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Comparison of Yolk Production in Seven Pyralid Moth Species

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Summary

The yolk proteins of six pyralid moths were analyzed and compared with the yolk proteins of *Plodia interpunctella* (Hübner). When cross-reacted in an Ouchterlony double immunodiffusion with antiserum raised to either total yolk proteins or purified vitellin from *P. interpunctella*, the yolk proteins of *Anagasta kuehniella* (Zeller), *Cadra cautella* (Walker), *C. figulilella* (Gregson), and *Ephestia elutella* (Hübner), closely related members of the subfamily Phycitinae, showed strong precipitation lines that consisted of four major yolk polypeptides (YPs). The yolk proteins from *Amyelois transitella* (Walker) were only weakly reactive, whereas yolk proteins from *Galleria mellonella* (L.) were not precipitated by either antiserum. Abdominal body walls (containing primarily fat body) from late pharate adult females were incubated *in vitro* and they secreted two major polypeptides that had molecular masses similar to the vitellogenins (YP1 and YP3) from *P. interpunctella.* In addition, ovarioles from late pharate adult females were incubated *in vitro*, and they secreted two major polypeptides that had molecular masses similar to YP2 and YP4 from *P. interpunctella.* When late pharate adult females were injected with ^{35}S-Met, the hemolymph of all species contained vitellogins that were secreted by their respective body walls *in vitro.* Ovarioles from injected females contained many labeled polypeptides, but there were four major bands that corresponded consistently to the vitellogenins secreted from the fat body and the two major polypeptides secreted from the ovarioles. These data show that the production of the major YPs in these closely related pyralid species is very similar, and that there is considerable conservation of immunological characters of yolk proteins in the subfamily Phycitinae.

Vitellogenesis, vitellogenins, yolk production, oogenesis, pyralid moths.

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Abbreviations: Ak = *A. kuehniella*, At = *A. transitella*, Cc = *C. cautella*, Cf = *C. figulilella*, Ee = *E. elutella*, Gm = *G. mellonella*, Pi = *P. interpunctella*, kDa = kilodaltons, SDS-PAGE = Sodium dodecyl sulfate polyacrylamide gel electrophoresis, TCA = trichloroacetic acid, YP = yolk polypeptide. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

Introduction

In moths, the majority of the proteins found in the yolk are produced by two sources: either the protein is produced in the fat body as a true vitellogenin that is transported via the hemolymph to the maturing oocytes for uptake and deposition as vitellin, or the protein is produced in the ovarioles, typically by the follicular epithelium, and is taken up directly by the oocytes [cf. 1–6]. Mature oocytes of the Indianmeal moth, *Plodia interpunctella* (Hübner), contain similar proteins [7]. By resolving the yolk proteins on SDS-PAGE, four major yolk polypeptides (YPs) were identified that had molecular mases of 153 kDa (YP1), 69 kDa (YP2), 43 kDa (YP3), and 33 kDa (YP4). Of the four major polypeptides in the oocytes, only YP1 and YP3 were synthesized by the fat body and secreted into the hemolymph. Isolation of the native proteins from the yolk showed that YP1 and YP3 were subunits of a protein considered to be a true vitellin. YP2 and YP4 were produced in the ovarioles by culturing the organ is *in vitro*, and when isolated under native conditions YP2 and YP4 were found to be the subunits of a second yolk protein that was unique from the vitellin. Vitellin (YP1/YP3) contributed approximately 40% of the protein to the yolk, and YP2/YP4 contributed an additional 40% of the protein.

Vitellogenin has been isolated and characterized from the hemolymph of pharate adult females and adult females of *Bombyx mori* (L.), *Hyalophora cecropia* (L.), and *Manduca sexta* (L.), and was composed of two subunits that had approximate molecular masses of 180 kDa and 45 kDa [8–11]. Deposition of vitellogenin as vitellin in the yolk granules in the oocyte occurred without major structural modification. In *B. mori* and *H. cecropia*, the two vitellogenin subunits were synthesized in the fat body as shown by measuring their secretion into organ culture medium [12–14]. The fat body of *H. cecropia* and *M. sexta* also produced a microvitellogenin (30 and 31 kDa, respectively) that was found in the hemolymph and was taken up into the oocytes [15–16].

In addition to vitellin, the eggs of *B. mori* and *H. cecropia* contained a protein component that was produced by the follicular epithelium [1–2]. Approximately 20% of the yolk protein was produced by the follicle cells, and the protein was designated egg-specific protein (55 kDa) in *B. mori* [1,17] or paravitellin in *H. cecropia* [2,15].

The process of yolk production in six species of pyralid moths from the subfamilies Phycitinae and Galleriinae was examined to determine if the proteinacious yolk in other moths of the pyralid family is similar to that of *P. interpunctella.* The YPs from the various moths were identified, and the sites of their synthesis were determined. As a measure of structural relatedness and phylogenetic relatedness the yolk proteins from each of the species were tested for immunocross-reactivity to antiserum raised to yolk proteins from *P. interpunctella.*

Materials and Methods

Animals and materials

Anagasta kuehniella (Zeller), *Cadra cautella* (Walker), *C. figulilella* (Gregson), *Ephestia elutella* (Hübner), and *P. interpunctella* were reared on diet according to Silhacek and Miller [18]; *Amyelois transitella* (Walker) according to Finney and Brinkman [19]; and *Galleria mellonella* (L.) according to Beck [20] with the addition of wheat germ to the diet. All insects were maintained at 27°C. in 60–80% relative humidity and 16:8 hr light:dark cycle. L-[^{35}S]-Methionine (^{35}S-Met) (specific activity $\geq$ 1,000 Ci/mmole) was obtained from New England Nuclear. Grace's insect medium was obtained from Grand Island Biological.

Gel electrophoresis

The tissues were homogenized in sodium dodecyl sulfate (SDS)-sample buffer [21] and resolved by SDS polyacrylamide gel electrophoresis (SDS-PAGE) (8–15% gradient) [7,22]. Protein molecular mass standards [myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine albumin (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa) (Sigma)] were included on each gel for estimation of molecular masses. The gels were stained for protein with Coomassie brilliant blue R (BioRad). To show the relative rate of protein synthesis between the species, equal quantities of radioactively labeled proteins were run in each lane and then an autoradiogram was made using Kodak X-Omat® AR X-ray film.

Immunocross-reactivity of yolk proteins

The antigens in the yolk of the various species were identified initially by Ouchterlony double immunodiffusion [23]. The ovarioles from newly eclosed adult females were homogenized in phosphate buffer (PB) [150 mM NaCl, 10 mM phosphate (pH 7.2)] (1 oocyte/μl PB) at 4°C and centrifuged at 12,000×g. Infranatant from the homogenate of each species was used as the antigenic material and was placed in a 5μl well cut in 1% agarose in PB. The yolk homogenates were diffused against either anti-yolk antiserum raised against total yolk proteins from *P. interpunctella* [7] or rabbit anti-vitellin antiserum raised against purified YP1/YP3 from *P. interpunctella* (Bean, Shirk and Bookes, unpublished). The precipitation lines were photographed by darkfield illumination.

The antigens in the YPs of each species cross-reacting with the anti-yolk antiserum from *P. interpunctella* were identified by SDS-PAGE resolution of ^{35}S-Met labeled yolk proteins that had been immunoprecipitated in solution. The samples were prepared for electrophoresis by homogenizing the ovarioles in PB and diluting the 12,000×g supernatant two-fold with 150 mM NaCl, 2 mM EDTA and 50 mM Tris/HCl (pH 7.6) (NET) plus 1% Triton-X 100. To this, 0.1 volumes of either anti-yolk antiserum or anti-vitellin antiserum from *P. interpunctella* were added, and the mixture was incubated 16 hr at 0°C. The antigen/antibody complexes were precipitated by addition of 0.1 volumes of protein A-Sepharose (Sigma) with continued incubation for 2 hr. The precipitates were centrifuged at 12,000×g for 10 sec and then washed three times with NET. The washed precipitates were dissolved in SDS-sample buffer, boiled and then centrifuged again before electrophoresis.

Labeling of YPs in vivo and in vitro

Pharate adult females 1 day before eclosion were injected with 10μCi of ^{35}S-Met in 1μl Weevers' saline [24]. After 6 hr at 27°C, the moths were bled, and the ovarioles were dissected. The ovarioles were either homogenized directly in SDS-sample buffer or homogenized in PB, immunoprecipitated with anti-yolk antiserum, and the precipitates were placed in SDS-sample buffer.

The ovarioles or fat body were incubated *in vitro* in the presence of ^{35}S-Met to identify the polypeptides produced by a specific tissue. The abdominal body walls, consisting primarily of fat body, from pharate adult females 1 day before eclosion were dissected under sterile conditions, washed several times with sterile Weevers' saline and then transferred to Grace's insect medium

plus ^{35}S-Met (0.5μCi/μl) and incubated in 100% O_2 at 27°C for 2 hr. The ovarioles from pharate adult females 1 day before eclosion were dissected under sterile conditions, cleaned of adhering fat body, washed several times with sterile Weevers' saline, and then transferred to Grace's insect medium plus ^{35}S-Met (0.5μCi/μl) and incubated in 100% O_2 at 27°C for 2 hr. The culture media and tissues were placed separately in SDS-sample buffer, homogenized, boiled and centrifuged before SDS-PAGE.

Results

Immunocross-reactivity of YPs to anti-yolk antiserum

As a measure of the apparent relatedness of these six species to *P. interpunctella*, the yolk proteins from the mature oocytes of adult females of each pyralid moth were immunoprecipitated with anti-yolk antiserum to *P. interpunctella* in an Ouchterlony double immunodiffusion. Substantial precipitation lines formed against the yolk proteins from *A. kuehniella*, *C. cautella*, *C. figulilella*, and *E. elutella* (Fig. 1A). However, the presence of spurs (indicated by the arrows, Fig. 1A) showed the yolk proteins of these species not to be totally homologous with the yolk proteins of *P. interpunctella*. The yolk proteins of *A. transitella* formed a faint precipitation line (Fig. 1B, arrow), but there was no evidence of cross-reactivity with the yolk proteins of *G. mellonella* (Fig. 1B). The yolk proteins of the various species also were diffused against antiserum to purified vitellin (YP1/YP3) from *P. interpunctella* (Fig. 1C,D). Again, major precipitation lines with minor spurs formed against the yolk proteins from *A. kuehniella*, *C. cautella*, *C. figulilella*, and *E. elutella*. The precipitation line for yolk proteins from *A. transitella* was defined clearly with the anti-vitellin antiserum (Fig. 1D, arrows), and again no precipitation line formed against the yolk proteins from *G. mellonella* (Fig. 1D).

Identification and synthesis of YPs

In *P. interpunctella*, the fat body previously was shown to synthesize and secrete two major YPs (YP1 and YP3) into the hemolymph and when incubated *in vitro* into the culture medium [7]. The body walls of females 1 day prior to eclosion were dissected and incubated in medium that contained ^{35}S-Met to identify the polypeptides being synthesizd and secreted by the fat body of the other six species. The body walls of all seven species synthesized primarily two major polypeptides that were similar to size to YP1 and YP3 that were secreted by the body walls of *P. interpunctella* (Fig. 2).

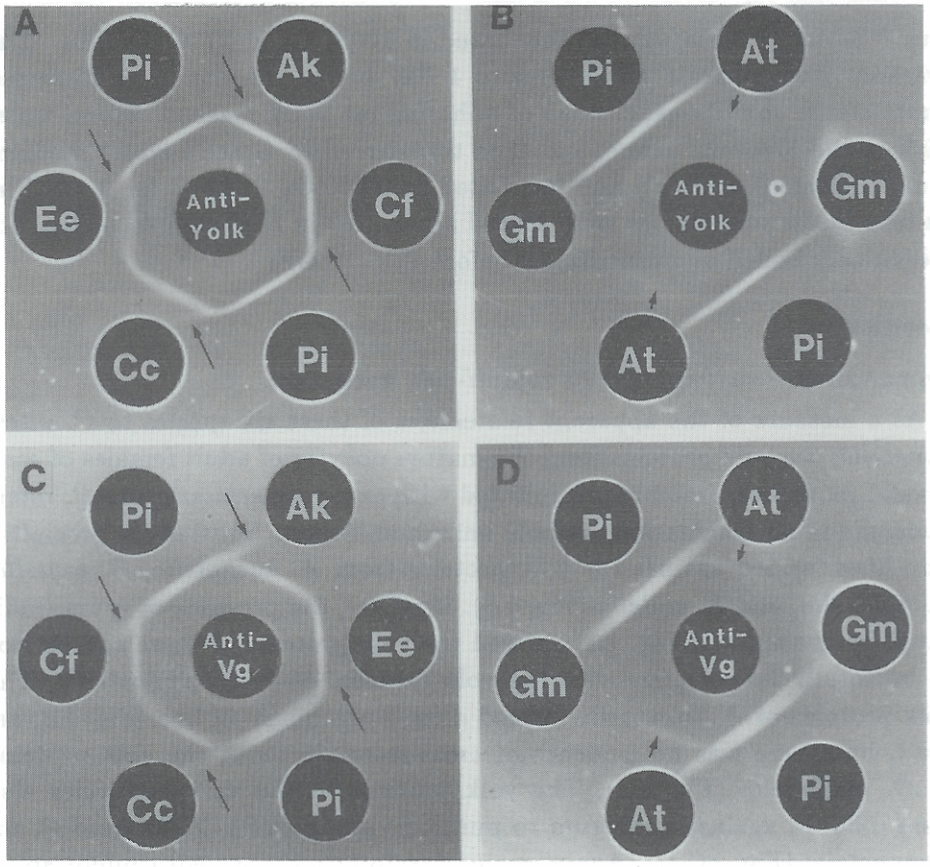

Fig. 1. Immunoprecipitation of yolk proteins from six pyralid species by antiserum to yolk proteins from *P. interpunctella* on Ouchterlony double immunodiffusion. A and B were diffusions against antiserum against total yolk proteins (anti-yolk) from *P. interpunctella*. C and D were diffusions against antiserum to purified vitellin (anti-vitellin) from *P. interpunctella*. The arrows indicate the presence of spurs due to non-homology of the yolk antigens with the yolk proteins of *P. interpunctella*. Abbreviations: Pi = *P. interpunctella*, Ak = *A. kuehniella*, At = *A. transitella*, Cc = *C. cautella*, Cf = *C. figulilella*, Ee = *E. elutella*, Gm = *G. mellonella*.

The molecular masses of the two major polypeptides were estimated, and the larger polypeptide ranged in size from 159 kDa for *G. mellonella*, to 167 kDa for *A. kuehniella* (Table I).

YP1 from *P. interpunctella* was smaller than those of the other species and had a molecular mass of 153 kDa. The other major polypeptide secreted by the body walls had a molecular mass of 44 kDa in *G. mellonella*, 46 kDa in *A. kuehniella*, *A. transitella*, *C. figulilella* and *E. elutella*, and 47 kDa in *C. cautella* (Table I). YP3 from *P. interpunctella* had a molecular mass of

48 kDa, which was larger than the estimates reported previously [7]. The difference in molecular mass appears to be the result of a shift in the migration of the egg albumin and YP3 on gradient gels and fixed percentage gels (see difference between Fig. 2 and Figs. 3 and 4 in Shirk et al. [7]).

Previous work also showed that the ovarioles synthesize two major YPs (YP2 and YP4) in *P. interpunctella* [7]. To identify the secretory products of the ovarioles from the other pyralid moths, the ovarioles for each species were dissected from females 1 day prior to eclosion and incubated in medium containing ^{35}S-Met. All of the species synthesized and secreted two major polypeptides close to the molecular masses of YP2 and YP4 from *P. interpunctella* (Fig. 3). The molecular masses were estimated, and the largest ranged in size from 66 kDa for *C. figulilella* to 74 kDa for *G. mellonella* (Table I). YP2 from *P. interpunctella* had a molecular mass of 69 kDa. The smallest major secreted polypeptide appeared to have a molecular mass of 33 kDa for all species except *G. mellonella* which was 37 kDa. YP4 from *P. interpunctella* had a molecular mass of 33 kDa. In addition to the major polypeptides described, there were several other bands appearing in the culture media from the other species that did not appear in the medium of *P. interpunctella.* The presence of heavily labeled bands in the 45–65 kDa molecular-mass range of most of the samples probably represent proteolytic cleavage products of the YP2 class polypeptides. We have observed specific proteolytic cleavage of YP2 that results in the appearance of additional bands between 45 and 60 kDA when YP2 is maintained in unpurified native conditions (Bean, Shirk and Brookes, unpublished), which appears to be similar to the phenomenon observed here.

TABLE I.
Molecular masses of YPs from pyralid moths.

Species	YP1	YP2	YP3	YP4
P. interpunctella	153 (9)[a]	69 (1)	48 (2)	33 (1)
A. kuehniella	167 (7)	70 (1)	46 (2)	33 (0)
A. transitella	161 (8)	70 (2)	46 (3)	33 (1)
C. cautella	160 (8)	67 (2)	47 (2)	33 (1)
C. figulilella	162 (7)	66 (2)	46 (2)	33 (1)
E. elutella	159 (6)	67 (2)	46 (1)	33 (1)
G. mellonella	159 (10)	74 (2)	44 (2)	37 (1)

[a] Molecular mass $\times 10^{-3}$ (standard deviation). Each value represents the average molecular mass estimate from two replicates from four different trials.

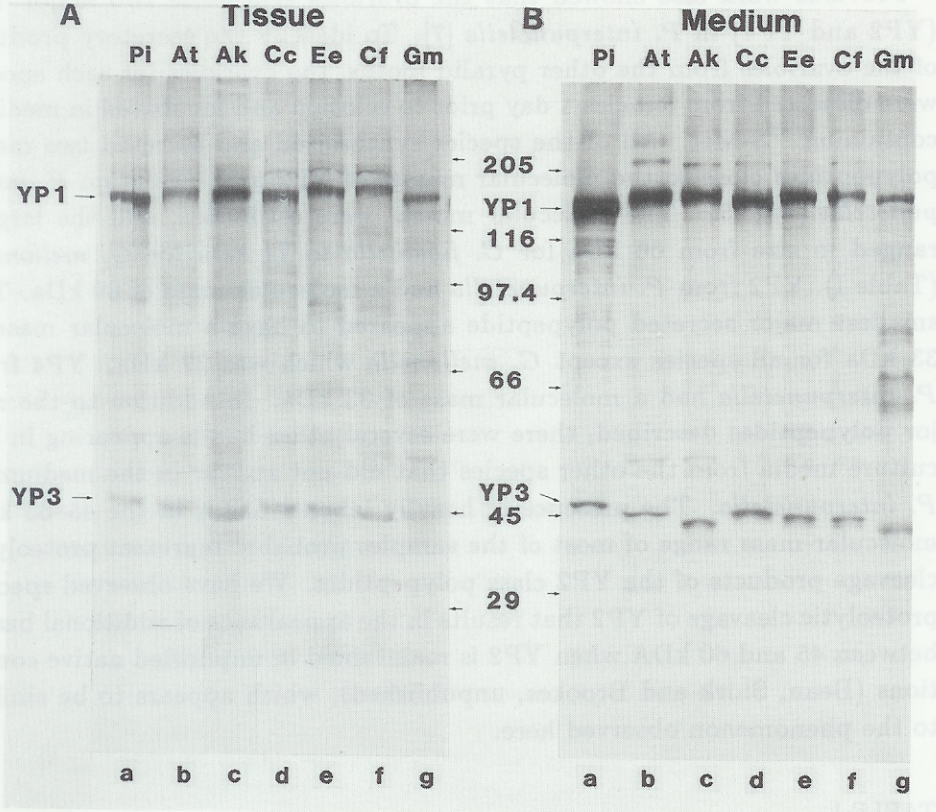

Fig. 2. Polypeptides synthesized and secreted by the abdominal body walls of pharate adult female pyralid moths incubated *in vitro*. The autoradiogram shows (A) the polypeptides synthesized in the tissues of the body walls, and (B) those polypeptides secreted into the medium during the incubation with ^{35}S-Met. The same number of TCA precipitable cpm were loaded in each lane. The positions of the molecular mass standards are shown in the center. Lanes for body wall tissues (A): (a) Pi, (b) At, (c) Ak, (d) Cc, (e) Ee, (f) Cf, (g) Gm. Lanes for medium (B): (a) Pi, (b) At, (c) Ak, (d) Cc, (e) Ee, (f) Cf, (g) Gm. (Symbols as defined in Fig. 1 legend.)

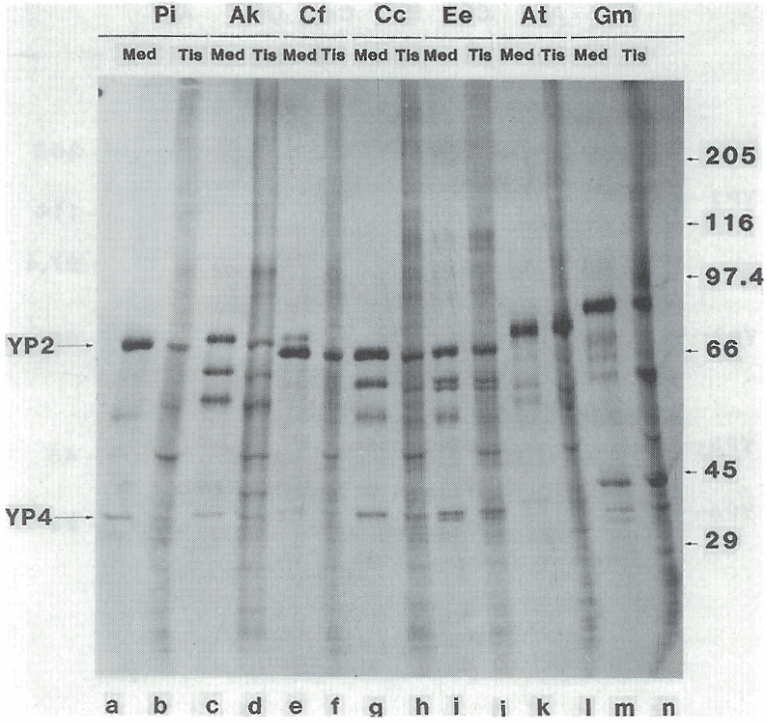

Fig. 3. Polypeptides synthesized and secreted by ovarioles from pharate adult females of pyralid moths incubated *in vitro*. The autoradiogram show as ^{35}S-Met labeled polypeptides that were secreted into the medium or found in the tissues of ovarioles. The same number of TCA precipitable cpm were loaded in each lane. The positions of the molecular mass standards are shown on the right. Lanes (a) Pi Med, (b) Pi Tis, (c) Ak Med, (d) Ak Tis, (e) Cf Med, (f) Cf Tis, (g) Cc Med, (h) Cc Tis, (i) Ee Med, (j) Ee Tis, (k) At Med, (l) At Tis, (m) Gm Med, (n) Gm Tis. Abbreviations: Med = medium; Tis = tissue. (Symbols as defined in Fig. 1 legend.)

To correlate the production of polypeptides *in vitro* with synthesis *in vivo*, females 1 day before eclosion were injected with ^{35}S-Met to label the newly synthesized proteins. Hemolymph and ovarioles were collected from each animal. The autoradiogram showed the presence of radiolabeled polypeptides in the hemolymph of each species that were equivalent to the polypeptides secreted by their respective body walls incubated *in vitro* (Fig. 4). The ovarioles contained numerous radiolabeled polypeptides, but there were consistently four major polypeptides accumulating in each species. The four polypeptides were similar in size to the two polypeptides appearing in the hemolymph of each species and to the two major polypeptides secreted by the cultured ovarioles. The radiolabeling patterns from the injected insects were not entirely consistent with the patterns from the cultured tissues. The

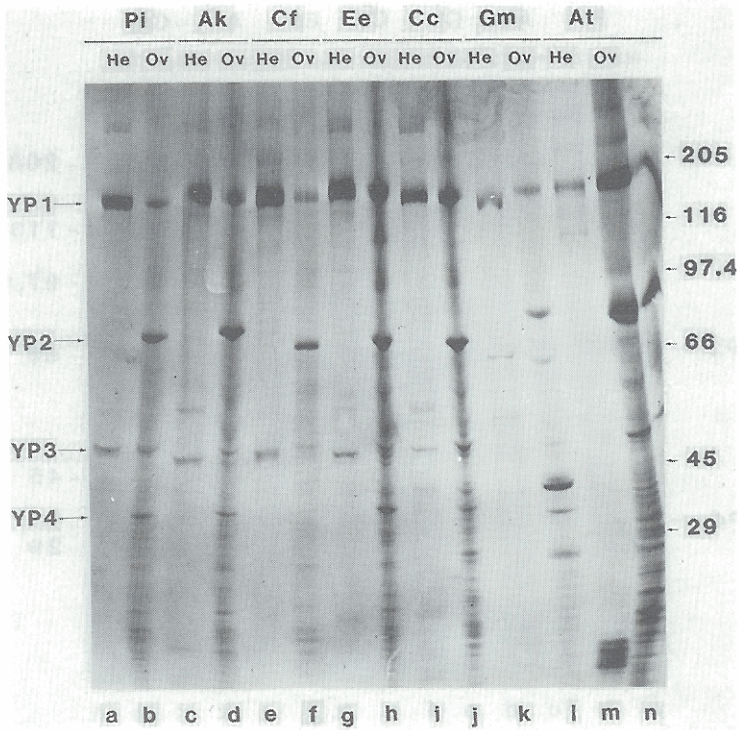

Fig. 4. Rapidly synthesized polypeptides appearing in the hemolymph and the ovarioles of pharate adult females of pyralid moths. The autoradiogram show as the labeled polypeptides that were secreted into the hemolymph or accumulating in the ovarioles after the injection of ^{35}S-Met intopharate adults. The same number of TCA precipitable cpm were loaded in each lane. The positions of the molecular mass standards are shown on the right. Lanes: (a) Pi He, (b) Pi Ov, (c) Ak He, (d) Ak Ov, (e) Cf He, (f) Cf Ov, (g) Ee He, (h) Ee Ov, (i) Cc He, (j) Cc Ov, (k) Gm He, (l) Gm Ov, (m) At He, (n) At Ov. Abbreviations: He = hemolymph; Ov = ovarioles. (Symbols as defined in Fig. 1 legend.)

ovarioles from *C. figulilella* and *A. transitella* did not show the presence of the 33 kDa polypeptide that appeared in the medium from cultured ovarioles (compare Fig. 3e with Fig. 4f and Fig. 3k with Fig. 4n). The absence of these polypeptides from the tissues may indicate significant shifts in the biosynthetic activity of the organs when placed in culture, but the phenomenon remains unexplained in this report.

Identification of YPs immunoprecipitated by antiserum

The radiolabeled yolk proteins from each species were precipitated from solution and resolved by SDS-PAGE to identify the specific polypeptides cross-reacting with the anti-yolk antiserum from *P. interpunctella.* The autoradiogram show as the total radiolabeled yolk proteins accumulating in the ovarioles and those polypeptides that were immunoprecipitable by the

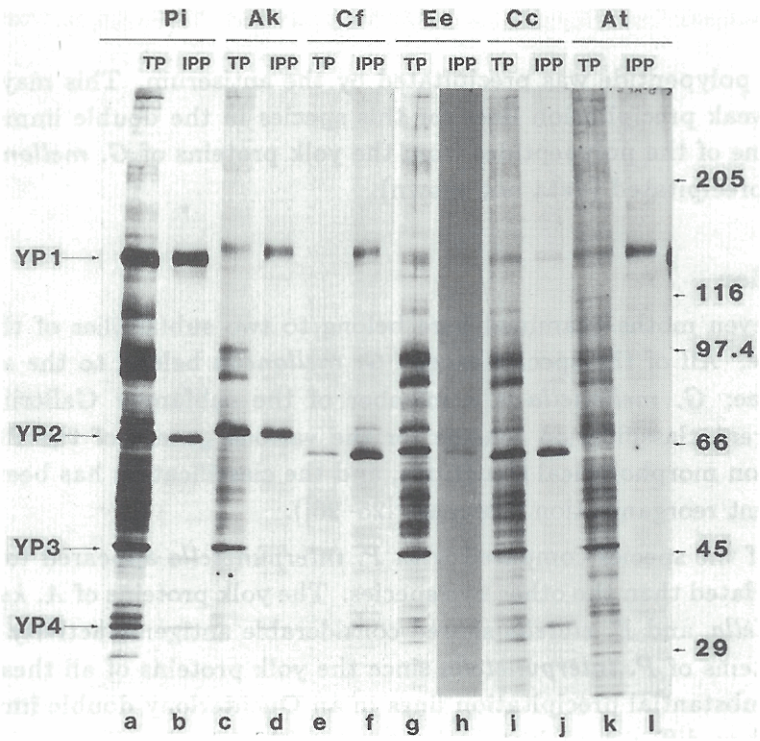

Fig. 5. Yolk polypeptides from oocytes of pyralid species that were immunoprecipitable by antiserum to total yolk from *P. interpunctella.* The autoradiogram show as the total labeled polypeptides and those that were immunoprecipitable by anti-yolk antiserum from ovarioles of pharate adult females that were injected with ^{35}S-Met. The same number of TCA precipitable cpm were loaded in each lane. All lanes were from the same autoradiogram, but lanes (a) and (h) were photographically enhanced. The positions of the molecular mass standard are shown on the right. Lanes: (a) Total Proteins Pi, (b) IPP YPs Pi, (c) Total Proteins Ak, (d) IPPs Ak, (e) Total Proteins Cf, (f) IPPs Cf, (g) Total Proteins Ee, (h) IPPs Ee, (i) Total Proteins Cc, (j) IPPs Cc, (k) Total Proteins At, (l) IPPs At. Abbreviations: TP = total proteins; IPP = immunoprecipitable proteins. (Symbols as defined in Fig. 1 legend.)

anti-yolk antiserum (Fig. 5). The polypeptides identified as major secretory products of the body walls and ovarioles of *A. kuehniella*, *C. cautella*, *C. figulilella*, and *E. elutella* were precipitated by the anti-yolk antiserum from *P. interpunctella* as were the four YPs from *P. interpunctella.* These observations agreed with the results of the Ouchterlony double immunodiffusions of the yolk proteins from these species where precipitation lines formed when diffused against the anti-yolk antiserum. The YP1 and YP2 class of polypeptides appeared to be precipitated more readily than were the YP3 and YP4 classes of polypeptides for all of these species. The immunoprecipitates of the yolk proteins from *A. transitella* showed that only the

160 kDa polypeptide was precipitated by the antiserum. This may account for the weak precipitation lines for this species in the double immunodiffusion. None of the polypeptides from the yolk proteins of *G. mellonella* were immunoprecipitated (data not shown).

Discussion

The seven moths examined here belong to two subfamilies of the family Pyralidae. All of the species except *G. mellonella* belong to the subfamily Phycitinae; *G. mellonella* is a member of the subfamily Galleriinae [25]. The current classification scheme for the various genera of the Phycitinae is based on morphological structures, and the classification has been subject to frequent reorganization (compare [25–26]).

Four of the species compared with *P. interpunctella* appeared to be more closely related than the other two species. The yolk proteins of *A. kuehniella*, *C. figulilella*, and *E. elutella* shared considerable antigenic activity with the yolk proteins of *P. interpunctella* since the yolk proteins of all these species formed substantial precipitation lines in an Ouchterlony double immunodiffusion when diffused against antiserum to either total yolk proteins or purified vitellin from *P. interpunctella* (Fig. 1). However, the yolk proteins from these four species were not identical to the yolk proteins from *P. interpunctella.* Precipitation spurs formed where the precipitation line from *P. interpunctella* intersected the precipitation line from another of these species (arrows in Fig. 1A,B), which indicated the two intersecting antigens lacked total homology.

The yolk proteins from *G. mellonella* were not precipitated by the antiserum to yolk proteins from *P. interpunctella* which suggests that there has been considerable divergence in the structure of the yolk proteins between these two species even though the size of the major polypeptides found in the yolk are of similar sizes (Table I). The formation of a weak precipitation line for the yolk proteins of *A. transitella* that is composed principally of the 161 kDa polypeptide shows the yolk proteins between *A. transitella* and *P. interpunctella* have diverged but not to the extent that all structural homology has been eliminated. The weak antigenic cross-reactivity shared between *A. transitella* and *P. interpunctella* is consistent with the classification scheme of Arnett [25] since *A. transitella* is considered to be a more distantly related member of the Phycitinae subfamily than are *A. kuehniella*, *C. cautella*, *C. figulilella*, *E. elutella*, and *P. interpunctella.*

The nature of the antigenic cross-reactivity of the yolk proteins from the six species examined to the antiserum to yolk proteins from *P. interpunctella* generally supports the current perception of the morphologically based classification for these species [25]. Placement of *A. kuehniella*, *C. cautella*, *C. figulilella*, *E. elutella*, and *P. interpunctella* in the Phycitinae subfamily has been indicated also on the basis of the chemical composition of the sex pheromone [27] and their similar natural histories [28]. All five of these species produce (*Z,E*)-9,12-tetradecadien-1-ol as the principal component of their sex pheromone. The placement of *A. transitella* within this subfamily could not be made on the basis of the sex pheromone composition since (*Z,Z*)-11,13-hexadecadienal has been identified as the primary sex pheromone component [29]. However, there was weak antigenic cross-reactivity between the yolk proteins which suggests that *A. transitella* should be included as a closely related species to the Phycitinae. The classification of this subfamily is supported by the various non-morphological characters that have been examined, but none of these characters is sufficiently accurate to define the evolutionary relationships between the members.

The production of yolk proteins by these seven pyralid species appeared to be similar to the processes described for the other moths that have been examined. Vitellogenin consisting of a large and a small subunit was synthesized by the fat body and secreted into the hemolymph (Figs. 2,4). The size of the large vitellogenin subunit appears to be slightly smaller than that reported for other moths. In the pyralid moths, the molecular mass of the larger subunit ranged from 153 to 167 kDa (Table I), but the equivalent subunit was about 180 kDa in other moths (c.f. [6]). In addition to the vitellogenins produced by the fat body, the ovarioles of all these pyralid moths produced polypeptides that were taken up by the oocytes (Figs. 3,4). In contrast to the pyralids, the follicular epithelium of *B. mori* and *H. cecropia* produced a single protein which was incorporated in the yolk granules [1–2]. In *P. interpunctella*, YP2 and YP4 were identified as the heterogeneous subunits of a major protein isolated from the yolk ([7]; Bean, Shirk and Brookes, unpublished). However, egg-specific protein from *B. mori* and paravitellin from *H. cecropia* were found in homogeneous proteins; egg-specific protein was shown to be a dimer of identical 55 kDa [17], and paravitellin was found to be a monomer of 70 kDa [15]. Even though the yolk proteins of *H. cecropia* contained a fourth major protein, microvitellin and the other pyralids examined here, similar in molecular mass to YP4 in *P. interpunctella*, microvitellin has not been found to form subunit associations with other yolk

proteins [15]. Even with these minor variations, the manner of yolk production within pyralid moths was consistent with processes described for other moths.

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