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NUTRITIONAL ECOLOGY*: LYMANTRIA DISPAR* AS A MODEL SYSTEM FOR STUDY OF SERUM STORAGE PROTEINS

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The lepidopteran family Lymantriidae contains about 200 genera and about 2,500 described species, mostly from the Old World Tropics (DeWorms, 1983). Of the 46 recognized species and subspecies of Lymantriidae in North America, 30% are considered pests (Ferguson, 1978). The pest species include three introduced species which have become established in North America, the gypsy moth, *Lymantria dispar* (L.), the browntail moth, *Euproctis chrysorrhoea* L. and the satin moth, *Leucoma salicis* (L.). These exotic species are of particular interest to us because of their differing life history strategies and varying levels of success in North America. The satin moth is a minor pest of native and exotic species of poplar, but has on occasion defoliated aspen forests in North America. (Wagner & Leonard, 1979; 1980). The browntail moth is currently a refugial species in several maritime localities in eastern North America (Schaefer, 1974), but populations have recently rebounded (Leonard, 1988). The most successful of the invading Lymantriids is the gypsy moth, which continues to spread in North America.

With economically important species of Lymantriidae, a wide variation in the degree of polyphagy exists, from the rather narrow host ranges of the nun moth, *Lymantria monacha* L., the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McD.), and the satin moth, to the more catholic appetites of the gypsy moth and browntail moth.

Within the wide range of hosts that gypsy moth will feed upon, performance and fitness can vary between species of hosts (Barbosa & Greenblatt, 1981; Barbosa *et al.,* 1981). The quality of a tree host species will differ according to foliage age, location of foliage on the tree, and site conditions. (Wallner, 1987 and references therein.). Leaf quality can decline in response to grazing by gypsy moth larvae (Lance *et al.,* 1986; Schultz & Baldwin, 1982; Valentine *et al.,* 1983; Wallner & Walton, 1979).

There is considerable current interest in insect-host interactions encompassing the emerging field of nutritional ecology. As noted by Slansky and Scriber (1985), the amount, rate, and quality of food consumed influences growth rate, developmental time, weight, dispersal ability, and probability of survival; thus, the processes of food consumption and utilization underlie and

link the physiological, behavioral,ecological,and evolutionary aspects of insect life. Nutrient requirements for insects, reviewed by Dadd (1975), include protein and amino acids, carbohydrates, minerals, water-soluble growth factors including B vitamins, lipogenic factors, ascorbic acids, nucleic acids, and lipid growth factors including essential fatty acids, sterols, and fat soluble vitamins. Host-plant selection by feeding insects involves attraction to a potential food plant, arrest or cessation of locomotion, and stimulation or deterrence of feeding (Hanson, 1983). Allelochemical composition of food can influence performance of an insect feeding on a particular food, and often act in a negative, antibiotic manner (Slansky & Scriber, 1985).

The complexities of biotic and abiotic factors relating to the nutritional ecology of insects have produced a large and growing literature (e.g. Slansky & Rodriguez, 1987; Slansky & Scriber, 1985; and references therein). As noted by our esteemed colleague, Professor Vincent Dethier (1987), "Knowledge of all these newly revealed capacities of plants and insects forces us to reexamine our ideas of ecological relationships and evolutionary hypotheses. We cannot fully comprehend what populations are doing without understanding individuals nor what individuals are doing without some understanding of relevant features of their physiology and behavior."

Our group is utilizing a unique approach to nutritional ecology by examining the major nutrient storage systems in insects: the serum, cuticular, and yolk storage proteins. Insect storage proteins are synthesized predominantly (c.f. Palli & Locke, 1987) by the larval fat body, accumulate primarily in the hemolymph, and their concentration increases enormously in the later larval instars (Levenbook, 1985). The existence and persistence of distinct groups of storage proteins suggests that they have important functional roles in the physiology of insects, and the absolute or relative amounts of these proteins would be diagnostic of the insects nutritional, developmental, and behavioral state.

We have focused our attention on proteins, rather than on carbohydrates and lipids. Nitrogen-containing compounds play an essential role in growth processes of insects; cellular growth, differentiation, internal and external structural components, enzymes, carrier molecules, pigments, and chromosomal material require and involve a large investment of nitrogen (Mullins & Cochran, 1983.). The female gypsy moth is remarkably efficient in utilizing nitrogen for egg production, incorporating nearly half of that assimilated by last stage larvae into eggs (Montgomery, 1982). Serum storage proteins are not only essential for development of larvae, pupae, and adults, but also serve as a nutritional link between generations. Yolk proteins are the primary nutrient resources in eggs (Kunkel & Nordin, 1985) and the nutritional quality of eggs is important to survival during embryogenesis, diapause, and survival of pre-feeding neonates. The Lepidoptera in particular have a complicated set of yolk proteins which include vitellogenin, microvitellogenin, and egg specific protein (lrie & Yamashita, 1983; Kawooya et al., 1986; Zhu et

al., 1986). This complexity of stored nutrient allows for a complex message between the maternal and offspring generation.

Animals are exposed to several thousand naturally occurring molecules in the food they ingest (Turunen, 1985). Our paradigm in studies of nutritional parameters is to examine how the nitrogenous compounds are utilized after being absorbed from the midgut of feeding larvae. From our prospective, the feeding gypsy moth larva is the ultimate biological filtering system, accomplishing the necessary steps in finding food, utilizing the appropriate behavioral and chemosensory repertoires required for ingestion, and in dealing with allelochemicals and other feeding-associated adaptations. Nitrogen-containing compounds absorbed through the midgut form the metabolic pool for biochemical and physiological processes necessary for development. From this pool the fat body will synthesize polypeptides and proteins and release them to the serum as storage proteins, the major internal pool of amino acid resource for development, growth and reproduction.

The gypsy moth is proving to be an excellent model system. The insect is readily cultured in the laboratory. Feeding and accumulation of nutrients occurs only in larvae, since adults are non-feeding. Reserves accumulated by the onset of the wandering stage in last instar larvae provide the store of amino acids and proteins for pupae, adults, and eggs. As perhaps the most studied of forest pests, field aspects of the biology and ecology of the gypsy moth are well documented, yet the underlying physiological and biochemical processes have received little attention.

PROTEINS ASSOCIATED WITH INSECT NUTRITION

Arylphorin (Ap).

The arylphorins are hexameric serum storage proteins which may also be stored in the fat body close to the time of metamorphosis (Tojo et al. 1978; 1980). Arylphorins have been described in several species of insects from several Orders, including an Ap isolated by our group from gypsy moth (Karpells, Leonard, & Kunkel, in ms). The Ap from gypsy moth is one of eight thus far described in lepidopteran species. The gypsy moth Ap is a native Mr 440,000 hexamer composed of nonidentical subunits of Mr 73,000 and 80,000. Ap has been suggested to supply the amino acids necessary for tissue remodeling during metamorphosis. Ap is high in aryl groups which may be related to the particular need by metamorphic stages for phenolic hard cuticle and tanning substrates (Munn & Greville, 1969; Levenbook, 1984). The greatest concentration of arylphorin occurs in hemolymph in the wandering stage, just prior to the prepupal stage. Ap may play a supportive role in supplying the conduit that amino acids must pass through in the hemolymph of the last stage larva in order to take part in the synthesis of vitellogenins in the fat body.

Female specific protein (FSP).

FSP is a hexameric storage protein described in five lepidopteran species. FSP, although structurally related to Ap, is compositionally distinct, lacking the high aryl content. FSP accumulates in the last larval instar and is cleared and stored prior to use in the fat body (Tojo *et al.,* 1981). FSP is more actively synthesized in females; in the best documented case, *Bombyx mori,* its disappearance from the hemolymph and fat body is correlated with the accumulation of vitellogenin (Tojo *et al.,* 1981). We are now reasonably certain that FSP is missing in *Lymantria dispar.* This absence is important, since lack of FSP puts more pressure on Ap as a storage vehicle for yolk protein synthesis.

Vitellogenin (Vg).

The vitellogenins are the maternal serum precursors of the major yolk proteins or vitellins (Vts), of the egg (Kunkel & Nordin, 1985). Vitellogenin is the generic name for a unique group of proteins produced in the maternal fat body in most insects and transferred to the developing oocytes through the hemolymph (Hagedorn & Kunkel, 1979). Most often there are multiple immunologically distinct Vts in the egg (Storella et al., 1985), and these may be used differentially during the embryonic development, such that one of them is being utilized by the embryo close to hatching (Kunkel & Nordin, 1985). Differential synthesis, or turnover, of one or multiple Vgs before storage in the egg could dramatically affect the nutritional supply by these proteins to the embryo or first instar larva. In lepidopterans which do not feed in the adult stage, such as the saturniid silk moths, Vgs are often synthesized starting in the late larval and the pupal stage (Pan et al., 1969). With gypsy moth we have found that synthesis begins early in the ultimate stage of female larvae, with Vg appearing in the hemolymph in small amounts on day three. Vg accumulates most rapidly as Ap and FSP decline in the last instar or prepupae of *Bombyx mori,* suggesting that these proteins are the major potential sources or precursors for VG synthesis (Izumi et al., 1989). In *Bombyx mori,* Vt is not essential for embryonic development (Yamashita & Irie, 1980); however, Vt may be an important nutritional reserve for the pharate first instar larva (Indrasmith et *al.,* 1987).

Egg-specific protein (ESP).

ESP is a yolk protein produced within the ovary of lepidopterans. Its use during embryonic development has been correlated with the early phase of embryogeny. ESP is synthesized in the ovary and may rely on other serum storage proteins, such as FSP or Ap in *L. dispar* to be transported into the ovary and serve as an amino acid source for egg proteins (Ono et al. 1975). The quantity of ESP in the egg may determine the size, vigor, and subtle behavior of the resultant larva since early embryonic development consumes the stored ESP of an egg. Surveying ESP levels may allow one to assess the nutritional state of

egg masses.

PROTEIN STUDIES OF GYPSY MOTH.

Production of antisera.

Antisera against purified proteins were obtained by immunizing male, white, New Zealand rabbits using a standard protocol (Kunkel, 1988). We have produced antisera for the storage proteins Ap, Lp, Vg, Vt, and for the ovarian protein, ESP.

Developmental profiles.

Profiles of Ap, Lp, and Vg have been developed for daily-staged larvae, and prepupae, using quantitative immunoelectrophoresis (QIEP) techniques outlined in Kunkel (1988). These profiles are based on individual animals reared at 24°C, 16:8 LD cycle, and fed *ad lib*. on the diet of Bell *et al.* (1982). Protein profiles of eggs have been determined by QIEP for Ap, Lp, and Vt and by gel electrophoresis for ESP using homogenates of individual eggs. For 1st and 2nd instar larvae we use whole tissue homogenates and for later stages we collect 1 ul of serum from a small puncture in a proleg. We sex larvae by dissecting and identifying testes or ovaries.

Maternal - egg profiles.

For correlations of maternal protein levels with those of their progeny, I ul of hemolymph from female prepupae is subjected to OTEP. The small puncture causes no apparent trauma, and we rear females to adulthood for mating and to obtain eggs to compare egg protein profiles with those of the maternal larva.

PROTEIN PROFILES OF GYPSY MOTH

Arylphorin.

During each larval stadium, the concentration of Ap (mg/ml) in the hemolymph shows a gradual increase, reaching the highest concentration at early apolysis. As apolysis progresses, Ap is rapidly cleared from the hemolymph, providing an amino acid source for the newly forming larval tissues. Newly molted larvae contain very low levels of Ap. The titers of Ap in male and female larvae of instars III and IV are similar until the metamorphic instar, V in males and VI in females. These stadia are longer in duration, and more Ap accumulates, reaching a level of 26.2 mg/ml in males and 44.8 mg/ml in females. The extra larval inslar in females results in a substantially greater absolute amount of Ap accumulation than in males. The titer of Ap in eggs is low.

Lipophorin.

Lipophorin also displays some of the same cycling as seen with Ap, but the concentration of Lp is much lower, and remains at constitutive levels throughout development. Lp reaches it's highest concentration of about 5 mg/ml in female prepupae and 4 mg/ml in prepupal males (Karpells, Leonard & Kunkel, in prep.) with the higher concentrations as a function of the longer duration of the ultimate instars. Concentration of Lp in eggs is highest in newly laid eggs.

Vitellogenin.

Vitellogenin appears in the hemolymph in low levels at day three, and accumulates rapidly during the later third of the ultimate instar of females. In wandering stage larvae, Vg levels are ca. 25 mg/ml, and increase to ca. 30 mg/ml in prepupae. Vt levels in eggs are highest in newly oviposited eggs, and our preliminary studies show about 1/2 of the Vt is utilized during embryogenesis, and about 90% is utilized by the time of eclosion of neonates.

Egg Specific Protein.

Titers of ESP are highest after eggs are deposited. At completion of embryonation ESP is completely utilized.

IMPACT OF NUTRITIONAL STRESS ON PROTEIN LEVELS

The raising of antisera to Ap, Lp, Vg and ESP and determination of their titer on daily stage animals fed ad lib. provides us with the opportunity to compare the base line levels of serum proteins of healthy animals with levels in animals that have been stressed in the laboratory or in field populations. These studies are just beginning but our initial results show that various nutritional stress factors affect concentrations of proteins in serum and chorionated eggs.

Defoliation-induced changes in leaf quality have been shown to affect the population quality of the gypsy moth (*e. g.* Capinera & Barbosa 1976, 1977; Lance et al. 1986; Rossiter et al. 1988; Schultz 1983; Schultz & Baldwin 1982; Valentine et al. 1983; Wallner & Walton 1979). Lance et al. (1986) found that the addition of tannin at 2.5% of the total dry weight of the diet fed to third and fourth stage gypsy moth larvae could induce behavioral changes that approximated those observed during the shift in diel periodicity in dense field populations of the gypsy moth. In our studies (Leonard, Montgomery & Kunkel, unpubl.) larvae fed continuously on 0.5% (wt/wt) diet with tannin until apolysis of instar IV had 3 to 4% of the amount of Ap found in larvae fed normal diet, levels too low for molting to occur. Larvae switched from control to tannin diet at stage IV and bled at apolysis IV had reductions in Ap and Lp of ca. 30% and

20% respectively of that in control-fed larvae.

Mid- and late-stage larvae show a reduction in Ap and Lp levels in the serum after a two day period of food deprivation. In ultimate stage female larvae, starvation after day four causes about a one-third reduction in the level of Vg in hemolymph of prepupae.

Leonard (summarized in 1974; 1981) described a series of qualitative changes in the gypsy moth, including variation in the rate of development, supernumerary molts, coloration (phase polymorphism), fecundity, and size and quality of eggs associated, in part, with nutrition. Nutritional stress will be manifested in the amount and quality of nutritional components (proteins) biosynthesized for the egg by late-stage female larvae of the previous generation. While the amount of storage proteins could clearly be a factor in determining the number of eggs produced by the female, it is our hypothesis that the absolute and/or relative amounts of the storage proteins may be causal or at least be correlated with the quality of the eggs of gypsy moth such that a different behavior is exhibited by the next larval generation.

We have initiated studies to determine protein profiles of metamorphic female larvae in the wandering or early prepupal stage after feeding has been completed, to correlate maternal reserves with fecundity and partitioning of Vt, ESP, and Lp in eggs. The role of vitellin reserves in newly hatched larvae relative to dispersal of neonates is of particular interest to us. Mason & McManus (1981) summarized their studies on dispersal, reviewed the research of others, and note that dispersal is an important process in the population dynamics of the gypsy moth and is still a subject of much controversy. The gate for dispersal is narrow. McManus (1973) considered dispersal as an innate tendency that larvae must satisfy before feeding. Collections of dispersing larvae show that they produce frass with little or no leaf constituents (Leonard, 1970a; 1971). Mason & McManus (1981) suggest that the "turnoff mechanism" for dispersal is likely associated with the expending of energy reserves, the inability to produce silk, and starvation. We believe that the tendency of a lst instar larva to disperse greatly affects its reproductive fitness in a given population level, and that this propensity is reflected by both physiological and behavioral adaptations. These factors would logically be influenced by the amount, quality, and utilization of nutritive reserves in the egg and in newly hatched larvae to sustain it during dispersal. The parameters relating to the nutritive condition of eggs are: (1) a function of synthesis storage proteins during late instars of female larvae since adults do not feed; (2) the quality and quantity of proteins available to the developing oocyte, and (3) the utilization of proteins during the egg stage.

The role of yolk reserves and dispersal of neonates remains to be resolved. Large gypsy moth eggs contain about twice the yolk content of small eggs (Capinera et al., 1977, Capinera & Barbosa (1967), Greenblatt & Barbosa (1979), Campbell (1981) and Lance & Barbosa (1981) consider that larvae from larger eggs have a higher tendency to disperse, whereas Leonard (1970) suggested that larvae from smaller eggs or larvae that had depleted yolk reserves

were more active and dispersed more readily. McManus & Mason (1983) suggest that physical factors probably exert greater control of the extent of dispersal than size and quality of individuals. Our technique of using QIEP to quantify protein levels in individual animals provides an opportunity to examine the nutritional status of dispersing larvae. Using the wind tunnel of Carde & Hageman (1979) to induce dispersal behavior of neonates, we have begun making direct measurements of Vt of larvae which disperse as well as those that remain on the platform in the airstream.

SUMMARY

We are utilizing a unique approach to examine the influence of nutritive parameters on the population biology of the gypsy moth by examining the major nutrient storage system in insects, the serum and yolk storage proteins. By development of specific antibodies for the major serum storage proteins, vitellogenin, lipophorin, and arylphorin, and the ovarian-produced egg-specific protein, we can examine changes in the titer and the profiles of these proteins during development, and determine the influence of stress factors such as the change in diel periodicity of larvae, and influence of leaf polyphenols such as tannin, on the quantity and quality of these proteins. One of the advantageous features of our study is the ability to obtain hemolymph samples for analyses of storage proteins without sacrificing the insect. This allows us to compare the profiles of prepupae of the previous generation which are diagnostic of the major pool of nutrient reserves available to the progeny via the egg. This approach will permit an examination of the influence of events occurring in the previous generation on fitness (nutritional) factors which are important in the survival, dispersal, and the dynamics of the succeeding generation. Interest in the identification of serum storage proteins is growing.

Relatively few have been identified from a limited number of insect species, but our efforts will add at least five from the gypsy moth. We know of no other research group, however, that has begun an intensive study of the role of storage proteins in biological and behavioral parameters relating to population quality or population trends. The gypsy moth lends itself as an excellent. model for such a study, because of the importance of nutritional status as it relates to behavior, dispersal, and host anti-herbivore responses. Equally exciting to us is the potential of using data generated from immunoelectrophoresis to develop discriminant functions to predict population trends.

Discriminant function analysis of biochemical parameters has not yet been applied to assess a physiological or behavioral class in insects.

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