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PROTEIN COMPOSITION OF THE DIFFERENT LIFE STAGES OF DOLANIA AMERICANA (EPHEMEROPTERA: BEHNINGIIDAE)

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Abstract. Proteins from the different life stages of Dolania americana were extracted by homogenizing the specimens in a solution containing 9.3 M urea, 5 mM K_2CO_3, 2% /v/v/ nonidet P-40 and 0.5% /w/v/ dithiothreitol. The homogenate was centrifuged and the resulting supernatant was subjected to two-dimensional gel electrophoresis /2-D PAGE/. The 2-D PAGE system resolved mayfly proteins into approximately 18 major and more than 50 minor polypeptides. The apparent MW of the polypeptides ranged between 18,000 to 140,000 and the pI's between 4 and 8. Comparison of polypeptide maps of male and female D. americana showed several differences in their polypeptide composition. For example, the males lacked three polypeptides with molecular weights around 18,000, 32,000 and 44,000. Likewise, three polypeptides with molecular weights around 22,000, 43,000 and 47,000 were absent in the female dark morph compared to the light morph. The light morph on the other hand lacked one polypeptide /MW 26,000/ that was present in the dark morph. The protein pattern of the eggs of both the light and dark morphs were similar and contained three major polypeptides with apparent molecular weights around 45,000, 53,000 and 140,000.

Electrophoresis, molecular weight, isoelectric point

Dolania americana is a burrowing mayfly occurring in relatively clean, circumneutral streams in the southeastern United States. The predaceous nymphs are the dominant mayflies in the Blackwater River in northwestern Florida, and in Upper Three Runs, a spring-fed blackwaterstream in South Carolina (Harvey et al. 1980). Females never moult to imagos and are polymorphic (Peters and Peters 1977). The female light morph has

predominantly white body while the dark morph has strikingly dark blackish-brown body. Data on the life history, habits of nymphs and adults, and emergence are found in Harvey et al. (1980), Tsui and Hubbard (1979) and Peters and Peters (1977).

The objective of the present study was to determine the protein composition of the different life stages of Dolania americana, and identify the difference in the protein composition of the female adult morphs and their eggs.

MATERIAL AND METHODS

Specimens used in this study were collected from the Blackwater River, northwestern Florida. The physical and chemical characteristics of the river were described by Beck (1973), and the flora and fauna by Peters and Jones (1973).

The adults and nymphs of D. americana were collected by light traps and standard handscreen, respectively. Both nymphs and adults were separately contained in plastic (Ziploc) bags and immediately placed in a cooler box with 2/3 full of ice. The eggs were extracted from the female adults by dissecting the abdomen under a field microscope. Eggs extracted from the light and dark female morphs were kept separately in different containers. All specimens were stored at -20° C until used.

Protein Extraction

The specimens (ten male imagos, ten each of light and dark adult female morphs, seven nymphs, and 200 - 300 eggs) were homogenized with 2 ml of a solubilization buffer containing 9.3 M urea, 5 mM K_2CO_3, 2% nonidet P-40 and 1.5% dithiothreitol. The homogenate was centrifuged at 20,000 g for 20 min and the resulting supernatant was subjected to two-dimensional polyacrylamide gel electrophoresis.

Two-dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE)

The protein extracts were subjected to 2-D PAGE following the methods of O'Farrell (1975) and Basha (1979). The first dimension consisted of isoelectric focusing in 4% (W/v) acrylamide gels using 2% (v/v) ampholines (pH 3.5 to 10; 5 to 7 and 9 to 11; 50 : 35 : 15 % respectively). The second dimension was sodium dodecyl sulfate gel electrophoresis in 10% acrylamide slab gels. Following electrophoresis, the proteins were fixed in 7% acetic acid and 40% ethanol and then stained with Coomassie blue.

Molecular Weight Estimation

The molecular weight (MW) of the polypeptides in SDS gels were estimated using proteins of known molecular weight. Protein standards used for calibration of the gel were thyroglobulin (334,500) β-galactosidase (130,000), phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000),

carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100) and lysozyme (14,300).

RESULTS AND DISCUSSION

Following 2-D PAGE, proteins of adult female dark morph resolved into approximately 75 polypeptide spots (Fig. 1a). The molecular weight of these polypeptides ranged between 20,000 to 140,000 and isoelectric points between 4 and 8. Of the total polypeptides 18 were major proteins and the rest were minor components. Figure 1b shows the polypeptide pattern of female light morph. The light morph also contained about 18 major polypeptide spots and several minor polypeptides. Most of the polypeptides were seen in the acidic pH region of the gel. Like the adult females, the protein pattern of male D. americana was also heterogeneous and contained at least 14 major and numerous minor polypeptides (Fig. 1c).

Based on the 2-D PAGE pattern, it appears that the majority of the polypeptides of the D. americana adults are acidic in nature and are composed of numerous complex polypeptides with varying isoelectric points and molecular weights.

Figure 1 also shows the polypeptide patterns of the eggs of female light morph (d) and dark morph (e). The egg protein patterns were simple and contained mainly three groups of polypeptides with apparent molecular weights of around 45,000 (A), 53,000 (B) and 130,000 (C). The polypeptide spots of the egg proteins were broad and diffused. This is probably due to the microheterogeneity in their composition resulting from the slight modifications in their amino acid composition and/or differential glycosylation. Such a pattern is typically seen with albumin and certain globulin type of proteins (Basha et al. 1980, Basha 1979).

The 2-D PAGE profile of nymphs was quite distinct from the adults (Fig. 1f). They contained five major acidic polypeptides with MW around 15,000 (A), 16,000 (B), 20,000 (C), 32, 000 (D), and 32,500 (E). The solubilization buffer used in this study appear to be less efficient in solubilizing the nymph proteins due to the presence of tough cuticle. Additional studies are in progress to devise a method for improved solubilization and better resolution of polypeptides from nymphs following 2-D PAGE.

Comparative Characterization

After establishing the 2-D PAGE pattern for female dark and light morphs, and male imagos, it was of interest to determine the compositional differences in their protein pattern. The comparative studies have revealed significant differences between the dark and light adult female morphs, and between male and female adults. In general, the light morph contained more polypeptides than the dark morph, and the male. For example the dark morph lacked the polypeptides (shown in arrows in

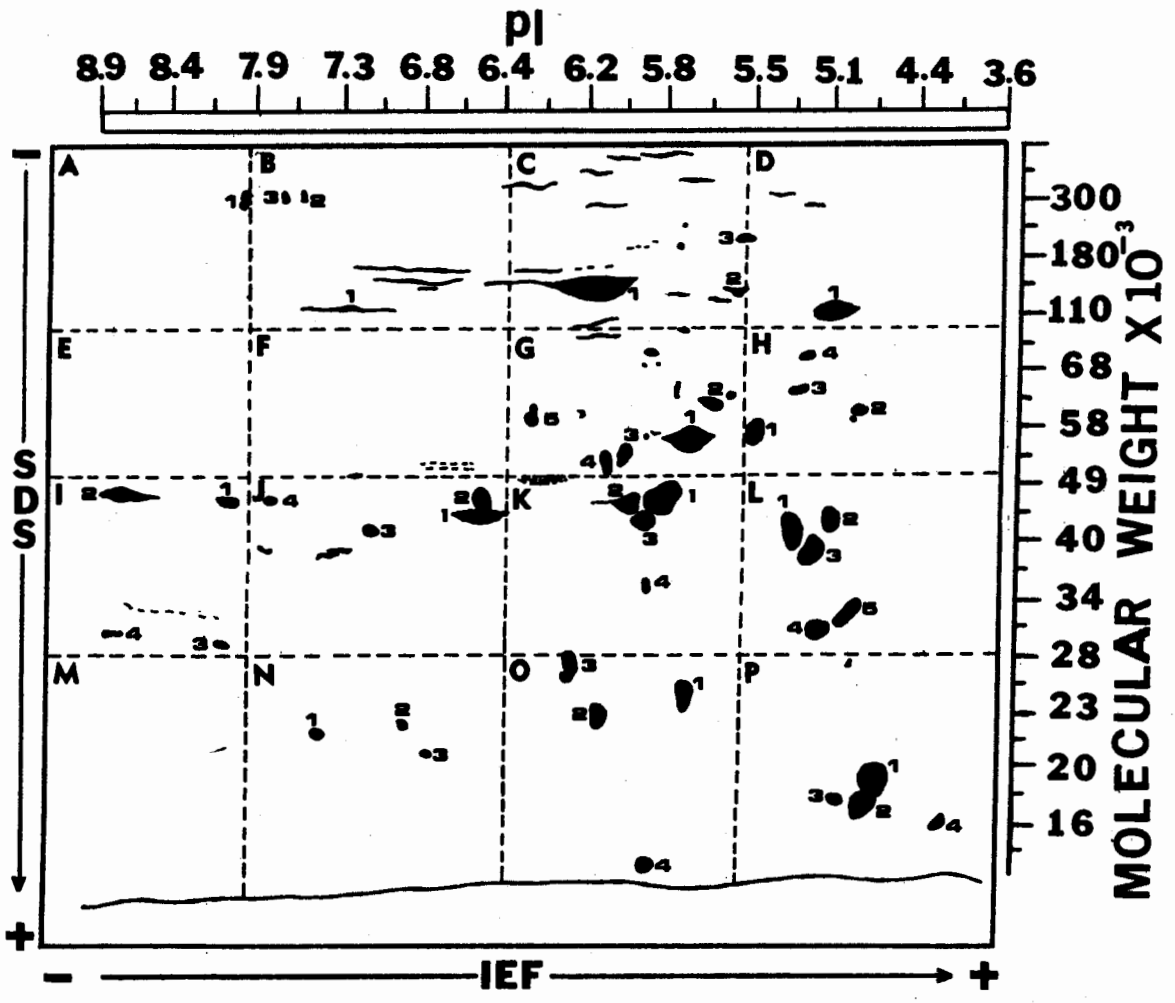

Fig. 1: Two-dimensional gel electrophoretic patterns of protein extracts from female dark morph /a/, female light morph /b/, male imago /c/, dark female morph eggs /d/, light female morph eggs /e/, and nymphs /f/ of Dolania americana. About 100-300 ug of protein was applied on each gel. The arrows in the adult protein patterns /a-c/ indicate missing polypeptides in the specimen, while the arrows in the eggs /d and e/ and nymph /f/ patterns indicate the molecular weights /given in the text/ of these polypeptides.

Fig. 2: Composite polypeptide map of adult D. americana protein extract which include all the polypeptides found in the female light and dark morphs, and the male imago. The polypeptide map has been divided into alphabetically labeled quadrants and the the polypeptides falling within each quadrant were assigned a number. Vertical bars represent different isoelectric points and the horizontal bars represent molecular weights.

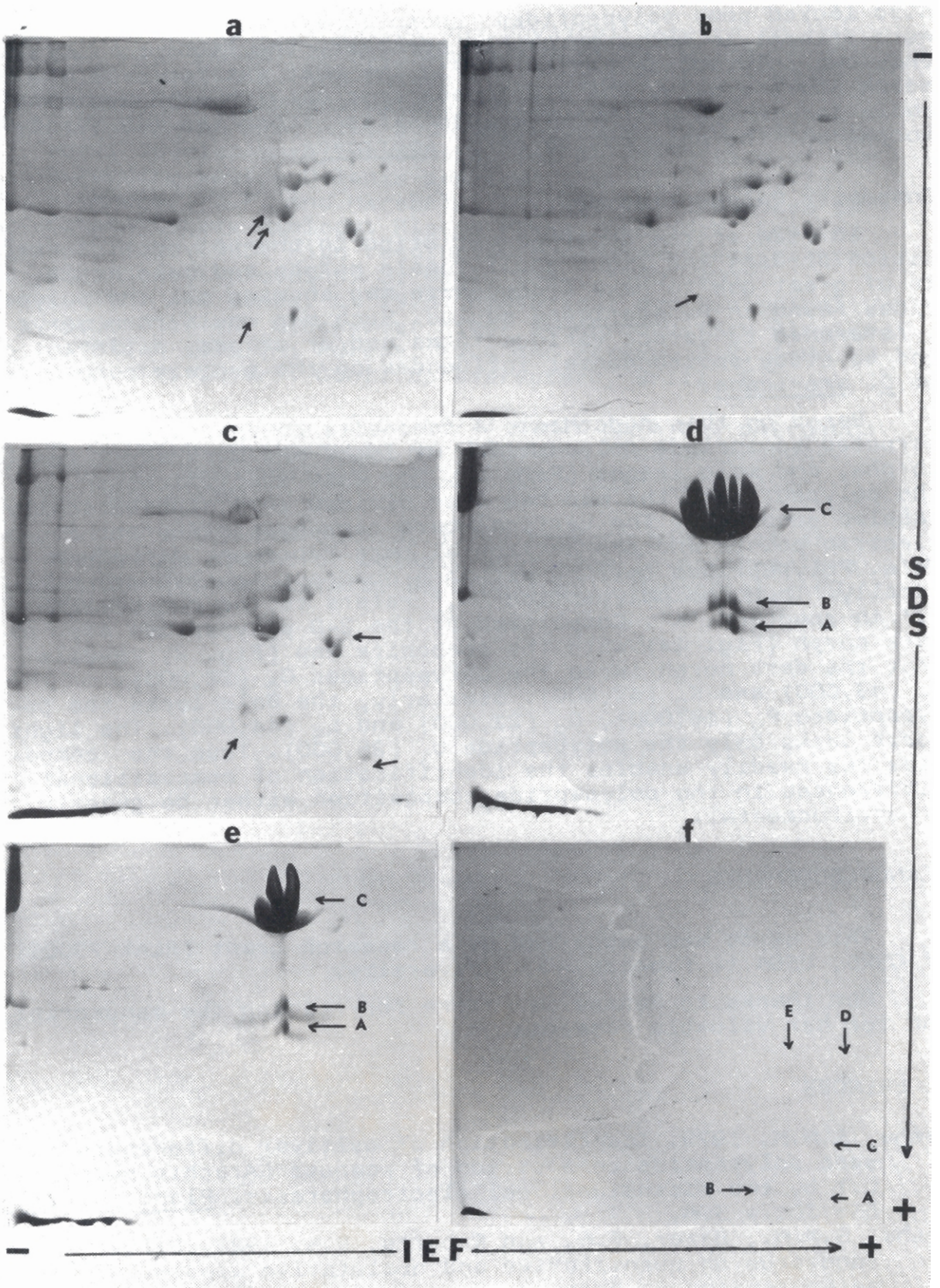
a
b
c
d
e
f
C
B
A
E
D
SDS
IEF
−
+

Fig. 1a) that were found in light morph. Similarly, the male also lacked some polypeptides (shown in arrows in Fig. 1c) that were present in white morph. In contrast, the light morph lacked only one polypeptide (arrow in Fig 1b) that was found in the dark morph and the male. The electrophoresis data thus showed inherent variation in the polypeptide composition among the two female morphs and the male adults of D. americana.

Composite Polypeptide Map

In view of the apparent variation in the polypeptide composition of dark and light adult female morphs and male adult, it is of great use to have a composite map showing all the polypeptides found in D. americana adults. Such map would then serve as a reference to detect various forms and/or stages, different sexes, and to study environmental influences on the protein composition of D. americana.

Based on this hypothesis a composite polypeptide map is constructed to include polypeptides of light and dark adult female morphs, and male imagos (Fig. 2). For easy identification of the polypeptides, the map has been divided into alphabetically labeled quadrants. The vertical bars represent different isoelectric points and the horizontal bars represent different MW's. Polypeptides falling within a quadrant are labeled numerically. Thus the polypeptide 'K_1' refers to the polypeptide '1' in quadrant 'K'. Comparison of the 2-D polypeptide map of dark morph (Fig. 1a) with the composite map (Fig. 2) reveals that the dark morph lacks the polypeptides O_2 (22,000), K_3 (43,000) and K_2 (47,000). Similarly, the male lacks the polypeptides P_2 (18,000), O_2 (22,000) and L_5 (44,000). The light morph lacks only the polypeptide O_3 (26,000). Thus the composite map readily enables the identification of compositional differences in the polypeptide composition either be genetic or environmental.

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REFERENCES

Basha, S.M.M. 1979. Identification of cultivar differences in seed polypeptide composition of peanuts (Arachis hypogaea L.) by two-dimensional gel electrophoresis. Plant Physiol., 63: 301 - 306.

Basha, S.M.M., Bazer, F.W. and Roberts, R.M. 1980. Effect of the conceptus on quantitative and qualitative aspects of uterine secretions in pigs. J. Reprod. Fert., 60: 41 - 48.

Beck, W.M., Jr. 1973. Chemical and physical aspects of the Blackwater River in northwestern Florida. p. 231 - 241. In: Peters, W.L. and Peters, J.G. (eds.). Proc. Ist Intern. Conf. Ephemeroptera, Brill, E.J., Leiden.

Harvey, R.S., Vannote, R.L. and Sweeney, B.W. 1980. Life history, developmental processes, and an energetics of the burrowing mayfly Dolania americana. p. 211 - 230. In: Flannagan, J.F. and Marshall, K.E. (eds.). Advances in Ephemeroptera Biology. Plenum Press, New York, London.

O'Farrel, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem., 250: 4007.

Peters, W.L. and Jones, L. 1973. Historical and biological aspects of the Blackwater River in northwestern Florida. p. 242 - 253. In: Peters, W.L. and Peters, J.G. (eds.). Proc. Ist Intern. Conf. Ephemeroptera, Brill, E.J., Leiden.

Peters, W.L. and Peters, J.G. 1977. Adult life and emergence of Dolania americana in northwestern Florida (Ephemeroptera: Behningiidae). Inter. Rev. Ger. Hydrobiol., 62: 405 - 438.

Tsui, P.T.P. and Hubbard, M.D. 1979. Feeding habits of the predaceous nymphs of Dolania americana in northwestern Florida (Ephemeroptera: Behningiidae). Hydrobiol., 67: 119 - 123.