Free Amino acid content and biosynthesis in eggs of the grasshopper Aulocara Elliotti Thomas during development
by Daryl Eugene Bunde

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Zoology
Montana State University

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Zoology

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MONTANA STATE COLLEGE
Bozeman, Montana

June, 1965

VITA

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In May, 1959, he was married to the former Marie Louise Erickson. They have three children, Steffen Maurice, age 5, Kermit Austin, age 3, and Jeannine Marie, age 1.

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ACKNOWLEDGEMENTS

The invaluable assistance and counsel that I have received from my advisor, Dr. James H. Pepper, throughout my doctoral studies is most gratefully acknowledged. I am indebted also to Professor Ellsworth Hastings for his aid in obtaining the experimental specimens and for his criticism in preparing this manuscript. The suggestions offered by Dr. George R. Roemhild during the laboratory work are appreciated greatly. To Drs. P. D. Skaar and K. J. Goering, I want to express my gratitude for their critical reading of this manuscript. I am grateful to Dr. Graeme L. Baker of the Department of Chemistry for permitting me to use some of the department's equipment, and I also want to thank Mr. Hans K. Hamann of the Department of Mathematics for the statistical analysis of the data.

This investigation was supported in part by a Predoctoral Fellowship, No. 4-F1-12,890-05, from the National Institutes of Health for which I am very grateful.

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ABSTRACT

Aulocara elliotti eggs from three wild and two greenhouse-reared populations were analyzed for free amino acid content and for *in vivo* synthesis of free amino acids from radioactive acetate. These analyses were performed on eggs in the prediapause, diapause, and postdiapause periods of development. Column chromatography, thin-layer chromatography, colorimetry, and liquid scintillation counting were used as analytical techniques. Fourteen free amino acids were present in each population egg sample throughout embryonic development. Five additional free amino acids and three unknown substances were found in some of the egg samples. The free amino acid content of each egg sample was found to increase considerably from the prediapause to the diapause period and then to decline during postdiapause development. Four amino acids were synthesized *in vivo* by all the egg samples during each period of development. Five additional ones were synthesized *in vivo* only in specific stages of development. All of the differences in free amino acid content and in the *in vivo* synthesis of free amino acids between the different egg samples at the same stage of development were not attributable to a direct effect of population density.

INTRODUCTION

The grasshopper species, Aulocara elliotti (Thomas), has been observed over an extended period of time to undergo considerable fluctuations in population density within a given habitat. Similar large periodic changes in population numbers have been recorded for other animals. Although various environmental conditions usually have been presumed to be the causative agents for these changes, more recent evidence indicates that intrinsic factors may be involved. In small mammal populations the stress of high densities has been suggested to be responsible for changes in the hormonal secretions within individuals, and also for changes in their behavior patterns. These in turn adversely effect the reproductive potential of the total population (Chitty, 1957, 1964; Christian, 1957, 1963 a, 1963b; Krebs, 1964).

Hastings and Pepper (1964) have indicated several factors including genetic segregation, physiological vigor, and environmental stress which may be associated with A. elliotti population fluctuations. They have also observed instances where mortality among newly hatched nymphs exceeded 99 percent with no apparent extrinsic cause for these high mortalities. This led them to conclude, (Pepper and Hastings, 1964), that the embryos from which the nymphs hatched were physiologically altered, and that this condition may have been due to stress imposed during the preceding adult stage which brought about a hormonal imbalance within the eggs. This imbalance in hormones, conceivably, could have effected the normal metabolsim of carbohydrate, lipid or protein materials

within the developing embryo. The result could be a physiologically deranged animal. Since the above was postulated, Svoboda (1964) investigated the lipid composition of A. elliotti eggs from different populations, but was unable to find any differences that could be correlated with population density; whereas, Van Horn (1963) followed the morphology of these eggs throughout embryonic development and discovered significant differences in the morphological variability and development rates in different populations.

Since the morphology of cells is associated with protein structure, it follows that abnormal morphological development may be the result of aberrant protein synthesis. In the cleiodic egg of a grasshopper the amino acids required for the synthesis of new embryonic proteins must either be synthesized by the embryo or come from the yolk material surrounding it. Thus the pattern of these unbound amino acid building blocks at any point in embryonic development represents an index of the protein metabolism of the egg at that stage of morphogenesis (Colombo et al., 1962).

Upon such a background, the present study was initiated to obtain some basic information on the free amino acid and protein metabolism of the egg. The pattern of distribution of the free amino acids throughout the development of the egg at prediapause, diapause, and postdiapause stages was investigated as were the patterns obtained from different wild and greenhouse-reared populations. Relationships were also sought between the free amino acid patterns and the density of the populations from which

the eggs were obtained.

The many investigations that have been done on the free amino acids extracted from mature insect tissues have been reviewed recently (Chen, 1962; Gilmour, 1961; Wyatt, 1961). These studies have shown that adult insect tissues contain a greater concentration of free amino acids than is normally found in other organisms. And also in insects, the level of concentration of each amino acids has been found to vary considerably both between different species and within one species examined at different times (Awapara, 1962; Duchateau and Florkin, 1955; Gilmour, 1961; Stephen and Steinhauer, 1959). Although special functions have been designated to this unique amino acid distribution, complete understanding of its significance is still lacking (Barton-Browne, 1964; Blackith, 1961; Bursell, 1963; Gilmour, 1961; Schoffeniels, 1960).

Additional evidence of the importance of free amino acids in the metabolism of insects has been found upon examination of immature stages. In the holometabolous insects, Hadorn and Mitchell (1955) studying the development of Drosophila melanogaster were the first to use paper chromatography to identify the free amino acids present in that species. Auclair and Dubreuil (1951) adapted this technique to the quantitative estimation of the free amino acids in the last larval instar of Galleria mellonella. Studies have also been reported on the free amino acids in immature forms of Aedes aegypti and Culex quinquefasciatus (Micks and Ellis, 1952), Bombyx mori (Drilhon and Busnel, 1952), C. pipiens (Chen, 1958), and D. melanogaster (Crone-Gloor, 1959).

Benz (1957), Chen and Hadorn (1955), Hadorn (1956, 1961), Hadorn and Stumm-Zollinger (1953), and Lewis (1954) established the usual pattern of occurrence of free amino acids in D. melanogaster throughout its embryonic development and detected deviations from this pattern in five lethal mutants, all of which died prior to reaching the adult stage. Similar evidence of the possible effect of free amino acids on embryonic development has been found in C. pipiens by Laven and Chen (1956) and in Ephestia kuhniella by Chen and Kuhn (1956). Various other studies have also illustrated other possible effects that alterations in free amino acid content can have upon specific physiological processes (Elvehjem, 1956; Lyman et al., 1964; March et al., 1964; Sidransky et al., 1964).

In the hemimetabolous insects, the presence of free amino acids in the grasshopper egg of Chortophaga viridifasciata has been shown by Shaw (1955). Fu (1957) noted the changes in concentration of sulfur containing amino acids in the developing grasshopper egg of Melanoplus differentialis. Twenty free amino acids were determined quantitatively by Colombo, et al., (1962) in eggs of the locust, Schistocerca gregaria, from ripe oocytes through to the time of hatching, and separately in yolk and embryos of later developmental stages. The results of these studies have shown that, in all of the holometabolous and hemimetabolous insects so far examined, the free amino acid content of the immature forms is high, and the pattern of distribution of these compounds varies with the stage of development.

To follow the biosynthesis of the amino acids in A. elliotti eggs and to determine which ones can be synthesized in vivo, various precursor compounds, containing radioactive isotopes, were introduced into intact organisms, and after varying lengths of time the free amino acids were examined for radioactivity. In recent reports, Kasting and McGinnis (1958, 1960, 1962) and Kasting et al., (1962 demonstrated the incorporation of C^{14} from uniformly labeled glucose into several amino acids in larvae of Agrotis orthogonia, Ctenicera destructor and Phormia regina. This technique was extended to other organisms using glucose-C^{14} and other radioactive precursors such as carbon dioxide-C^{14}, glycerol-C^{14}, and acetate-C^{14} (Awapara and Campbell, 1964; Mohri, 1964; Rothstein, 1963; Schaefer, 1964; Strong and Sakamoto, 1963). Generally, less than 11 of the common 20 amino acids were found to be synthesized by any organism.

To accomplish the purposes of this investigation into the amino acid metabolism of the A. elliotti eggs, the techniques of column chromatography, thin-layer chromatography, colorimetry, and radioactive tracing were selected.

PROCEDURE AND RESULTS

Biological samples

The eggs used in this study were obtained from three geographically separated wild populations, and from additional ones reared under greenhouse conditions at two different densities. The wild populations were located south of Chinook, in northcentral Montana, west of Simms, in central Montana, and northwest of Townsend, in central Montana. During the summer of 1964 the Simms population was the lowest in density while the Chinook one was the highest and the Townsend population was intermediate between the two.

The grasshoppers that were reared in cages in the greenhouse were collected as nymphs in late June from the population near Townsend. Twelve cages in all were used. Each had a bottom area of one square foot and was constructed with a circular wooden floor, cylindrical lucite wall, and a cheesecloth top. A removable nine inch aluminum pan, filled with soil in which the grasshoppers could oviposit, was inserted through a hole in the bottom of the cage. On alternate days fresh cuttings of Agropyron smithii, the preferred foodplant of A. elliotti (Anderson, 1964), were placed in three small vials positioned in the cage floor.

Eight cages were included in one series of tests with five pairs of adult grasshoppers in each cage. In a second series 15 pairs of adults were placed in each of four cages. On alternate days throughout July and August the soil in the pans was sifted and the egg pods removed. These pods were positioned vertically in vials partially filled with soil and

stored at 25°C.

Egg pods from the wild populations were collected in late August and stored at 25°C. in petri dishes containing soil taken from the collection site. In the first part of October these pods along with those from the greenhouse were transferred to a room held at 8°C. They were returned to the 25°C. chamber in early December. Each week throughout this period the pods were watered lightly to prevent dessication.

Samples of eggs from the three wild populations and the two greenhouse groups were analyzed for free amino acid content at prediapause, diapause, and postdiapause stages of embryonic development. One hundred eggs were used for each sample except those from the Simms population which were limited to 50 eggs. Only eggs which appeared viable and were in the desired stage of development were included in the sample. Diapause eggs were processed in the 8°C. room. They were immersed in distilled water and observed under a dissecting scope to determine the developmental stage of the embryo. This was accomplished by comparison with the morphological characteristics for each stage as outlined by Van Horn (1963).

Table I shows the numerical developmental age of the A. elliotti eggs from the different populations at the time of analysis. The use of two samples at each stage of development will be explained later. It will be noted from Table I that during the diapause and postdiapause periods, the numerically designated stage of development is quite

TABLE I

	Numerical Development Age				
	GH-5	GH-15	Chinook	Simms	Townsend
Prediapause	*11,12	12,13	15,16,17		16,17
	**12,13	12,13	13,14,15	13,14,15	14,15,16
Diapause	*18,19	18,19	18,19		18,19
	**18,19	18,19	18,19	18,19	18,19
			18,19***		
Postdiapause	*25,26	24,25	25		25
	**23,24,25	23,24,25	24,25	25	24,25

* Samples used in colorimetry analysis.

** Samples used in radioactive analysis.

*** Sample used in separation of yolk and embryo.

consistent for all populations. It was not possible, however, to obtain eggs from all the populations at the same stage during prediapause because there was no way of knowing when the wild eggs were deposited.

After the stage of development was determined, the eggs were air dried on filter paper for approximately 15 minutes and then weighed. The results are recorded in Table II.

Free amino acid extraction

After weighing, each sample was triturated in a microhomogenizer for five minutes with cold 80 percent ethanol. This mixture was heated for a few minutes to ensure denaturation of the protein and the total homogenate centrifuged at 5,000 rpm for 15 minutes. After decanting the supernatant into another tube, the residue was extracted with 50 percent ethanol and then with distilled water. This procedure was modified from that suggested by Schaefer (1963).

The supernatant and the two additional extractions were combined and passed through a short ion exchange column of Dowex 50Wx8 (H^+) following the steps outlined by Corrigan and Kearns (1963), Furuholmen et al., (1964), and Schaefer (1963). The samples containing the amino acids were then eluted with 50 milliliters of 4 N NH_4OH, dried in vacuo to near dryness, and made up to two milliliters with distilled water. All samples were stored in the deep freeze until assayed. A total of thirty samples were prepared.

TABLE II

Weight per 100 Egg Sample (gram)					
	GH-5	GH-15	Chinook	Simms	Townsend
Prediapause	*0.6406	0.6977	0.7593		0.6974
	**0.8935	0.8466	0.7005	0.9210	0.7737
Diapause	*1.0513	1.0800	0.9753		1.0277
	**1.0765	0.9664	0.9838	0.8280	1.0864
			1.0370***		
Postdiapause	*1.0728	1.1303	0.9644		1.0555
	**1.0588	1.0506	0.9887	0.8670	1.0134
Totals	5.7935	5.7766	6.4090	2.6160	5.6541
Averages	0.9656	0.9628	0.9156	0.8720	0.9424

* Samples used in colorimetry analysis.

** Samples used in radioactive analysis.

*** Sample used for separation of yolk and embryo.

Thin-layer chromatography

Paper chromatography was employed initially to separate and identify the free amino acids. This procedure, however, was discontinued in favor of thin-layer chromatography which is less time consuming and of greater sensitivity. The method of thin-layer chromatography as described by Randerath (1963a) was pursued. L-amino acid standards, all of reagent grade, were purchased from Mann Research Laboratories, New York, and from Sigma Chemical Company, St. Louis.

At first, two cellulose adsorbents (Cellulose MN 300 and MN 300 G, Brinkmann Instruments, Westbury, New York) were tried using the solvents proposed by Wollenweber (1962). Ten other solvents of the author's own derivation also were tested with these adsorbents. One-dimensional runs were satisfactory with a few of these solvents, but none produced usable results two-dimensionally.

The adsorbent Silica Gel G (Brinkmann Instruments) and the solvent systems developed by Brenner and Niederwieser (1960) and Fahmy et al., (1961) were tried also. Since the author was not able to reproduce the separation pattern using the two solvents that were especially recommended (Fahmy et al., p. 2022), several modifications were attempted. Finally, a more adequate two-dimensional separation of 24 amino acid standards was achieved by using chloroform-methanol-17 percent ammonia (CMA, 1:3:1 v/v) as the first solvent in combination with phenol-water (PW, 75:25 w/w) as the second solvent. Figure 1 illustrates this separation pattern. R_f

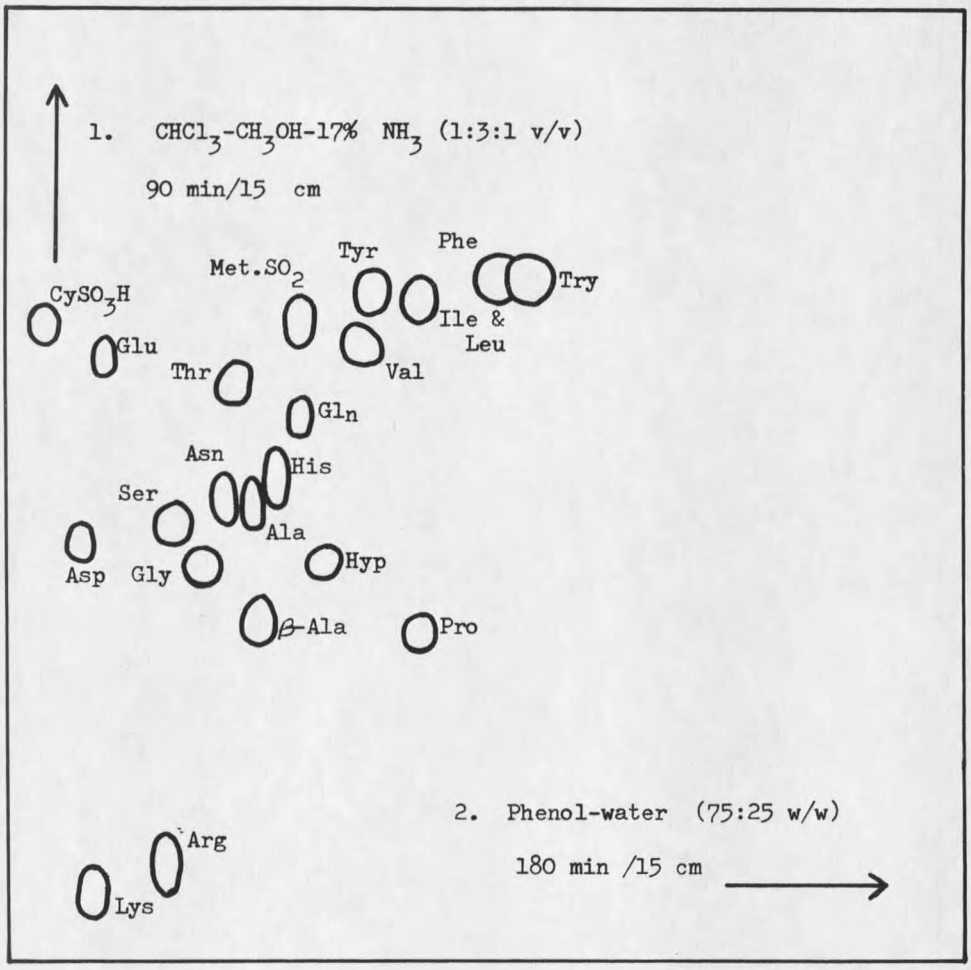

Figure 1. Two-dimensional separation of a mixture of 21 amino acids.

values for each amino acid in the CMA solvent is given in Table XI in the appendix.

Although the separation pattern produced by these two solvents (CMA and PW) was adequate, further attempts were made to improve the method. Another adsorbent, Anasil B, (Brinkmann Instruments) was tested with solvents reported by Cruickshank and Sheehan (1964). The results were unsatisfactory. A very useful separation pattern was finally obtained by substituting the solvent methyl ethyl ketone-propionic acid-water (4:3:2 v/v) used by Knight (1962) for the CMA solvent as the first dimension and phenol-water (75:25 w/w) as the second dimension with the silica gel adsorbent (Figure 2). The use of this combination was recently reported by Schaefer (1964). In all cases reagent grade solvents were employed but only one commercial phenol reagent (Mallinkrodt 88 percent phenol, liquefied, preservative free) was used. Richardson and Tolbert (1964) reported that with paper chromatography the commercial phenol reagent caused glycine to form stable condensation products.

Representative chromatographic distributions of free amino acids isolated from Chinook eggs at prediapause, diapause, and postdiapause development stages are shown in Figures 3, 4, and 5. In order to stay within the limits of the colorimetric quantitative test, the amount of sample used to obtain the chromatograph in Figure 3 was four times that in Figure 4 and two times that in Figure 5.

To detect and identify the individual amino acid spots on the

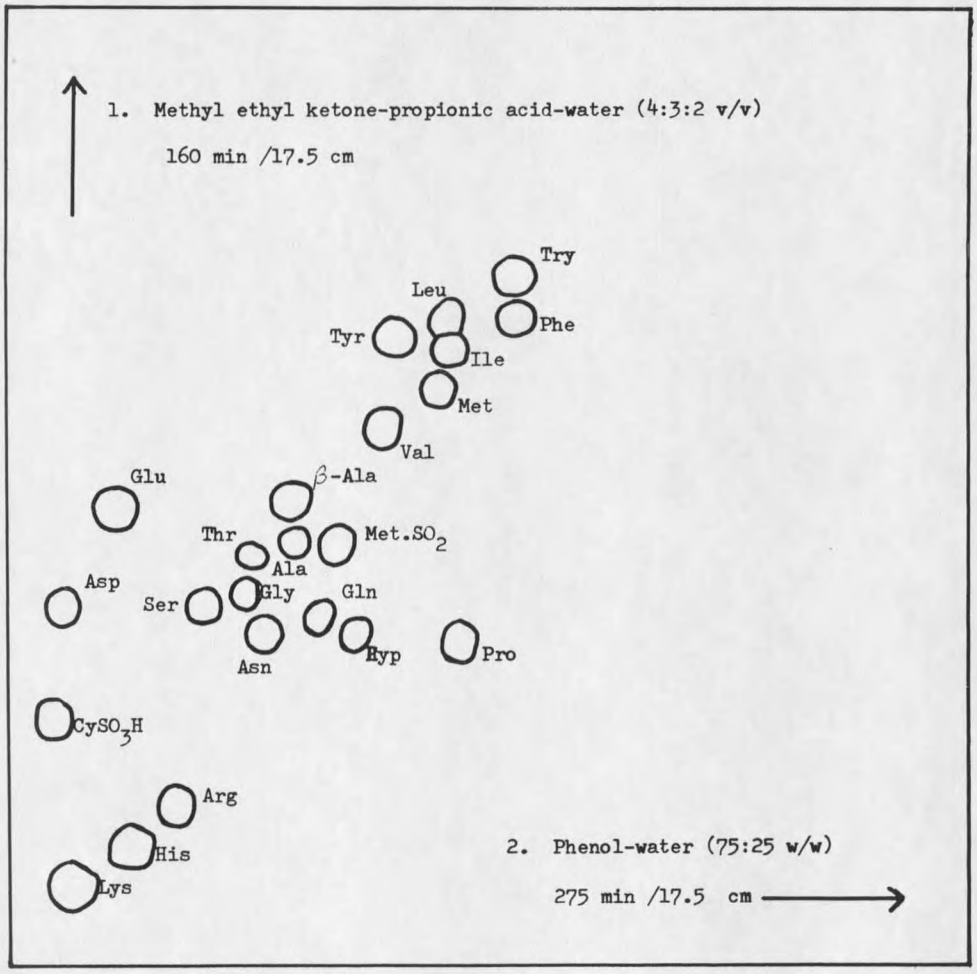

Figure 2. Two-dimensional separation of a mixture of 23 amino acids.

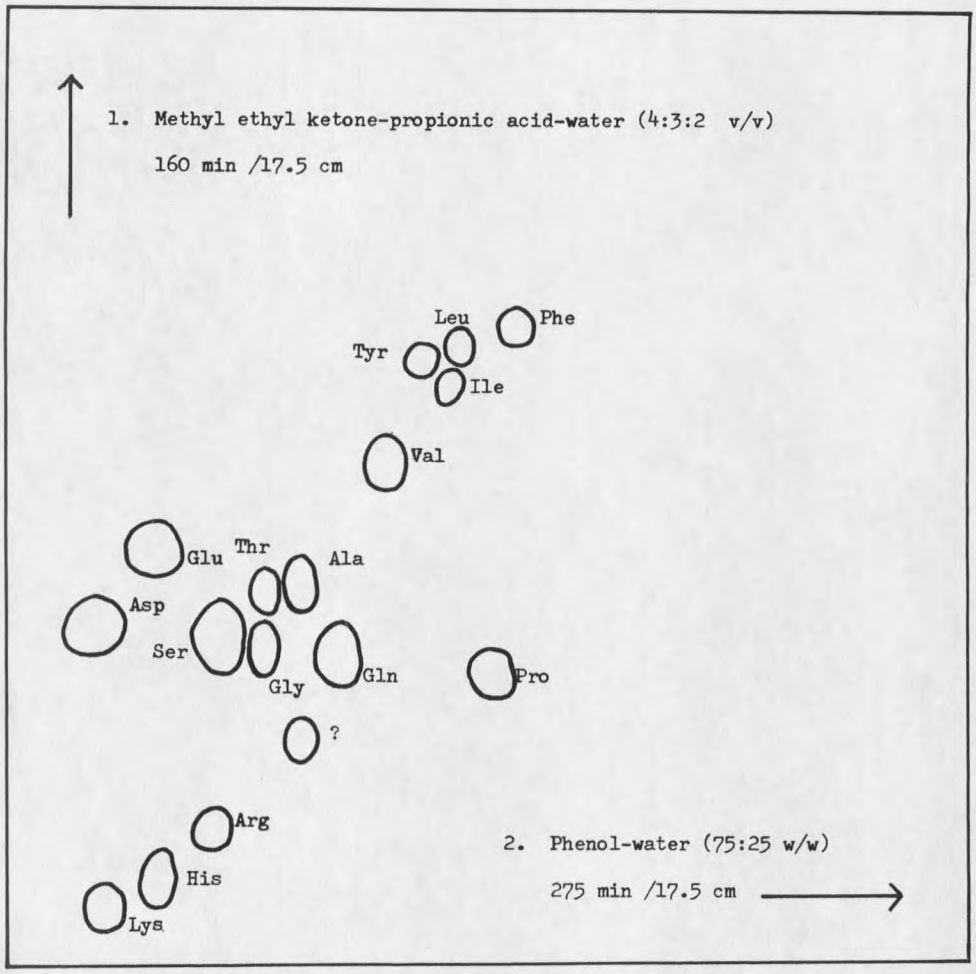

Figure 3. Two-dimensional separation of 16 free amino acids and one unknown substance from a sample of Chinook prediapause eggs.

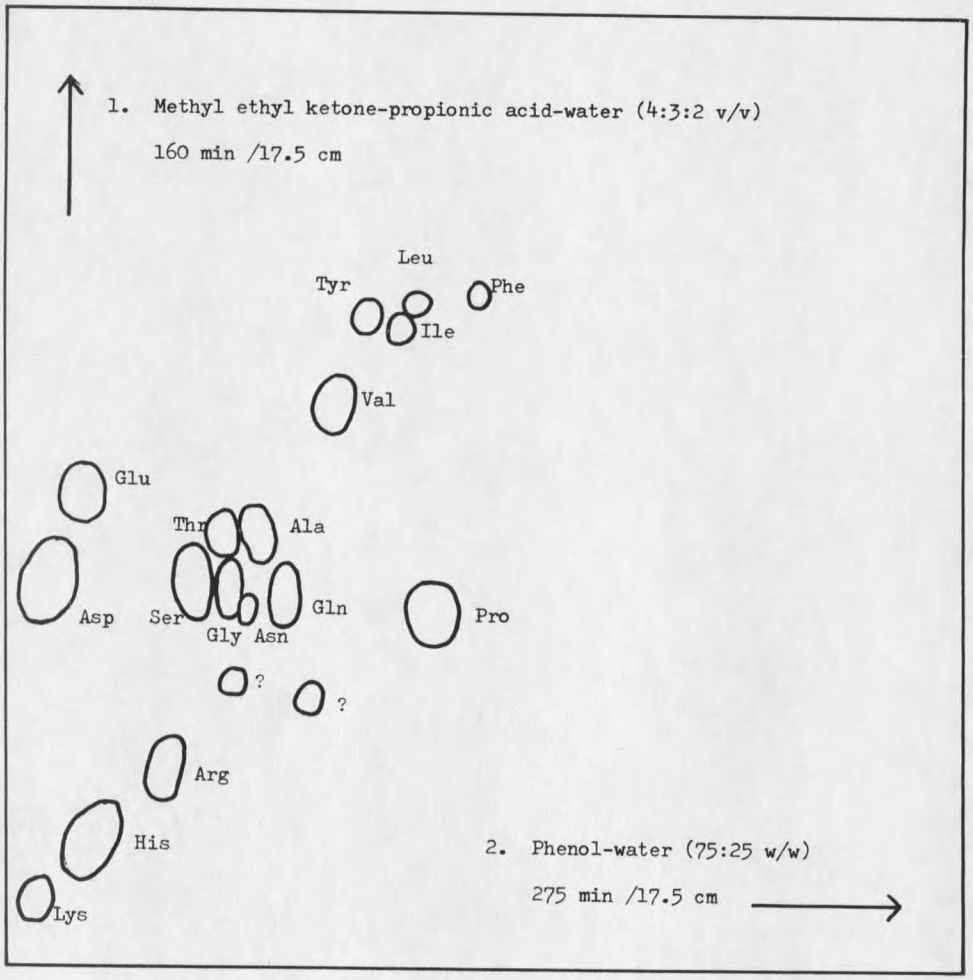

Figure 4. Two-dimensional separation of 17 free amino acids and two unknown substances from a sample of Chinook diapause eggs.

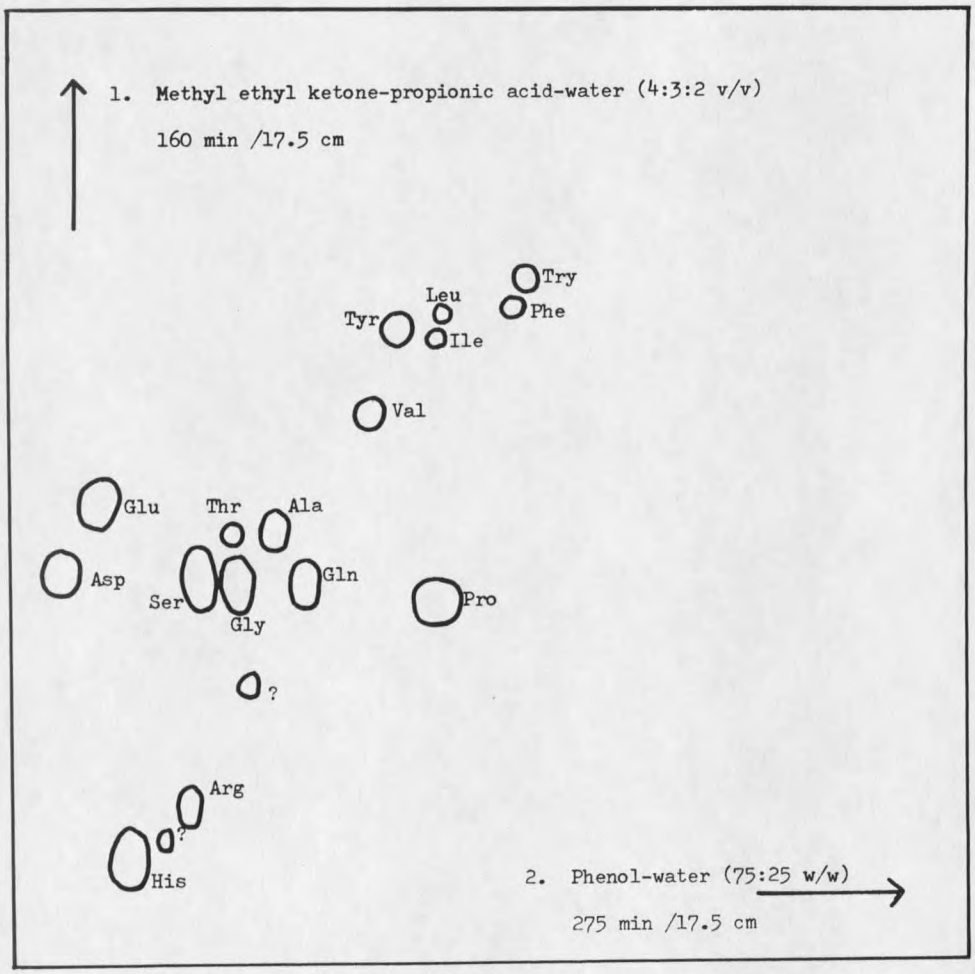

Figure 5. Two-dimensional separation of 16 free amino acids and two unknown substances from a sample of Chinook postdiapause eggs.

chromatoplate, the ninhydrin reagent of Moffat and Lytle (1959) was applied. The ninhydrin positive spots appearing on the chromatoplates were distinguished by comparison with the R_f values of the amino acid standards in the two-dimensional separations as well as the distinctive colors of this staining reagent.

Colorimetry

Quantitative determinations of the individual free amino acids present in each sample were made by the colorimetry method of Price (1963) and Shaw (1961). Their modified ninhydrin reagent (0.5 percent ninhydrin in absolute ethanol) was used as it gave more uniform color results. Each ninhydrin positive spot on the chromatoplate was outlined with a sharp pointer and the gel within the area scraped into a centrifuge tube. Three milliliters of 50 percent ethanol was then added and the mixture stirred thoroughly in a cyclo-mixer. After centrifuging to spin down the silica gel which would otherwise remain in suspension and distort the results, the supernatant was decanted into a colorimeter tube. Quantitative measurements on each amino acid were read at a wavelength of 570 millimicrons except for proline which was read at a wavelength of 350 millimicrons.

All readings were taken using a Bausch & Lomb Spectronic 20 Colorimeter and the amount of each amino acid determined by reference to standard curves. Values derived from three separate chromatographs in which known amounts of standard amino acids were developed were used for

locating each point on the reference curve. Amino acids from zero to 20 micrograms were determined within an error of ± 5 percent. All samples were analyzed in triplicate.

The free amino acid content of eggs from the different populations of A. elliotti as determined by the procedures described are presented for prediapause, diapause, and postdiapause stages in Tables III, IV, and V respectively. Nineteen free amino acids were found in the whole eggs. Three additional ninhydrin staining spots were detected, but they were not identified since they did not correspond to any of the standard amino acids. Possibly, these three spots were short chain peptides.

The values in Tables III, IV, and V are averages of three determinations on each sample, and are listed as micromole x 10^{-3} of amino acid per egg. Amino acids present in amounts too low to be measured in the colorimeter are shown as "traces". The absence of some of the amino acids in certain stages is indicated by a dash.

The histograms, presented in Figures 6, 7, 8, 9, and 10, show the concentrations of amino acids from each population in the three stages studied. The quantity of each acid is presented as a percentage of the total free amino acid content for that particular sample. The values presented in Tables III, IV, and V are shown graphically in Figure 11 to illustrate the change in total content of amino acids in the prediapause, diapause, and postdiapause stages.

In order to get an indication of the difference in free amino acid

TABLE III

Average Micromoles x 10^{-3} per Egg of Free Amino Acids
Prediapause

Amino acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	61.1	52.9	13.3	62.2	18.7
Arginine	16.4	8.92	3.40	Tr	3.50
Aspartic acid	37.7	38.9	33.3	50.8	26.7
Glutamic acid	50.3	47.7	28.4	56.8	30.4
Glutamine	20.1	27.8	5.66	1.01	7.33
Glycine	9.84	54.0	18.5	Tr	13.5
Histidine	26.8	15.9	Tr	Tr	11.1
Isoleucine	13.4	14.1	4.92	7.55	5.33
Leucine	16.4	15.8	7.44	13.5	7.44
Lysine	17.9	20.0	18.5	Tr	11.1
Phenyl-alanine	13.7	13.7	8.95	14.7	8.95
Proline	31.3	26.2	29.9	55.6	27.6
Serine	51.1	45.5	35.5	54.9	25.5
Threonine	32.0	26.9	15.2	23.2	17.4
Tyrosine	12.5	13.8	8.36	16.7	8.88
Valine	27.8	25.6	13.7	27.8	10.4
(Unknown)	?	?	?	?	?
(Unknown)	?	-	-	-	-
Totals	438.34	447.72	245.03	384.76	233.83

TABLE IV

Average Micromoles x 10^{-3} per Egg of Free Amino Acids
Diapause

Amino Acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	38.2	40.4	40.4	38.2	38.2
Beta-Alanine	-	-	-	-	Tr
Arginine	19.9	8.00	17.4	10.3	19.3
Asparagine	Tr	Tr	Tr	Tr	-
Aspartic	55.5	55.5	58.0	51.0	55.5
Glutamic	68.6	100.	76.1	80.0	75.0
Glutamine	9.36	9.67	8.32	1.04	9.15
Glycine	46.9	2.66	13.3	15.9	7.98
Histidine	63.4	54.1	58.7	58.7	37.4
Isoleucine	5.49	5.49	3.92	6.10	6.10
Leucine	15.8	22.0	11.5	11.8	13.7
Lysine	32.0	19.9	23.2	25.9	21.3
Phenyl-alanine	15.4	12.1	12.1	8.48	11.6
Proline	80.8	95.2	98.4	73.0	85.6
Serine	68.1	68.1	80.8	43.8	74.2
Threonine	31.8	27.5	34.9	22.5	22.2
Tryptophan	-	Tr	-	-	13.7
Tyrosine	16.5	17.2	14.7	14.7	12.8
Valine	26.2	32.4	22.8	19.7	20.5
(Unknown)	-	?	?	?	-
(Unknown)	?	?	?	-	?
Totals	593.95	570.22	574.54	481.12	524.23

TABLE V

Average Micromoles x 10^{-3} per Egg of Free Amino Acids
Postdiapause

Amino acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	25.1	35.0	26.0	28.7	20.2
Arginine	10.1	9.60	8.48	8.00	10.7
Aspartic	55.5	60.1	55.8	48.1	52.6
Glutamic	53.0	38.0	38.0	39.4	39.4
Glutamine	Tr	Tr	Tr	Tr	Tr
Glycine	Tr	Tr	Tr	Tr	2.66
Histidine	42.0	44.3	27.7	44.3	25.8
Isoleucine	1.52	Tr	1.52	Tr	1.52
Leucine	8.80	6.40	8.48	7.01	7.62
Phenyl-alanine	-	-	Tr	Tr	-
Proline	114.	88.0	55.2	56.6	47.9
Serine	49.4	46.8	47.5	43.8	39.5
Threonine	22.2	Tr	21.1	21.8	Tr
Tryptophan	Tr	Tr	Tr	Tr	9.36
Tyrosine	14.6	10.1	13.0	11.0	14.6
Valine	15.0	15.7	21.4	18.7	16.0
(Unknown)	?	?	?	?	-
(Unknown)	?	-	-	?	-
(Unknown)	?	?	?	-	?
Totals	411.22	354.0	324.18	327.41	287.86

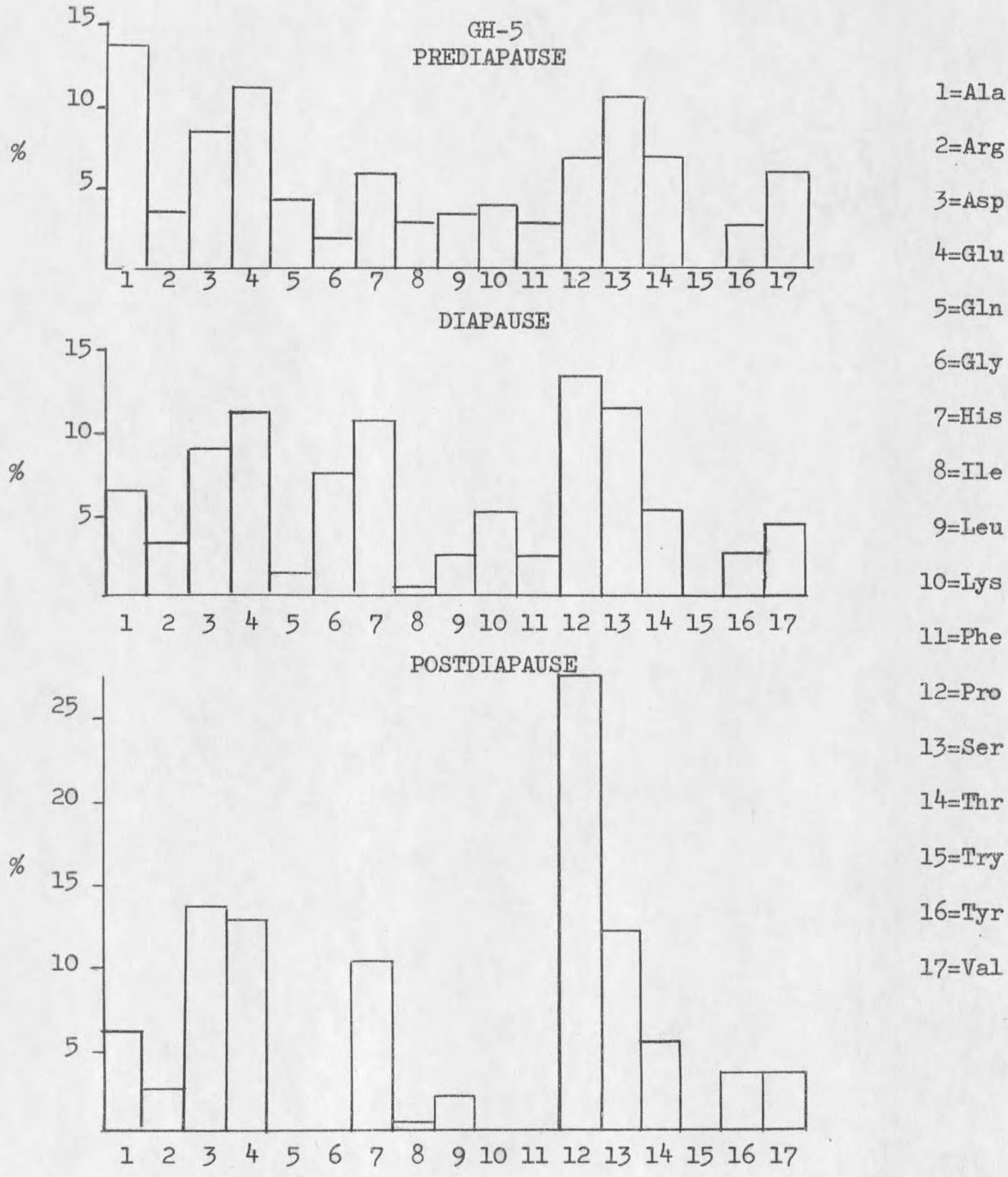

Figure 6. Percentages in total content of free amino acids from GH-5 eggs analyzed at the prediapause, diapause, and postdiapause stages of development.

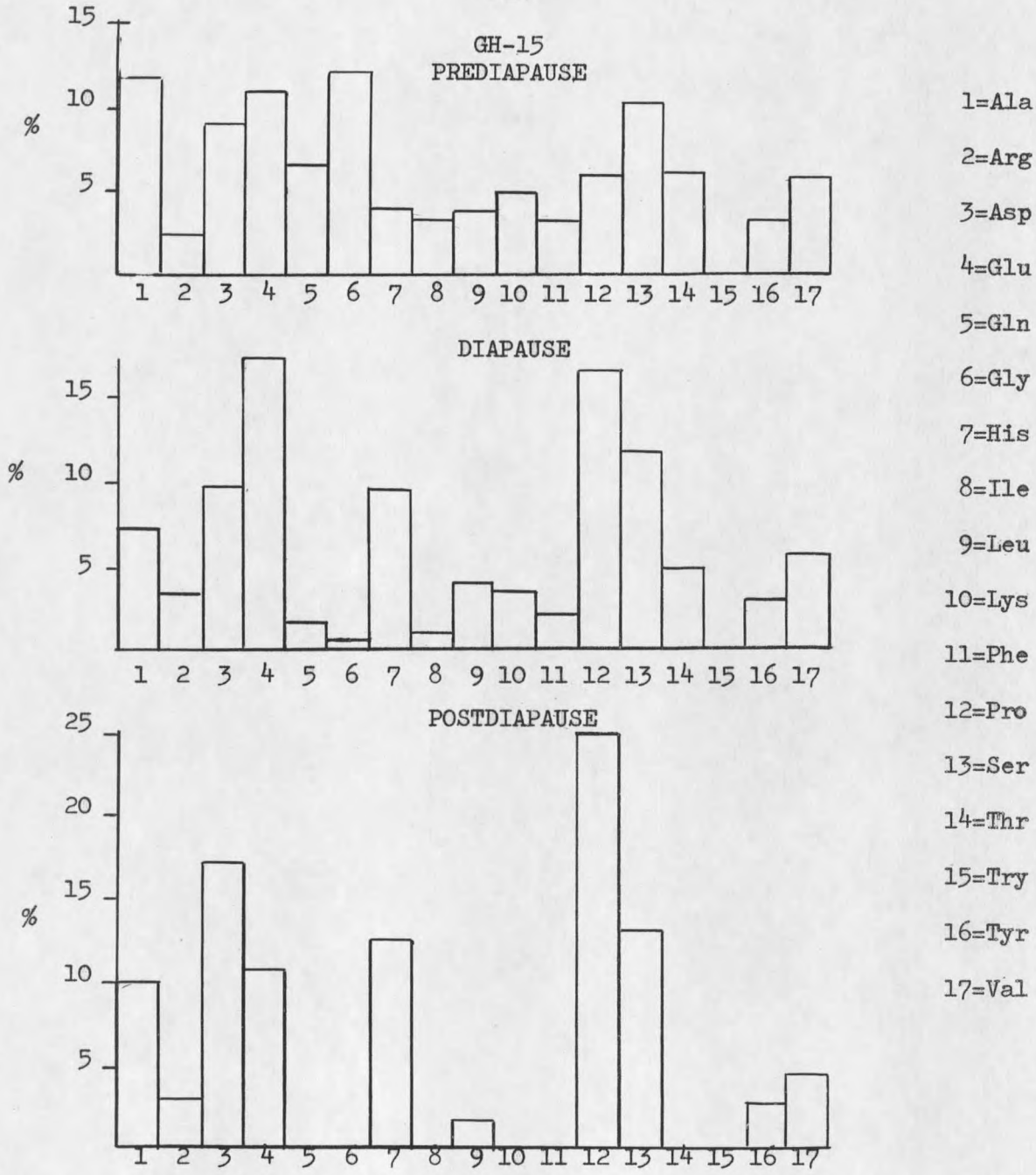

Figure 7. Percentages in total content of free amino acids from GH-15 eggs analyzed at the prediapause, diapause and postdiapause periods of development.

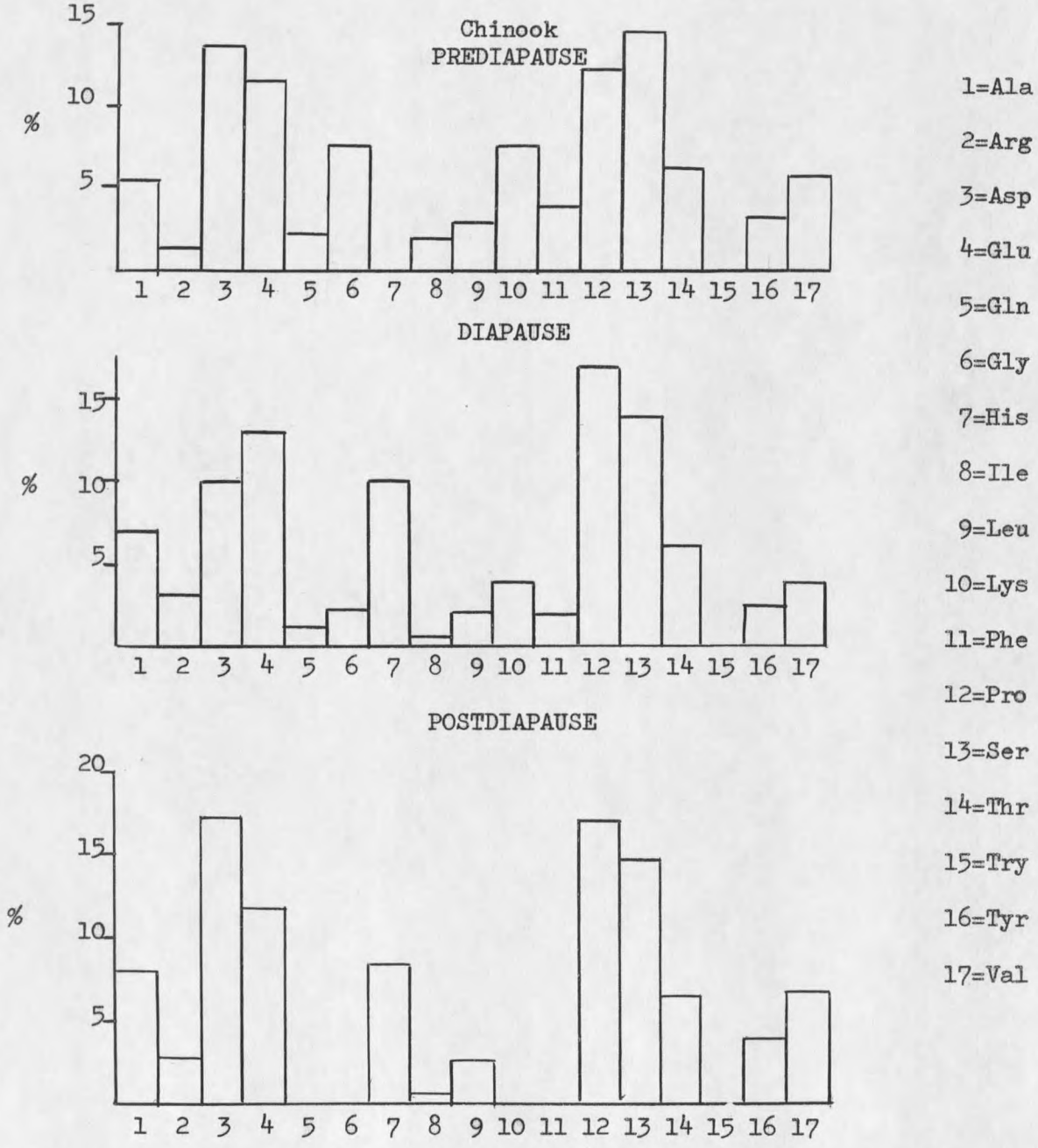

Figure 8. Percentages in total content of free amino acids from Chinook eggs analyzed at the prediapause, diapause, and postdiapause periods of development.

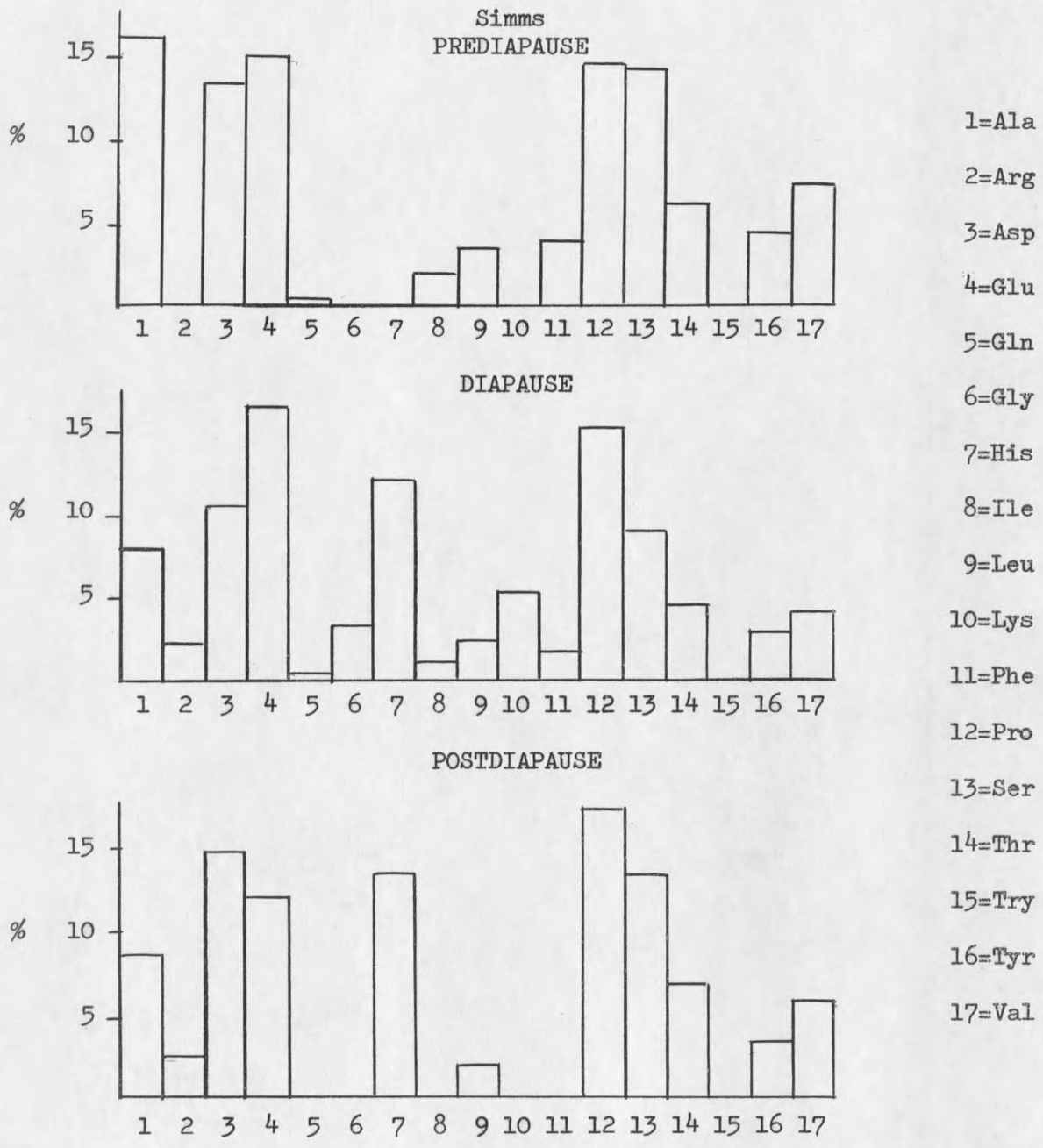

Figure 9. Percentages in total content of free amino acids from Simms eggs analyzed at the prediapause, diapause, and postdiapause periods of development.

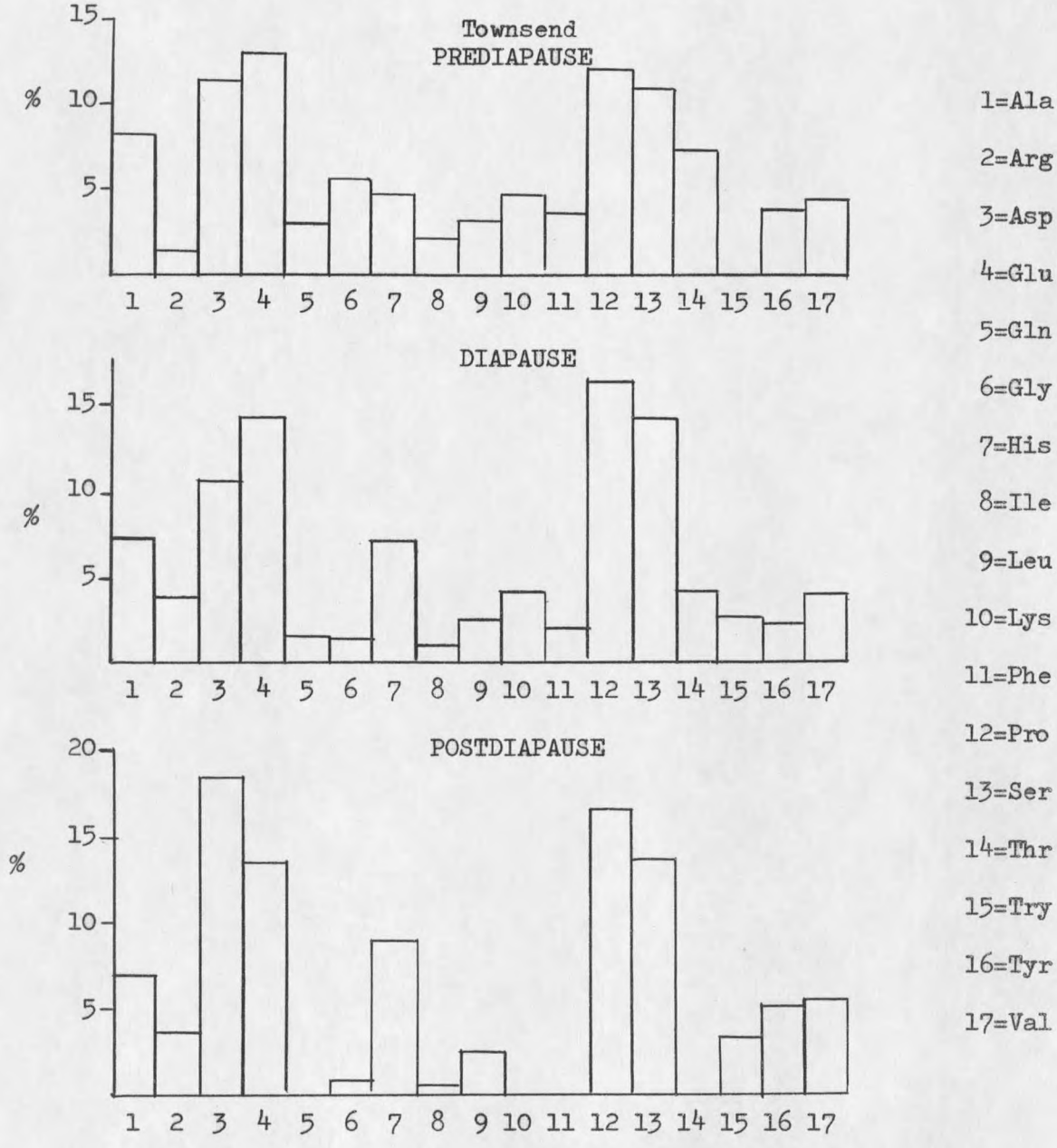

Figure 10. Percentages in total content of free amino acids from Townsend eggs analyzed at the prediapause, diapause, and post-diapause periods of development.

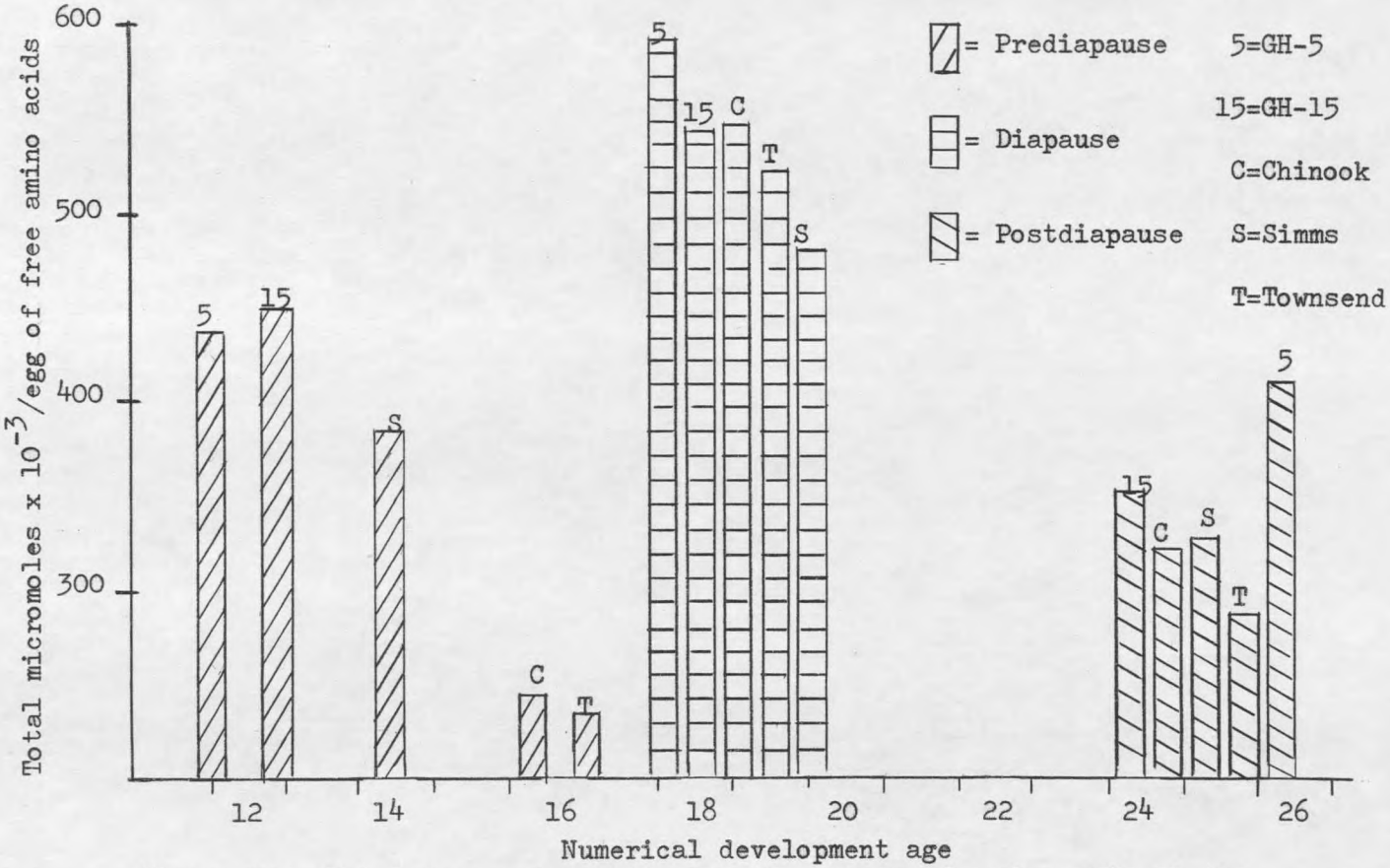

Figure 11. Total free amino acid content per egg of each sample at the different numerical stages of development.

content between embryos and their surrounding yolk during the diapause stage, one sample of Chinook eggs in this stage was prepared in the following manner. One hundred eggs, suitable for study, were removed from their protective pods, divided into groups of ten, and immersed in distilled water previously chilled to the ambient temperature of the cold room. The eggs were then opened and, as meticulously as possible, the yolk material separated from the embryo. This material was left in the distilled water and the embryos transferred to a container into which 50 percent ethanol was added. The yolk-water mixture was evaporated to near dryness and absolute alcohol added to bring the mixture to 80 percent alcohol by volume. The free amino acids then were extracted from the samples of yolk and embryos in the manner previously described. Identical amounts of yolk and embryo extracts were pipetted onto separate chromatoplates and developed as before. The results are shown in Figures 12 and 13.

Radioactive tracing

In general, radiochemicals are introduced into an animal either by including them in the subject's diet or by injecting them directly into the animal (Mohri, 1964; Strong and Sakamoto, 1963). Including a radioisotope in the diet has no application with the cleidoic eggs of A. elliotti. The injection technique also has limitations since the procedure itself causes a wound that could possibly alter the embryonic amino acid metabolism. Roemhild (1963) employed a technique in which an entire sample of eggs was

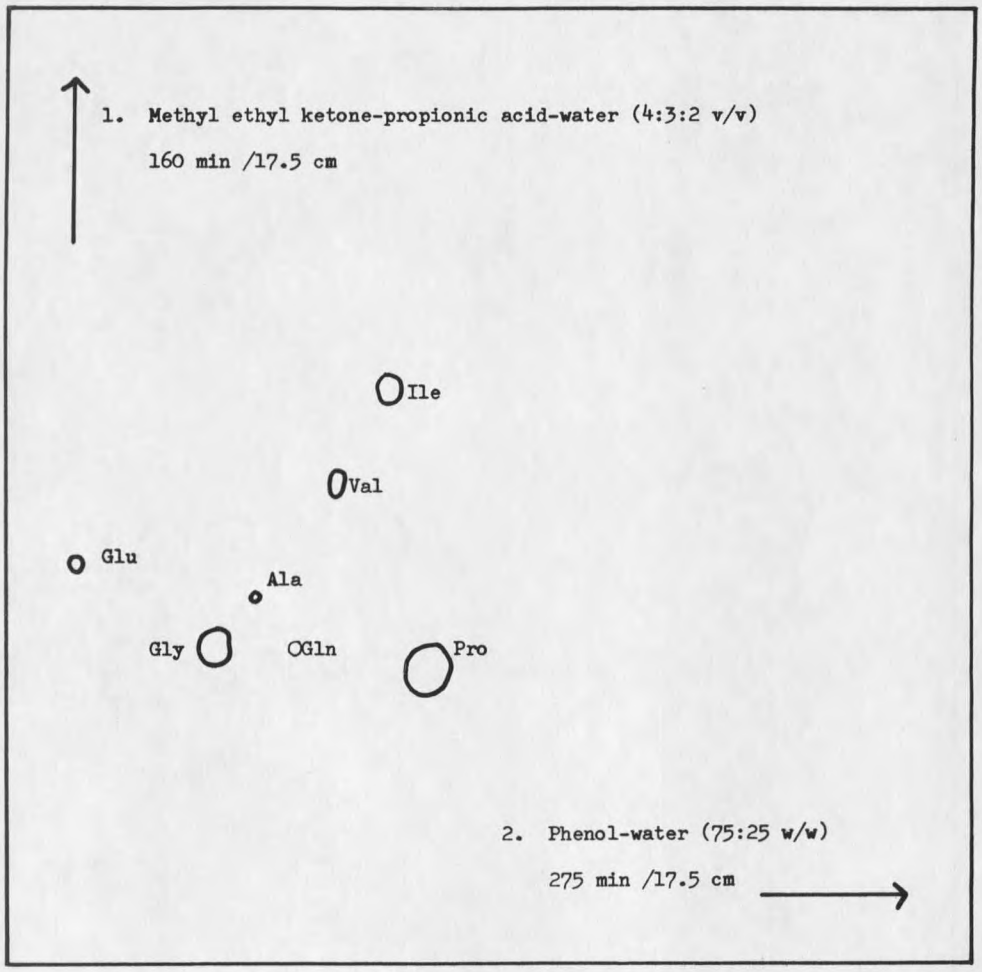

Figure 12. Two-dimensional separation of 7 free amino acids from embryos of Chinook diapause eggs.

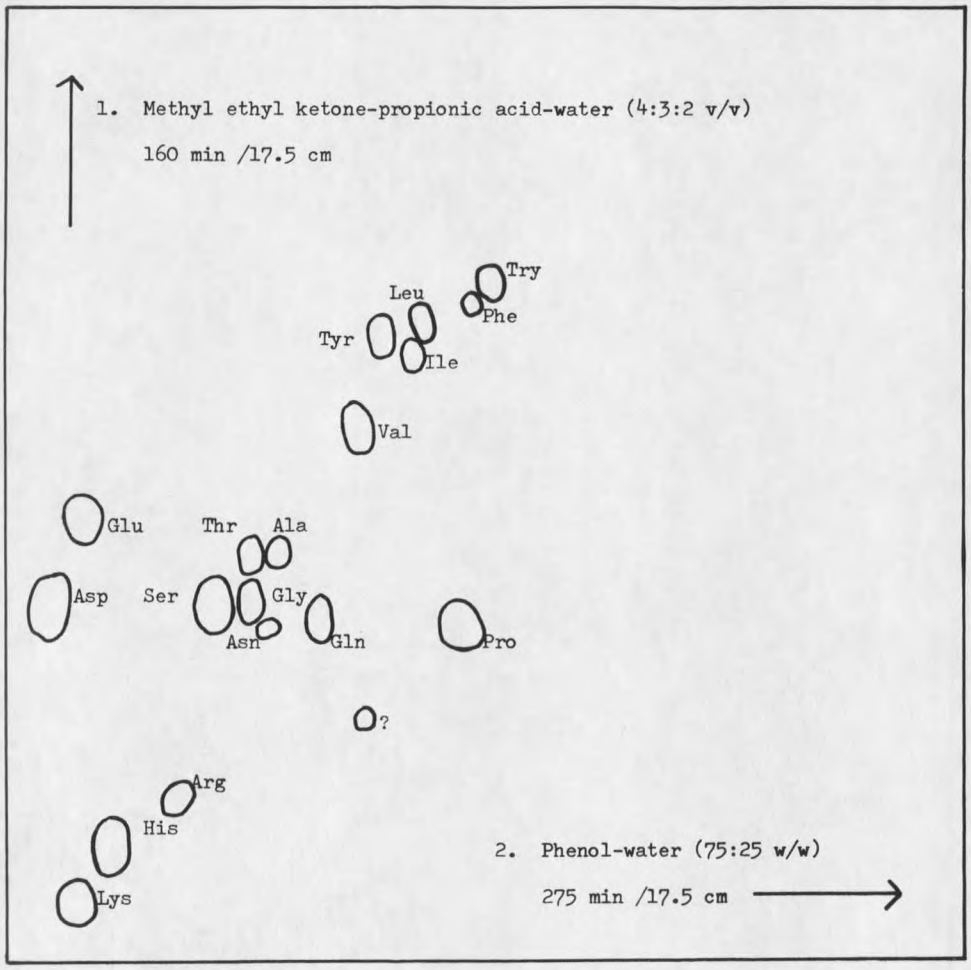

Figure 13. Two-dimensional separation of 18 free amino acids extracted from yolk material of Chinook diapause eggs.

dessicated for a few days and then allowed to absorb water in which a radiochemical had been dissolved.

In the present study 50 microcuries of acetate-1-C^{14} (specific activity 12.1 mc/mm) were dissolved in two milliliters of water. When approximately the same percentage of total weight was lost by dessication from each of the samples, the radioactive acetate (19,120 cpm per egg) was added. After an arbitrarily selected five day absorption period, the free amino acids were extracted from the eggs. The total weight of each egg sample was recorded daily throughout the dessication and absorption periods. The average weight loss per day per egg during dessication and the corresponding average weight gain per day per egg during the absorption period for each sample was calculated and is shown in Table VI.

For all populations, except Simms, two samples were prepared, one for quantitative colorimetry and one for radioactive analysis. There was only enough material from Simms for one 50 egg sample at each of the developmental stages analyzed so the colorimetry test was run also on the radioactive sample.

To determine if C^{14} was incorporated into the free amino acids extracted from eggs to which acetate-C^{14} was added, the samples first were chomatographed two-dimensionally with the different solvent systems. Locations of individual amino acids were made by spraying the plates lightly with 0.02 percent ninhydrin in absolute ethanol (Schaefer, 1963,

TABLE VI

Change in Egg Weight during Dessication and Absorption Periods

Populations	Average Weight Loss per Day per Egg	Average Weight Gained per Day per Egg
	Prediapause	
Chinook	0.050 mg	0.593 mg
Simms	0.088	0.414
Townsend	0.412	0.218
GH-5	0.038	0.891
GH-15	0.055	0.956
	Diapause	
Chinook	0.013	0.109
Simms	0.023	0.104
Townsend	0.033	0.121
GH-5	0.037	0.056
GH-15	0.046	0.093
	Postdiapause	
Chinook	0.006	0.094
Simms	0.020	0.020
Townsend	0.024	0.112
GH-5	0.021	0.067
GH-15	0.044	0.032

1964). Each spot then was scraped into a scintillator vial and liquid scintillation fluid consisting of a mixture of diphenyloxazole, toluene, and methanol was added (Birks, 1953; Roemhild, 1963). Radioactive emissions from each amino acid sample were counted in a liquid scintillation apparatus (Packard TriCarb Liquid Scintillation Spectrometer, Series 314a) and continued until a counting error of only five percent was attained (Aronoff, 1956).

The study was undertaken to determine if there were differences between the populations in their ability to utilize acetate in synthesizing amino acids. Each sample was analyzed in triplicate. Radioactivity, above the normal background counts for the free amino acids which incorporated the acetate, is listed in Tables VII, VIII and IX. The total radioactivity of each sample at the different developmental stages was graphed and is shown in Figure 14.

Free amino acids in A. smithii from Chinook

No studies have been reported on the ability of adult A. elliotti to synthesize amino acids nor have any been reported on the presence of these compounds in this grasshopper's preferred food plant. Thus it seemed desirable to determine which free amino acids were available to the adult grasshoppers through their diet and subsequently available to the embryos as nutritive materials contained in the yolk.

In early September fresh cuttings of A. smithii were collected from the habitat of A. elliotti near Chinook. Ten grams of this grass were

TABLE VII

Average CPM-Bkgd per Egg of Free Amino Acids
Prediapause

Amino acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	38.5	18.8	8.3	23.2	6.2
Aspartic acid	9.2	5.2	9.0	11.6	1.1
Glutamic acid	111.6	50.2	55.8	91.7	33.3
Glutamine	32.8	9.7	20.1	28.9	5.7
Glycine	4.2	-	-	-	-
Lysine	0.9	1.2	1.0	1.9	1.4
Proline	14.4	2.8	25.4	27.8	6.4
Serine	1.7	-	-	4.7	-
Threonine	3.0	1.5	-	-	-
Totals	216.3	89.4	119.6	189.8	54.1

TABLE VIII

Average CPM-Bkgd per Egg of Free Amino Acids
Diapause

Amino acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	4.9	5.9	1.7	3.6	1.8
Asparagine	-	-	0.8	1.4	-
Aspartic acid	5.2	2.6	2.2	4.0	1.3
Glutamic acid	37.0	28.6	35.4	30.1	27.8
Glutamine	8.0	5.4	5.8	9.2	4.5
Glycine	0.9	0.9	1.2	0.7	1.1
Proline	0.9	2.2	1.8	1.4	-
Serine	0.8	0.8	0.6	1.6	0.2
Totals	57.7	46.4	49.5	52.0	36.7

TABLE IX

Average CPM-Bkgd per Egg of Free Amino Acids
Postdiapause

Amino acids	GH-5	GH-15	Chinook	Simms	Townsend
Alanine	3.1	7.2	6.5	4.9	12.1
Aspartic acid	1.2	1.2	3.8	2.1	6.6
Glutamic acid	13.4	14.9	23.3	19.9	40.1
Glutamine	9.1	7.5	13.4	8.1	26.8
Glycine	0.9	1.8	2.4	1.6	3.5
Proline	2.7	5.2	8.0	1.8	4.0
Serine	0.4	1.3	0.4	2.0	1.0
Totals	30.8	39.1	57.8	40.4	94.1

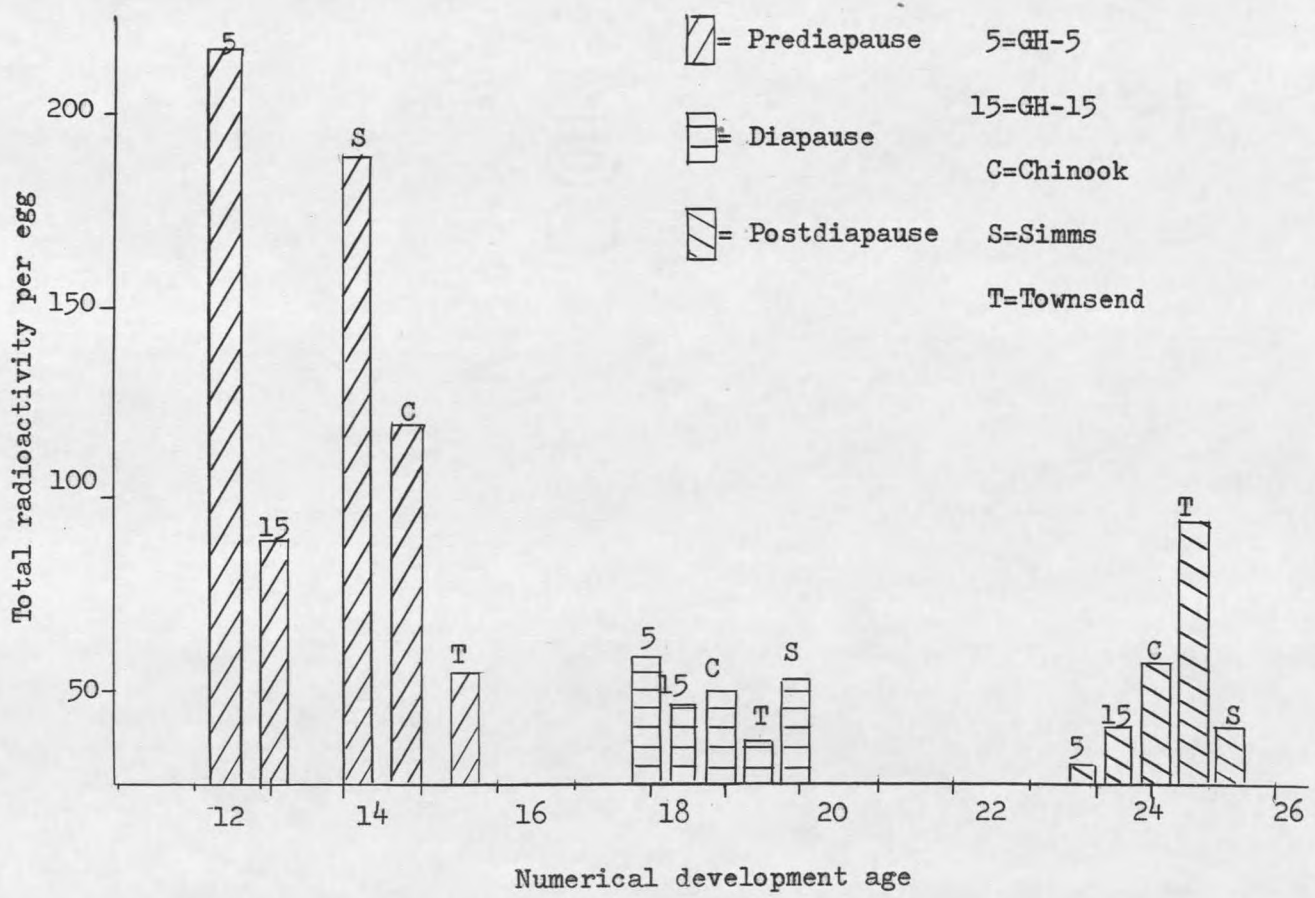

Figure 14. Total radioactivity per egg of each sample at the different stages of development.

triturated with 80 percent ethanol in a Hamilton Beach blender (Model 6) and the free amino acids extracted and determined as described earlier.

A chromatograph of the distribution of individual amino acids from the Chinook grass is shown in Figure 15. Quantitative results, based on three separate analyses, are presented in Table X. Seventeen amino acids were identified from the grass and two unknown spots appeared.

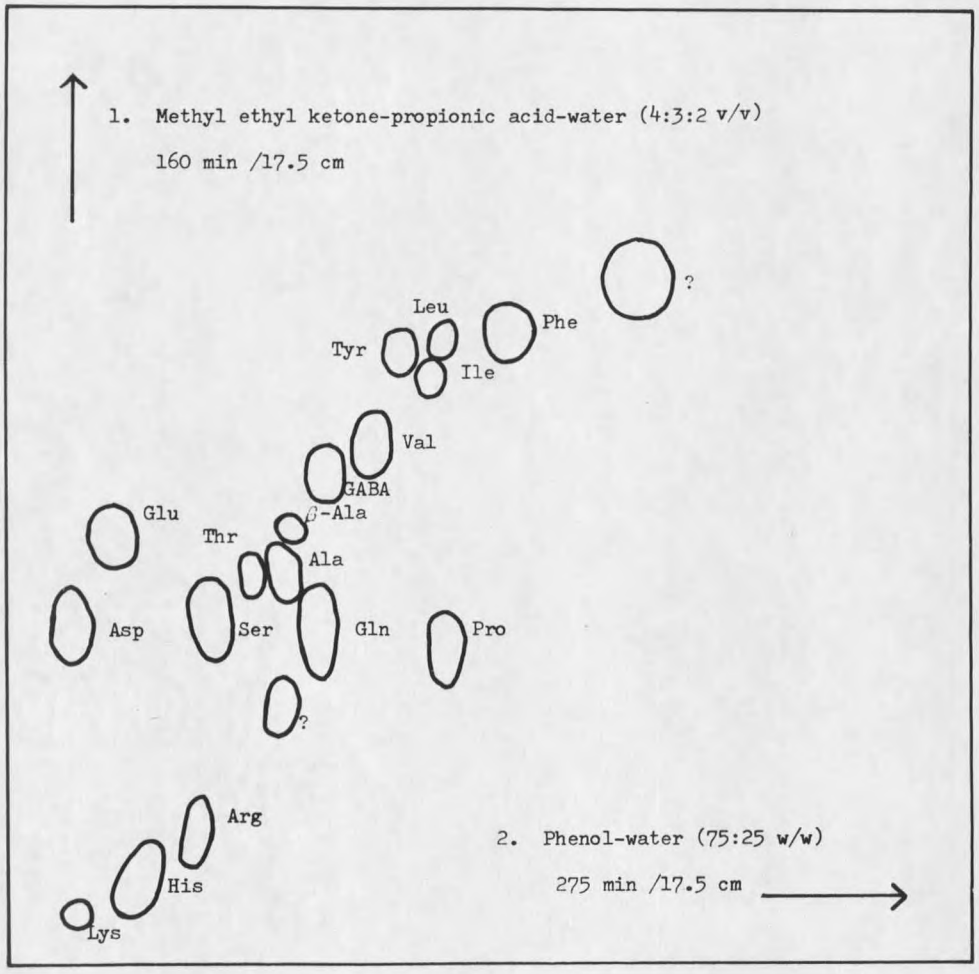

Figure 15. Two-dimensional separation of 17 free amino acids and two unknown compounds extracted from a sample of Agropyron smithii grass.

TABLE X

Free Amino Acid Content of *Agropyron smithii*

Amino acids	Average micromoles x 10^{-3} per 10 mg
Alanine	3.92
Alanine	Tr
Arginine	Tr
Aspartic acid	Tr
Glutamic acid	4.13
Glutamine	3.81
Histidine	2.72
Isoleucine	0.61
Leucine	1.41
Lysine	Tr
Phenylalanine	2.14
Proline	4.78
Serine	4.78
Threonine	Tr
Tyrosine	1.89
Valine	2.03
GABA*	?
(Unknown)	?

*GABA=gamma amino-*n*-butyric acid

DISCUSSION AND CONCLUSIONS

Fourteen free amino acids were found in each sample of A. elliotti eggs from the Chinook, Simms, and Townsend populations and in the greenhouse populations of five and 15 pairs of adults per cage, henceforth referred to as GH-5 and GH-15, respectively. These 14 acids were all present in the prediapause, diapause, and postdiapause developmental stages, and include alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, proline, serine, threonine, tyrosine, and valine (Figures 3, 4, 5; Tables III, IV, V). Two additional amino acids, lysine and phenylalanine, were present in prediapause and diapause stages of all egg samples but were not found in the postdiapause stage in any sample. Asparagine appeared in the diapause stage in eggs of all populations except those of Townsend. Those from this area only contained beta-alanine in the diapause stage. Tryptophan was found in diapause eggs from the Townsend and GH-15 groups and in postdiapause eggs from all populations.

These results are in general agreement with those of other workers. Auclair and Dubreuil (1951) found the same free amino acids plus phenylalanine in the last larval instar of Galleria mellonella. Micks and Ellis (1952) isolated and identified 18 free amino acids in different developmental stages of Aedes aegypti and Culex quinquefasciatus. Two of these amino acids, taurine and methionine, were not found in A. elliotti eggs; however, asparagine, glutamine, and phenylalanine, not found by Micks and Ellis, were present in A. elliotti eggs. Eggs of the grasshopper,

Chortophaga viridifasciata, were found to contain 15 free amino acids (Shaw, 1955) not including alanine, glutamine, histidine, lysine, and valine which were identified in eggs of A. elliotti. Colombo et al., (1962) extracted 20 free amino acids from eggs of the locust, Schistocerca gregaria, but did not report finding any asparagine.

No sulfur containing amino acids were found in any of the A. elliotti egg samples although test chromatographs of standard amino acids (Figures 1,2) included cysteic acid, methionine, and methionine sulfone. Cysteic acid and methionine sulfoxide were found in G. mellonella (Auclair and Dubreuil, 1951), methionine was found in A. aegypti and C. quinquefasciatus (Micks and Ellis, 1952) and cysteine, cystine, and methionine were identified in S. gregaria eggs (Colombo et al., 1962). According to Chen (1962), out of a total of 20 different species of insects examined, cysteine was isolated and identified from two species, cystine from nine species, methionine from 16 species, and methionine sulfoxide from two species.

Cysteine can be oxidized to cystine and cysteic acid, and methionine can be oxidized to methionine sulfoxide and methionine sulfone in the presence of phenol vapors (Aronoff, 1956; Shaw, 1955). Since phenol was used as a solvent in the chromatographic separations, cysteic acid and methionine sulfone were included in the initial test separations of standard amino acids. Additional tests also were run to establish R_f values for cysteine, cystine, and methionine sulfoxide in the different solvents used. The R_f values of the unknowns in Tables III, IV, and V did not conform to

those of cysteine, cystine, or methionine sulfoxide.

The quantitative results (Tables III, IV, V; Figures 6, 7, 8, 9, 10) show that there were differences in both the individual and total free amino acid content between eggs from different populations analyzed at the same stage of development. These differences were most noticeable in the prediapause stage (Table III). The total free amino acid content of eggs in this stage was highest in those from GH-5 and GH-15, somewhat lower in Simms eggs, and lowest in those from Chinook and Townsend.

The differences in total free amino acid content between eggs in the diapause stage from different populations (Table IV) were not as great as they were in the prediapause stage. In the diapause stage Simms eggs contained the lowest total acids and GH-5 eggs contained the highest while concentrations in the Chinook, GH-15, and Townsend eggs were intermediate between the high and low values.

The total free amino acid content in eggs from various populations varied the least in the postdiapause stage (Table V). The acid content was highest in GH-5 eggs at this stage and lowest in Townsend eggs while those of GH-15, Chinook, and Simms contained intermediate amounts.

The quantitative values in Tables III, IV, and V were analyzed statistically using the F-Test to determine the variance in the free amino acid content of eggs from the various populations at their different developmental stages. This F value was 6.843 for prediapause eggs (significant at the 99.5 percent level), 2.113 for diapause eggs (significant

at the 90 percent level), and 1.919 for postdiapause eggs (no significance). The data in Table I show that eggs from the wild populations and those from the greenhouse were in different stages of embryonic advancement according to the numerical staging criteria developed by Van Horn (1963) for A. elliotti embryos. The differences between populations in total free amino acid content of prediapause eggs therefore may have been the result of variations in developmental age between the populations. No correlations between the free amino acid content and the weight of each sample was evident from this study (Table II).

The diapause egg samples from the five populations were significantly different (at the 90% level) from one another in total free amino acid concentration. The two experimental groups, GH-5 and GH-15, were collected as nymphs from the same wild population and were reared under identical conditions in the greenhouse with the exception of population density. The histograms (Figures 6-10) show that, in diapause, the percentage concentration of each amino acid was similar for all samples with the exception of glycine in those from the GH-5 and GH-15 populations. Between these two samples the ratios of glycine content to the total amino acid content of each sample differed by 7.4 percent. This difference between the two greenhouse populations could have contributed significantly to the 90% correlation. While the wild populations from which the eggs were collected, differed from one another in population density, additional factors other than this may have masked differences in free amino acid

content that might otherwise have appeared. Such factors as food, developmental age, genetic differences, etc., could be responsible. Very significant differences in free amino acid composition were found between normal and mutant (lethal-meander) larvae of D. melanogaster, Chen and Hadorn (1955). They discovered that the total free amino acid content of normal larvae was two to three hundred percent higher than in the mutant larvae of the same age. No such differences were found between the populations reported in this study.

The data in Figure 11 illustrate the change in total free amino acid content of A. elliotti eggs as development proceeds from prediapause to postdiapause. It will be noted that a decrease in total concentration of the acids occurred from Stage 11 to Stage 17 in prediapause eggs. Noticeable decreases in individual amino acid concentrations during this stage occurred in alanine, glutamic acid, glutamine, leucine, serine, threonine, and valine. Although these measurements were made on samples from different populations, they may indicate what happens to the free amino acid content of eggs from a given population during prediapause development. The possibility exists that during the prediapause stage free amino acids are utilized for protein synthesis in embryonic histogenesis faster than they can be replaced by either yolk protein hydrolysis or by embryonic synthesis. Chen (1962) stated that, during the embryogenesis of insects, protein metabolism is especially intensive and involves mainly the breakdown of yolk materials with conversion of

the products into new organ-specific proteins. Shulov (1957) demonstrated that proteolytic enzymatic activity occurred in the yolk in various embryonic stages of the eggs of Locusta migratoria.

A striking increase in total free amino acid concentration from the prediapause to diapause periods was noted in all egg samples (Figure 11). The acids that increased the most in concentration in all samples during this period were aspartic acid, glutamic acid, histidine, proline, and serine (Tables III, IV). While the relative concentration of glutamic acid increased in all egg samples from the prediapause to the diapause stage, the relative concentration of glutamine decreased. Crone-Gloor (1959) noticed that, during the embryonic development of D. melanogaster, the concentration of glutamine was inversely proportional to that of glutamic acid. Colombo et al., (1962) reported that in eggs of S. gregaria the glutamic acid content dropped at the end of embryonic development while the glutamine content increased.

The high concentrations of the total free amino acids in eggs from all populations in the diapause stage may be related to a decrease in embryonic incorporation of protein during this period. Van Horn (1963) stated that the diapause period of A. elliotti usually begins when individuals reach Stage 19 at which time most embryos enter a period of retarded development. While a decrease in morphological development during diapause would be associated with a decrease in an embryonic protein synthesis, the hydrolysis of yolk protein reserves to yield free

amino acids may still continue thus resulting in an accumulation of these compounds by the end of the diapause. The chromatographs in Figures 12 and 13 show that the Chinook egg sample, in diapause, contained a greater concentration of free amino acids in the yolk than in the embryo. That the highest concentration of total free amino acids in all egg samples in the diapause stage was due to a continuation of yolk degradation was indicated also by the decrease in total radioactivity of free amino acids from the prediapause to the diapause stage (Figure 14; Table VII, VIII). This decline in total radioactivity indicates that the ability of the embryos to utilize acetate as a precursor for the synthesis of free amino acids is decreased from the prediapause to the diapause stage.

When diapause is broken, development is resumed. It should be noted that the total radioactivity of free amino acids did not increase from the diapause to postdiapause stage but remained at approximately the same level. Thus it would seem that the accumulation of free amino acids during diapause was sufficient to provide the embryos with the necessary amino acid building blocks for postdiapause protein synthesis and histogenesis without an increase in embryonic amino acid synthesis during the postdiapause stage.

By Stage 25 of postdiapause development, dorsal closure of the embryo is completed, and there is no more extraembryonic yolk (Van Horn, 1963). Since most of the postdiapause egg samples were analyzed for free

amino acid content at Stage 25 (Table I), the analyses reflect the embryonic free amino acid content and not that of the yolk. Therefore, it should be noted that the concentrations of free amino acids in all the postdiapause egg samples were considerably lower than in the corresponding samples during diapause (Figure 11; Tables IV, V). Glutamic acid, glycine, isoleucine, lysine, phenylalanine, and serine showed the greatest decrease in concentration in the postdiapause samples. The histograms (Figures 6-10) indicate that the concentrations of individual amino acids were similar in the various postdiapause samples except for small differences in concentration of glutamic acid, proline, threonine and tryptophan.

Colombo et al., (1962) published data on changes in the free amino acid composition throughout the embryonic development of nondiapausing eggs from a single population of S. gregaria. They analyzed laboratory reared S. gregaria eggs at eight different developmental stages ranging from the time the eggs were in the oviducts to the time they were ready to hatch. If the development of the external morphology is the same in embryos of S. gregaria and A. elliotti, perhaps the free amino acid content of eggs of the latter, can be compared with that of S. gregaria eggs at similar morphological stages.

Colombo and his co-workers determined that the total free amino acid concentration of the nondiapausing whole eggs of S. gregaria was 53.01 micromoles x 10^{-3} per egg after one day of incubation (Stage I),

74.48 micromoles x 10^{-3} per egg after two days of incubation (Stage 5, 6), 1570.20 micromoles x 10^{-3} per egg after three days of incubation (Stage 8, 9), 1536.0 micromoles x 10^{-3} per egg after six days of incubation (Stage 16, 17), 1959.42 micromoles x 10^{-3} per egg after nine days of incubation (Stage 20), and 870.23 micromoles x 10^{-3} per egg after twelve days of incubation (Stage 23). The total free amino acid content of whole eggs of A. elliotti ranged from 233.83 to 447.72 micromoles x 10^{-3} per egg in the prediapause period (Stage 11-17), from 481.12 to 593.95 micromoles x 10^{-3} per egg in the diapause period (Stage 18, 19), and from 287.86 to 411.22 micromoles x 10^{-3} per egg in the postdiapause period (Stage 23-25). This comparison shows that the total free amino acid content in both A. elliotti and S. gregaria eggs followed approximately the same trend during embryonic development even though one is a diapause species, while the other is not. Comparatively speaking, the free amino acid concentration was low in early development, increased in later embryonic stages and then decreased just before embryonic development was complete.

The same trend in free amino acid concentration during development was described in D. melanogaster by Chen and Hadorn (1955), Hadorn and Stumm-Zollinger (1953), and Stumm-Zollinger (1954). Deviations from this pattern were noted in populations of certain lethal mutants of this species. For example, Hadorn and Stumm-Zollinger (1953) determined that in the blood of normal D. melanogaster larvae the concentration of amino acids decreased as the animal approached metamorphosis; while in the blood of

the lethal-translucida mutant, the concentrations of these compounds did not decrease but remained at the same level that was reached in earlier development. Even though the difference between the two populations was the result of a genetic mutation and was not due to an effect of population density stress, this illustrates the possible differences in free amino acid content that can exist between two populations of the same species.

All egg samples of A. elliotti from the prediapause through the postdiapause stage contained high concentrations of aspartic acid, glutamic acid, proline, and Serine (Tables III, IV, V). During this same period, histidine increased while glutamine, isoleucine, leucine, lysine, and phenylalanine decreased. By comparison the eggs of S. gregaria, from Stages 8 to 23, were noted to have high or increasing concentrations of alanine, beta-alanine, glutamine, serine, and tyrosine while the concentrations of arginine, aspartic acid, glutamic acid, and leucine declined during this period (Colombo et al., 1962). Micks and Ellis (1952) found large amounts of aspartic and glutamic acids in eggs of A. aegypti. Crone-Gloor (1959) discovered that in eggs of D. melanogaster the quantities of aspartic and glutamic acids fell rapidly during embryonic development while the concentrations of alanine and glutamine showed a distinct increase. Laven and Chen (1956) and Chen (1958) reported that the content of proline was especially high in the blood of C. pipiens larvae. Although tryptophan is a precursor for the phenoxazine pigments in insects (Gilmour, 1961), the quantity of free tryptophan was low in eggs of A. elliotti (Tables IV, V, VI) as it was in the eggs of S.

gregaria (Colombo et al., 1962) and in the hemolymph of Ephestia kuhniella larvae (Chen and Kuhn, 1956).

The egg samples to which radioactive acetate was added incorporated C^{14} into nine free amino acids in the prediapause stage (Table VII), eight in the diapause stage (Table VIII), and seven in the postdiapause stage (Table IX). Four radioactive free amino acids, alanine, aspartic acid, glutamic acid, and glutamine, were isolated from all samples in all stages. In the prediapause stage all samples also contained radioactive lysine and proline. In addition prediapause eggs from GH-5 contained radioactive glycine, serine, and threonine, those from GH-15 contained radioactive threonine, and those from Simms contained radioactive serine. The amount of C^{14} incorporated in the free amino acids was greater in the prediapause than in the diapause stage. In the latter stage all of the egg samples incorporated C^{14} into two additional amino acids, glycine and serine. In the same stage radioactive asparagine was isolated from Chinook and Simms egg samples while radioactive proline was found in all samples except those from Townsend. No qualitative differences were found in the incorporation of C^{14} into free amino acids between the different population samples in the postdiapause stage. During this stage radioactivity was found in alanine, aspartic acid, glutamic acid, glutamine, glycine, proline, and serine.

The total radioactivity of the free amino acids in each sample differed quite markedly in the prediapause stage (Table VII, Figure 14) while the diapause and the postdiapause egg samples were similar in total radioactivity

(Table VIII, IX). Comparison of Figures 11 and 14 shows that the pattern of total radioactivity between prediapause samples parallels closely the pattern of total free amino acid content between different population samples, with the exception of the GH-15 sample. The discrepancy in this latter sample between total radioactivity and total content of free amino acids may be due to the method used in introducing the radioisotope into the eggs. With the method used, there was no way of determining if each sample absorbed the same amount of acetate-C^{14}. The differences in radioactivity between populations in the prediapause samples (Figure 14) may be due to the differences in developmental age between the egg samples since the numerical stage of development was not identical in each of them (Table I).

Some reports have appeared in the literature on the incorporation of radioisotopes into the free amino acids in insects. Schaefer (1964) working with the adult sawfly, Neodiprion pratti, found that C^{14} from glucose-U-C^{14} was incorporated into alanine, glutamic acid, glutamine, glycine, histidine, proline, and serine. Strong and Sakamoto (1963) demonstrated the utilization of C^{14} in synthesizing alanine, aspartic acid, glutamic acid, glycine, cystine, and serine in the aphid, Myzus persicae. Kasting and McGinnis (1960, 1962) and Kasting et al., (1962), in a series of papers, demonstrated that C^{14} was incorporated into alanine, aspartic acid, serine, and proline in larvae of Phormia regina, into alanine, aspartic acid, cysteic acid, glutamic acid, glycine, proline, and serine in Ctenicera destructor. The general pattern of utilization of C^{14} in synthesizing free amino acids is the same between eggs of A. elliotti and other insect species.

Seventeen free amino acids were isolated and identified from samples of A. smithii, a preferred host plant of A. elliotti, taken from the Chinook eggs collection site (Figure 15, Table X). No sulfur containing amino acids were found in the grass although two unknowns were present, the R_f values of which did not match those of any amino acid in the standards used (Figures 1, 2, 15). The location of one of these unknowns, however, corresponded with the location of an unknown from the A. elliotti eggs (Figures 3, 15). The seventeen acids identified from A. smithii included sixteen which were found in the eggs of A. elliotti and gamma-amino-n-butyric acid, which was not in their eggs. Asparagine, glycine, and tryptophan were not found in A. smithii but were present in the grasshopper eggs. These three amino acids plus others such as the sulfur containing ones could still be present in the grass in the form of amino acid residues within proteins, and thus be available to the adult grasshopper.

This investigation has shown that the free amino acid composition of A. elliotti eggs, changed qualitatively and quantitatively as embryonic development proceeded from the prediapause through the postdiapause stage. While the free amino acid content differed between eggs from various populations during development, these may be correlated with differences other than the density of the populations from which the eggs were collected. It is of interest that Svoboda (1964) found that differences in fatty acid content between A. elliotti eggs from different populations during the diapause stage could not be correlated with population density. Neither could the

ability of A. elliotti eggs to incorporate acetate-C^{14} be correlated with population density differences.

SUMMARY

The free amino acid composition of A. elliotti eggs from three wild populations and two greenhouse-reared groups was determined quantitatively in the prediapause, diapause, and postdiapause periods of development. The free amino acids were extracted from each sample of eggs by trituration, centrifugation, and column chromatography. A two-dimensional thin-layer chromatographic system was developed to isolate and identify the individual free amino acids from the column eluate. The quantities of free amino acids present in each sample were determined colorimetrically.

A total of 19 free amino acids were identified from A. elliotti eggs during embryonic development. Fourteen of these acids were present in each population egg sample throughout development. Five additional acids and three unknown compounds were found in some of the egg samples. The free amino acid content of each egg sample was found to increase from the prediapause to the diapause period and then to decline during postdiapause development. The increase in free amino acids during the diapause period was suggested to be the result of yolk protein hydrolysis continuing during diapause while embryonic protein synthesis decreases during this stage.

The ability of A. elliotti eggs to synthesize free amino acids from radioactive acetate also was investigated. Radioactive acetate was incorporated into separate samples of eggs from the five populations during the three periods of embryonic development. A total of nine acids were found to contain radioactivity. Four free amino acids were synthesized in vivo in each sample of eggs during all developmental periods. Five

additional ones were synthesized *in vivo* only in specific stages of development. The total radioactivity measured in each sample was found to decrease from the prediapause to the diapause stage. A slight increase in total radioactivity of each sample was recorded from the diapause to the post-diapause periods. The decrease in radioactivity in all samples during the diapause stage was considered to indicate a decreased *in vivo* synthesis of free amino acids during this stage.

Agropyron smithii, a grass from one of the grasshopper collection areas, was analyzed quantitatively for free amino acid composition. Seventeen acids plus one unknown substance were found. Sixteen of these acids were the same as those found in *A. elliotti* eggs while three of them, present in the eggs, were not found in the grass.

Differences in free amino acid content and in *in vivo* synthesis of free amino acids were found between eggs from the different populations of *A. elliotti* analyzed at the same stage of development. The greatest difference was found in the content of glycine between the samples from the two experimental populations which differed in density.

APPENDIX

TABLE XI

R_f Values of Some Amino Acids in the CMA solvent

Amino acids	R_f values
Alanine	.54
Beta-alanine	.40
Arginine	.09
Asparagine	.55
Aspartic acid	.49
Cysteic acid	.77
Cystine	.72
Glutamic acid	.70
Glutamine	.65
Glycine	.48
Histidine	.55
Hydroxy-proline	.49
Isoleucine	.79
Leucine	.79
Lysine	.07
Methionine	-
Methionine sulfone	.75
Phenylalanine	.78
Proline	.39
Serine	.52
Threonine	.67
Tryptophan	.81
Tyrosine	.76
Valine	.74

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