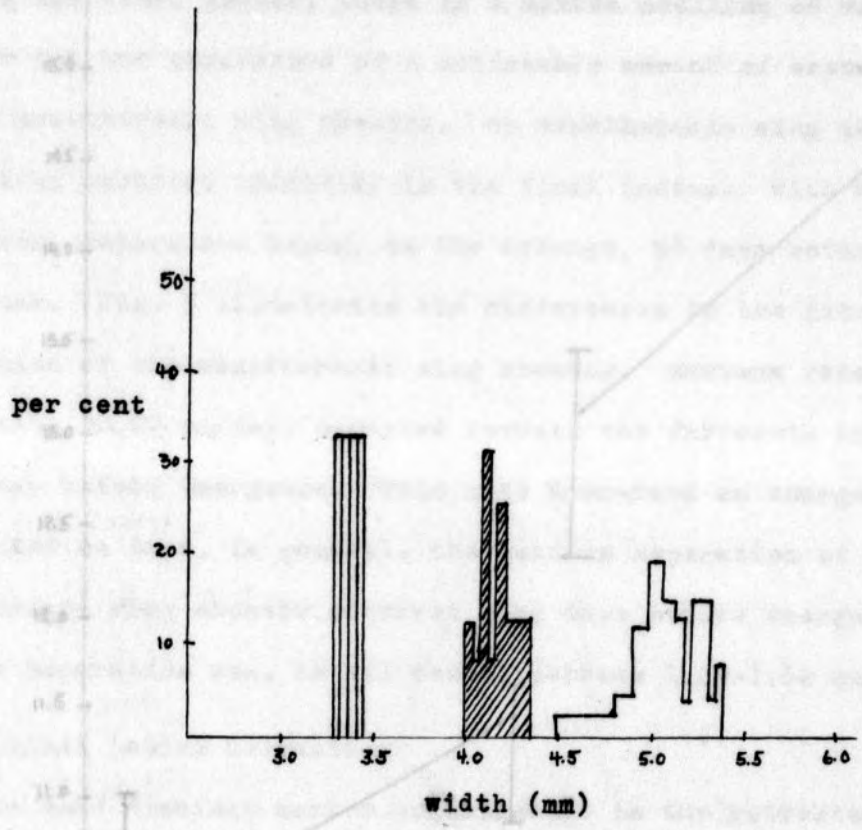

Fig. 3. Head width frequency histograms of the last three instars. Vertical lined= antepenultimate instar. Diagonal lined= penultimate instar. Clear= ultimate instar.

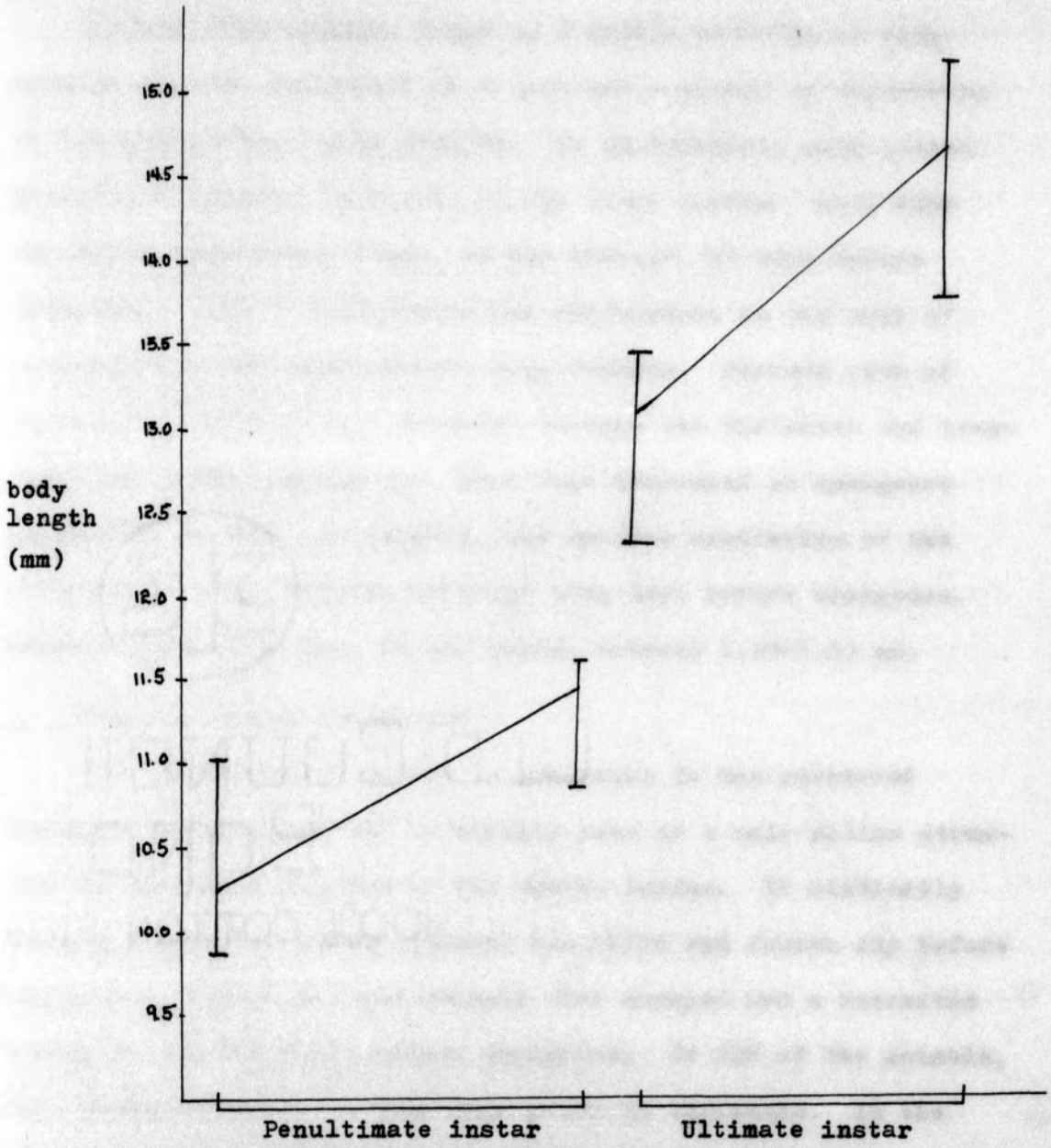

Fig. 4. Average body lengths of animals in the last two instars. Ranges are indicated by vertical lines.

C. Wing-sheath Separation

In the final instar, there is a marked swelling of wing sheaths and the occurrence of a noticeable amount of separation of the mesothoracic wing sheaths. No mesothoracic wing sheath separation occurred initially in the final instar. With wide variation, separation began, on the average, 58 days before emergence. Fig. 5 illustrates the differences in the rate of separation of the mesothoracic wing sheaths. Maximum rate of separation (0.07 mm/day) occurred between the fifteenth and twentieth day before emergence. This rate decreased as emergence approached so that, in general, the maximum separation of the mesothoracic wing sheaths occurred nine days before emergence. Maximum separation was, in all cases, between 1.29-1.62 mm.

D. Imaginal Labium Retraction

The most distinct marker to emergence is the retracted imaginal labium which can be sharply seen as a pale yellow structure on the inner surface of the larval labium. It distinctly becomes a separate entity between the third and fourth day before emergence. 43% of all the animals that emerged had a retracted labium on the third day before emergence. In 21% of the animals, the labium retracted at four days prior to emergence. In the remainder of the animals, labial retraction occurred one, two, or five days before emergence.

E. Eye Development

In the final instar, the brown pigment on the dorsal surface of the eye began to grow mesially and posteriorly until the two

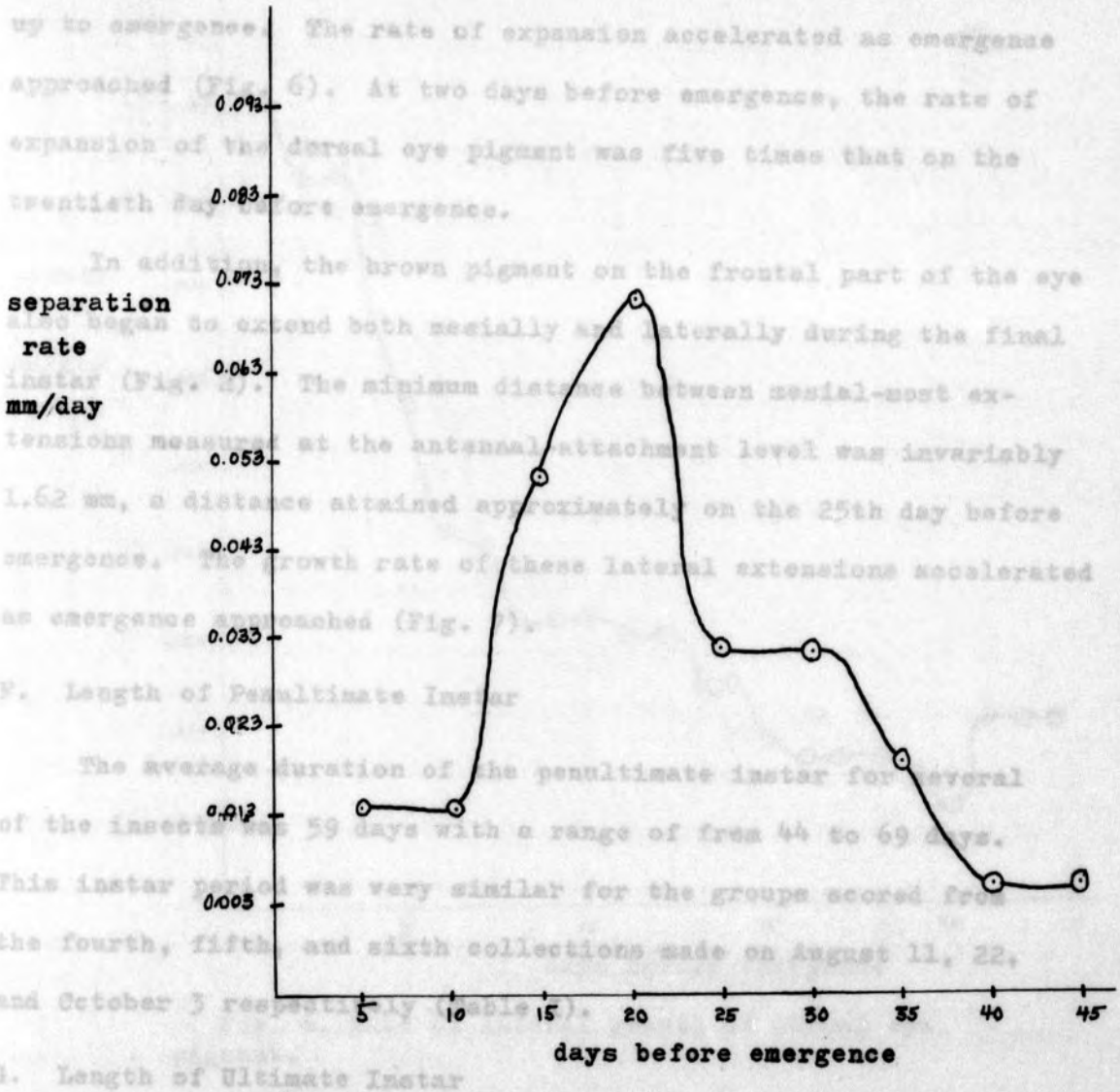

Fig. 5. Rate of separation of mesothoracic wing sheath in ultimate instar.

extensions met near the midline of the head. The rate of this expansion was essentially uniform, with the two extensions touching approximately fourteen-sixteen days before emergence. In addition, the lateral extension of this dorsal eye pigment continued up to emergence. The rate of expansion accelerated as emergence approached (Fig. 6). At two days before emergence, the rate of expansion of the dorsal eye pigment was five times that on the twentieth day before emergence.

In addition, the brown pigment on the frontal part of the eye also began to extend both mesially and laterally during the final instar (Fig. 2). The minimum distance between mesial-most extensions measured at the antennal-attachment level was invariably 1.62 mm, a distance attained approximately on the 25th day before emergence. The growth rate of these lateral extensions accelerated as emergence approached (Fig. 7).

F. Length of Penultimate Instar

The average duration of the penultimate instar for several of the insects was 59 days with a range of from 44 to 69 days. This instar period was very similar for the groups scored from the fourth, fifth, and sixth collections made on August 11, 22, and October 3 respectively (Table I).

G. Length of Ultimate Instar

The average duration of the ultimate instar of the group collected on June 11, 1965 was 61 days except for one retarded insect that had an ultimate instar of 121 days. The groups collected on July 22 and subsequently, however, showed much longer

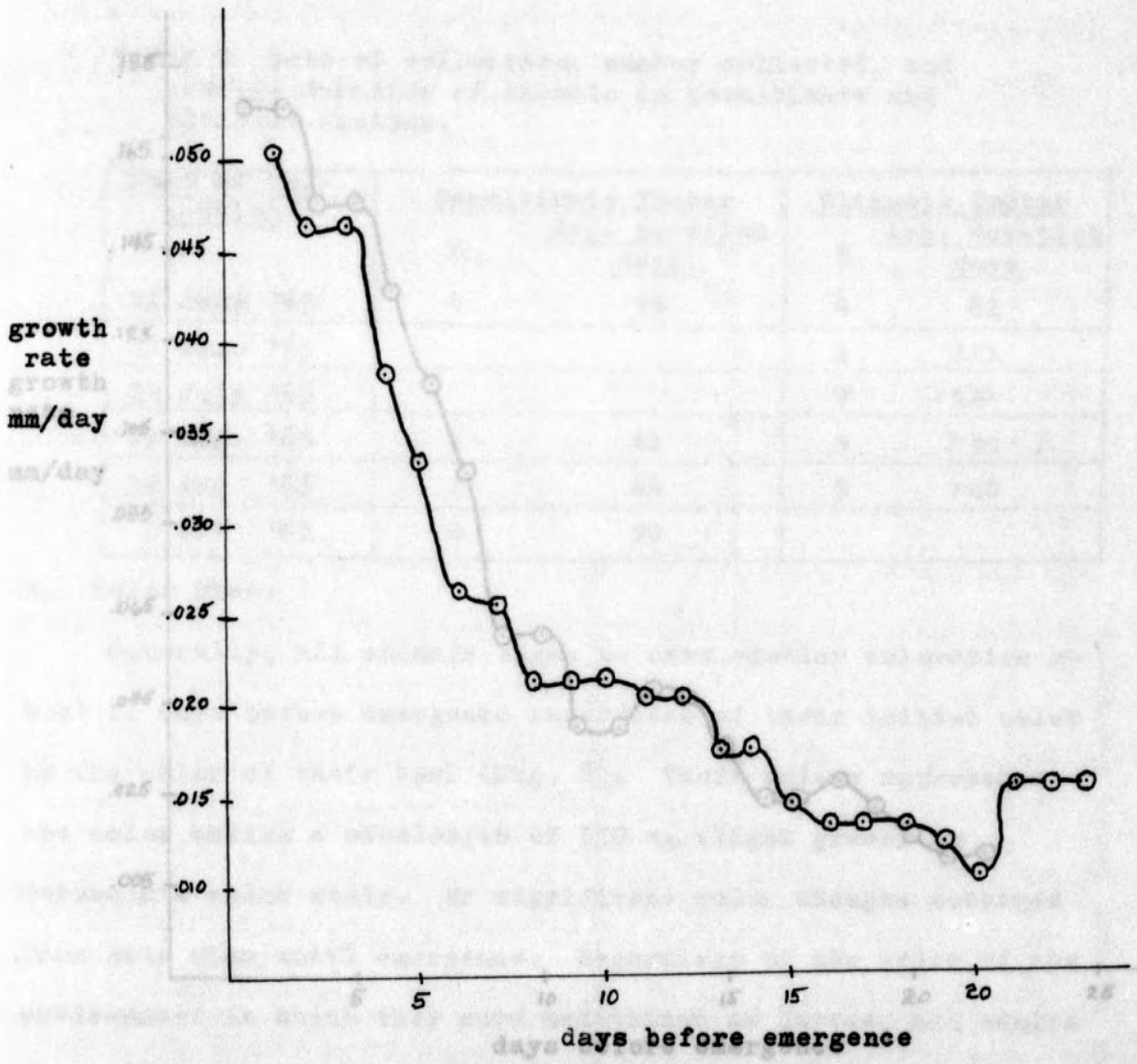

Fig. 6. Rate of lateral growth of dorsal eye pigment.

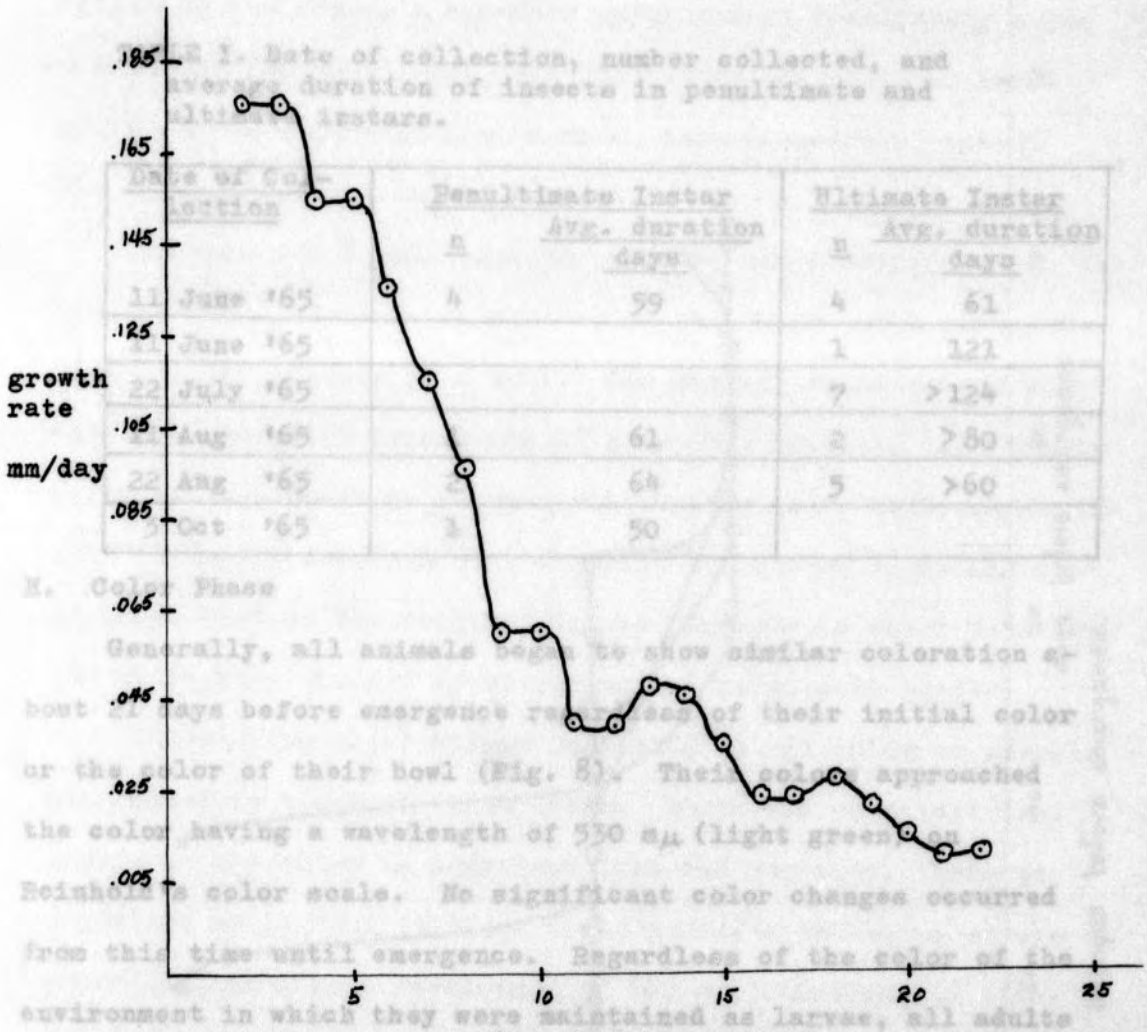

Fig. 7. Rate of lateral extension of frontal eye pigment.

ultimate instar periods. None of the animals have emerged to date from this group, and the average length of this instar has already been 139 days (Table I).

TABLE I. Date of collection, number collected, and average duration of insects in penultimate and ultimate instars.

Date of Collection	Penultimate Instar		Ultimate Instar	
	n	Avg. duration days	n	Avg. duration days
11 June '65	4	59	4	61
11 June '65			1	121
22 July '65			7	>124
11 Aug '65	1	61	2	>80
22 Aug '65	2	64	5	>60
3 Oct '65	1	50		

H. Color Phase

Generally, all animals began to show similar coloration about 21 days before emergence regardless of their initial color or the color of their bowl (Fig. 8). Their colors approached the color having a wavelength of 530 mμ (light green) on Reinhold's color scale. No significant color changes occurred from this time until emergence. Regardless of the color of the environment in which they were maintained as larvae, all adults were of the same color. These adults were the same color as adults occurring in nature.

Striking color changes were obtained with larvae reared in black and white environments. In the Munsell color system black has a value of one and white a value of nine or ten. Prior to the experiments, larval body colors varied considerably from light greens to browns. When placed in black bowls the larval

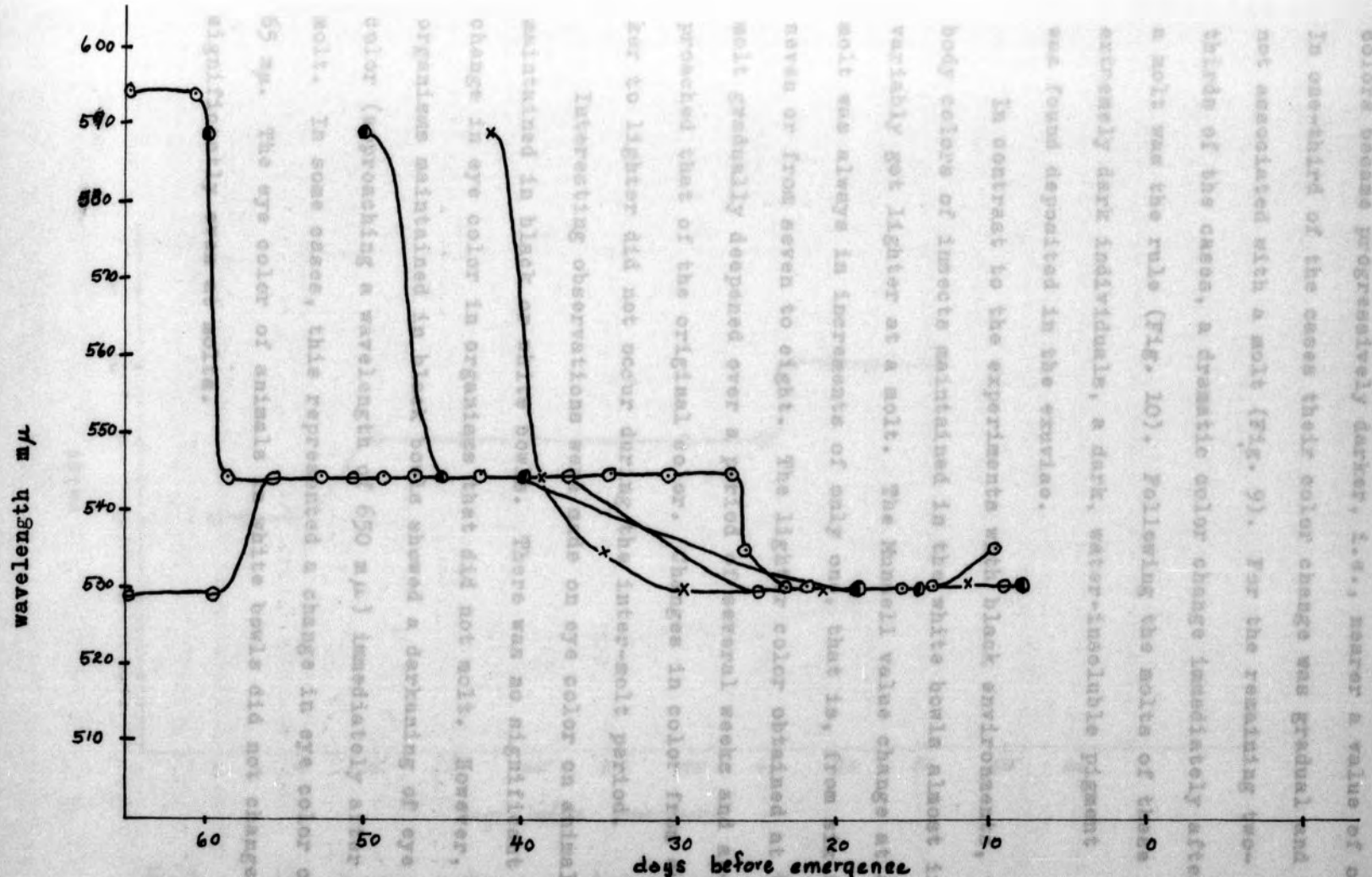

Fig 8. Color changes before emergence.

colors became progressively darker, i.e., nearer a value of one. In one-third of the cases their color change was gradual and was not associated with a molt (Fig. 9). For the remaining two-thirds of the cases, a dramatic color change immediately after a molt was the rule (Fig. 10). Following the molts of these extremely dark individuals, a dark, water-insoluble pigment was found deposited in the exuviae.

In contrast to the experiments with black environments, the body colors of insects maintained in the white bowls almost invariably got lighter at a molt. The Munsell value change at a molt was always in increments of only one, that is, from six to seven or from seven to eight. The lighter color obtained at the molt gradually deepened over a period of several weeks and approached that of the original color. Changes in color from darker to lighter did not occur during the inter-molt period.

Interesting observations were made on eye color on animals maintained in black or white bowls. There was no significant change in eye color in organisms that did not molt. However, organisms maintained in black bowls showed a darkening of eye color (approaching a wavelength of 650 mμ) immediately after a molt. In some cases, this represented a change in eye color of 65 mμ. The eye color of animals in white bowls did not change significantly even at molts.

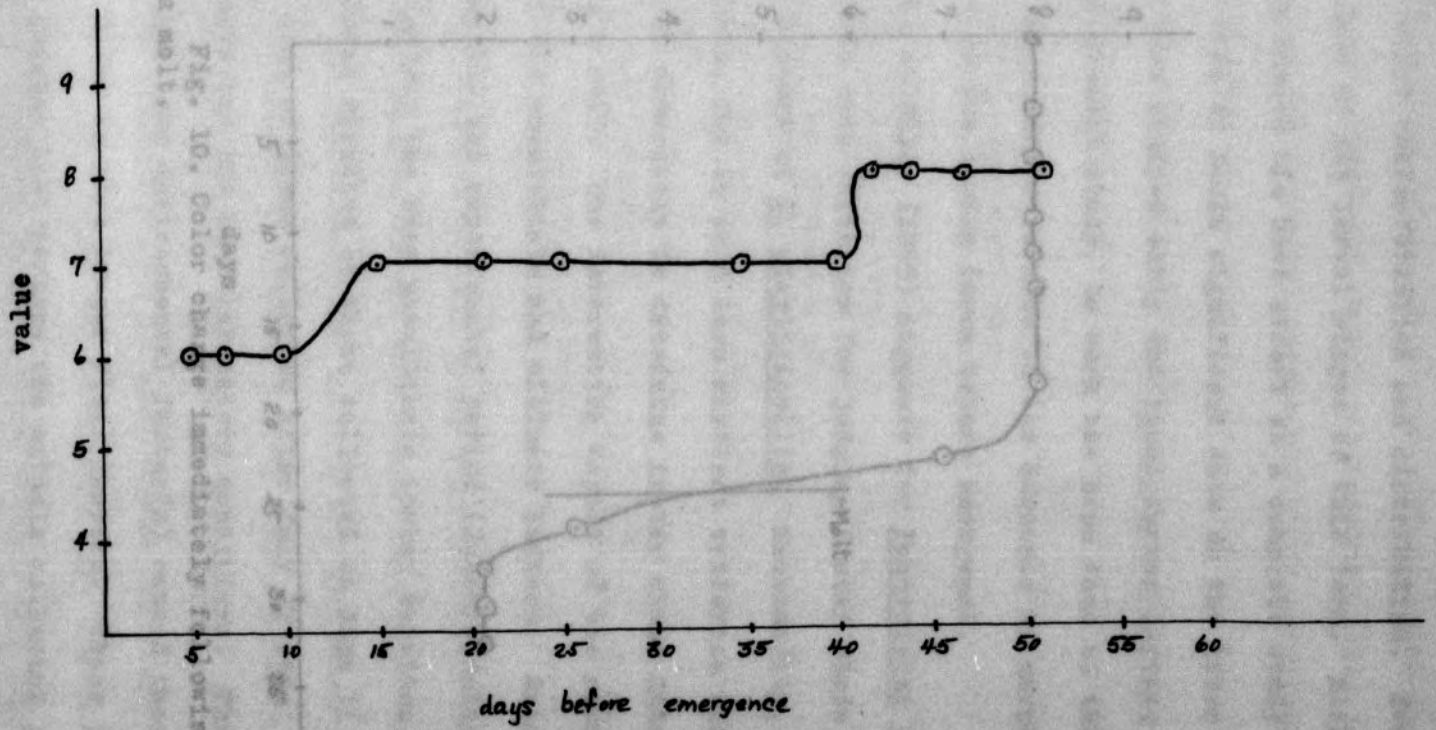

Fig. 9. Color change not associated with a molt.

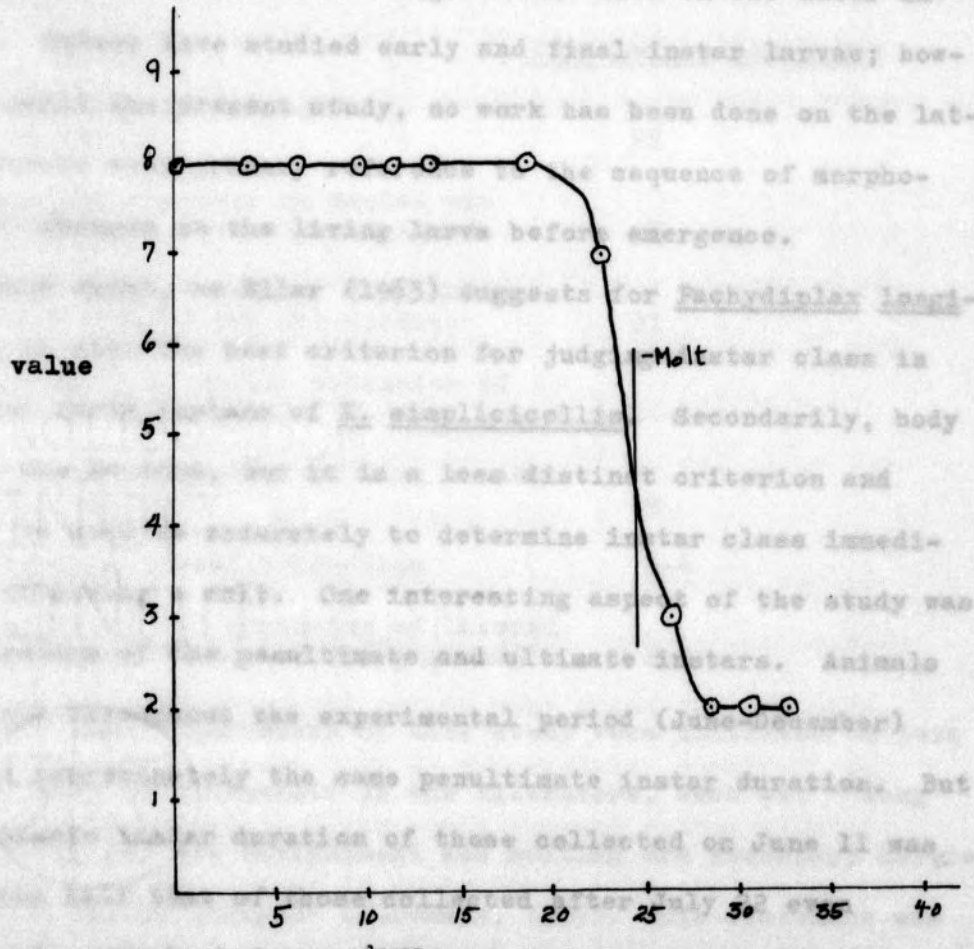

Fig. 10. Color change immediately following a molt.

Although Erythemis simplicicollis is a well-known dragonfly species, only one significant paper (Williamson, 1923) has been written on the adult characteristics and distribution. Even less work has been done of the larval stages of this form. Bick's work (1941) represents the best effort at a complete study of the larva, but even it lacks significant data on the later instars. Others have studied early and final instar larvae; however, until the present study, no work has been done on the latter instars with primary reference to the sequence of morphological changes on the living larva before emergence.

Head width, as Eller (1963) suggests for Pachydiplax longipennis is also the best criterion for judging instar class in the last three instars of E. simplicicollis. Secondarily, body length can be used, but it is a less distinct criterion and cannot be used as accurately to determine instar class immediately following a molt. One interesting aspect of the study was the duration of the penultimate and ultimate instars. Animals collected throughout the experimental period (June-December) all had approximately the same penultimate instar duration. But the ultimate instar duration of those collected on June 11 was less than half that of those collected after July 22 even though all animals had the same laboratory conditions. These data indicate that some environmental factor(s) caused those animals collected later to have longer final instars. These preliminary results suggest that perhaps the animals collected later were experiencing diapause induced by the naturally-occurring summer solstice.

The present study indicates that an animal in the latter parts of the final instar exhibited a very definite sequence of morphological events before emergence, each of which seems to be rather accurately timed and highly predictable. This sequence consists of the following events and their approximate time before emergence:

Event	Days before emergence
Beginning of wing sheath separation	58
Minimum distance on mesial extension of frontal eye pigment	25
Beginning of similar body coloration (530mμ) for all insects	21
Touching of mesial extension of dorsal eye pigment	14-16
Maximum separation of wing sheaths (1.29-1.62mm)	9
Imaginal labial retraction	3-4
Rapid rate of expansion of lateral extension of dorsal eye pigment	2-3

The color experiments of this study were initiated to test the validity of statements in the literature, such as: "long exposure to the new environment and molting are necessary before the color can be changed" (Portmann, 1959). His statement was found not always to be true in the case of Erythemis. Within days, some of the animals placed in black bowls underwent marked darkening of body color not associated with a molt. Furthermore, many underwent gradual color changes not associated with a molt. In many cases though, marked color changes were

only associated with a molt, thus agreeing with Portmann's findings.

The animals in black and white bowls showed significant darkening or lightening respectively. This phenomenon of homochromy (i.e., changing of body color to more nearly match the color of the environment) is of definite adaptive advantage for the organism which is preyed upon by other animals in its environment. Thus, this protective coloration is an extra camouflaging agent in addition to general body shape for the larval dragonfly.

The appearance of uniform coloration in all animals about 21 days before emergence is the result of the formation of the adult color pigments. The adult color, which is bright chartreuse, shows through the slightly brown cuticle and gives the light muted green color which is observed.

The literature makes no mention of the coloration in the lower part of the eye of E. simplicicollis. Variations in eye color from dark brown to light yellow were noted both in animals in nature and in those used in experimental studies. Eye color of animals placed in black bowls changed rather significantly, whereas those reared in white bowls retained their original eye colors. The eye color change and body color change seemed to be associated; generally, when the animal's body color became darker, so did its eye color. The possible adaptive advantage of the darker eye pigment is suggested by Fingerman (1952) in a study on Drosophila melanogaster. His data indicate that visual acuity is increased as the eye pigment becomes

darker. Since the dragonfly's nutrition is closely associated with visual acuity, an increase in visual acuity would be a definite advantage in capturing food.

In these preliminary studies on the species of dragonfly Erythemis simplicicollis, development proved to be a complex phenomenon. Rate of development in the penultimate instar was very uniform, while ultimate instar developmental rate was markedly decreased for insects collected after July 22. Significant markers used to pinpoint time of emergence were noted and included such events as wing sheath separation, coloration patterns, distance and rate of extensions of eye pigments, and imaginal labial retraction.

Color changes in both body and eyes as a response to different color immediate backgrounds was significant only in the cases of black or white environments. Some of the color changes were both marked and induced after only several days in the environment. Other changes were more gradual and not necessarily associated with a molt.

SUMMARY

1. The last three instars of the larval stages of Erythemis simplicicollis had a complex developmental history under laboratory conditions. The penultimate instar duration was generally uniform. The duration of the ultimate instar varied considerably in experimental animals collected at different times. These variations suggest that there might have been a faculative diapause in this species.

2. Morphological events were shown to be predictable criteria for anticipating the "day" of emergence; these formed a sequence leading to emergence.

3. Head width and body length were used as criteria for classifying larvae into the last three instar classes.

4. Significant color changes in both body and eye colors were induced as a response to black and white environments. Color change in the black environment was rapid at a molt and permanent during the following instar; while in the white environments, color change was gradual and only temporary.

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