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Original article

Protein profiles of testes, seminal vesicles and accessory glands of honey bee pupae and their relation to the ecdysteroid titer*

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Abstract – Protein content and pattern were determined for testes, seminal vesicles and accessory glands of drone pupae and compared to the ecdysteroid titer in hemolymph. In testes of young pupae, the protein titer increases in parallel with increasing ecdysteroid levels, but maximal protein titers are reached in late pupae, after the hormone titer has dropped. Protein titer then decreases at the end of pupal stage, coinciding with the time reported for spermatozoa migration to seminal vesicles and onset of testes degeneration. In seminal vesicles and accessory glands, the protein titer increases continuously during pupal stages, the maximal titer coinciding with low ecdysteroid levels in late pupae. Two new polypeptides appeared in accessory glands of late pupae, when the ecdysteroid level was low, and were not detected in ecdysteroid-treated pupae, thus indicating that some of the final steps of differentiation of the drone reproductive system are negatively regulated by ecdysteroids.

Apis mellifera **/ drone reproductive system / testes / seminal vesicles / accessory glands / ecdysteroids**

1. INTRODUCTION

The differentiation of the reproductive system in honey bee drones has received little attention when compared to that of queens and workers. Our understanding on this topic is mainly based on early histological studies of testes development and spermatogenesis during the larval and pupal stages (Meves, 1907 and Zander, 1916 in Snodgrass, 1956; Bishop, 1920). The reproductive system of drones includes three organs or structures that share a common mesodermal origin: somatic tissue of the paired testes, a pair of seminal vesicles formed by enlargement of respective deferent ducts and a pair of accessory or mucus gland (Snodgrass, 1956). The structural units of testes, or testioles, are already formed in the third larval instar, and germ cell clusters were observed in the fourth larval instar (Tozetto, 1997). The onset of spermatogenesis was detected by Louveaux (1977) in the third larval instar. Maturation divisions and spermiogenesis occur during the pupal stage, when testes attain their maximal development, and spermatogenesis is terminated before the adult drone emerges (Bishop, 1920; Silveira, 1972; Kerr and Silveira, 1974; Louveaux, 1977; Tozetto, 1997). The spermatozoa then enter the pre-vesicular portion of the deferent ducts and subsequently reach the seminal vesicles where they are temporarily stored. Following spermatozoa migration to the seminal vesicles, the testes shrink and become flattened (Snodgrass, 1956). These are evident signs of testes degeneration.

In sexually mature drones, accessory glands play an important role during mating and

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sperm transfer (Woyke and Ruttner, 1958; Koeniger et al., 1989). Following sperm injection into the genital opening of the queen, a discharge of accessory gland content is transferred (Koeniger, 1984). This proteinaceous secretion is the most prominent component of the mating sign left in the abdomen of the queen (Koeniger et al., 1996; Colonello and Hartfelder, 2005). In addition to sperm transfer, accessory gland secretion has been related to a variety of functions in insects in general, including sperm protection, storage and activation (Gillot, 2003). Gland secretion was also associated with the production of a spermatophore in many primitive insect species (Davey, 1985) and to physiological and behavioral changes in mated female. In *Drosophila* females, the most extensively studied insect with respect to accessory gland function, postmating responses such as egg production and laying, and reduced receptivity to remate were demonstrated to be induced by specific components of gland secretion (Swanson, 2003; Kubli, 2003).

Aspects of development and differentiation of the male reproductive system have been demonstrated to be hormonally regulated. Ecdysteroids stimulate mitotic divisions of spermatogonia and subsequent meiosis in many species of Diptera, Hemiptera, Orthoptera and Lepidoptera (Hagedorn, 1985). Jacob (1992) reported that fragments of testes from a beetle (*Oryctes rhinoceros*) required ecdysteroids for the occurrence of normal mitosis and meiosis in incubations in vitro. Ecdysteroids also have been shown to play a regulatory role in accessory gland maturation and activity in a number of insect species, including the honey bee (Happ, 1992; Ismail and Gillot, 1995; Gillot, 1996). Injection of 20-hydroxyecdysone (20E) in newly emerged drones impaired the normal increase in accessory gland protein content during the first days of adult stage. The injected hormone also caused the persistence of the gland protein pattern typical of newly emerged drones for a prolonged period of time (Colonello and Hartfelder, 2003). These results provide evidence that ecdysteroids negatively interfere in the maturation of these glands. However, this cannot be generalized, since both, inhibitory and stimulatory effects of ecdysteroids on protein synthesis in accessory glands have been reported for different insect species (Sridevi et al., 1988; Ismail and Dutta-Gupta, 1990; Ismail and Gillot, 1995, 1997). In addition to ecdysteroids, juvenile hormone has been cited and reviewed as of fundamental importance for accessory gland function (Raikhel et al., 2005). Neurosecretory factors were also demonstrated to participate in the regulation of protein synthesis and secretion of these glands (Wyatt and Davey, 1996; Regis et al., 1987; Cheeseman and Gillot, 1988).

The focus of the current study is on the differentiation of the drone reproductive system during the pupal stage. We were interested in studying the protein content and pattern during the development of the reproductive system in relation to the fluctuation of the ecdysteroid titer that regulates pupal development. To this aim, we established criteria to subdivide the drone pupal stage into thirteen phases. Protein content and pattern of testes, seminal vesicles, and accessory glands were determined for each pupal phase and were analyzed in comparison with the ecdysteroid titer measured in hemolymph samples by radioimmunoassay. Treatment of pupae with 20E was also performed to investigate whether differentiation of accessory glands is functionally related to the ecdysteroid levels.

2. MATERIALS AND METHODS

2.1. Drone pupae collection and staging

Drone pupae were collected from combs of Africanized bees maintained in the experimental apiary of the University of São Paulo in Ribeirão Preto, SP, Brazil. Developing pupae were staged according to the color of the compound eyes and onset and intensity of thoracic cuticle pigmentation, criteria established by Rembold et al. (1980) and Michelette and Soares (1993).

2.2. 20E-treatment

20E (Fluka) was first dissolved in ethanol (20 mg/mL) and then diluted in Ringer solution for

insects to a final concentration of 10 μg/μL immediately before treatment. This solution (1 μL) was injected between the 3rd and 4th tergite of newly-metamorphosed pupae (1 day-old pupae, P1). Control pupae, in the same stage, were injected with 1 μL of ethanol diluted in Ringer or were left untreated. They were then placed in Petri dishes and kept in an incubator at 34 °C and 80% RH until the end of the pupal stage. The progress of pupal stage was monitored in 24 h intervals.

2.3. Hemolymph collection

Hemolymph was collected from an incision in the dorsal abdominal cuticle of staged drones. As only small quantities could be obtained from each individual, hemolymph samples of pupae at the same stage were pooled. After centrifugation at 7 000 *g* for 5 min at 10 °C, supernatants were used for ecdysteroid quantification using a radioimmunoassay.

2.4. Radioimmunoassay (RIA)

Ecdysteroids extracted from the hemolymph samples of untreated pupae were quantified by RIA, as previously described (Feldlaufer and Hartfelder, 1997; Pinto et al., 2002). Briefly, 10 μL of hemolymph samples were mixed with 990 μL of cold methanol (to precipitate proteins), vortexed, and kept at 4 °C for 24 h before being centrifuged at 5 000 *g* for 15 min at 4 °C. The supernatants containing ecdysteroids were transferred to fresh tubes, and methanol was then removed by vacuum centrifugation. An antiserum prepared against a hemisuccinate derivative of ecdysone was used in ecdysteroid quantifications (Bollenbacher et al., 1983). [23,24-^{3}H(N)] ecdysone (NEN, spec. act. 1750 GBq/mmol) served as labeled ligand. Standard curves were obtained using 20E as nonradioactive ligand. Results were expressed as 20E equivalents (pmol/mL hemolymph).

2.5. Preparation of testes, seminal vesicles and accessory glands for protein quantification and SDS-PAGE

Two or three pairs of testes, seminal vesicles or accessory glands from staged drone pupae were rapidly dissected and rinsed in Ringer. Each pair was homogenized in 100 μL of 0.25 M Tris-HCl buffer (pH 6.8) containing 2% SDS. Pairs of accessory glands from 20E-treated pupae (n = 13) and their respective controls (ethanol/Ringer-treated pupae, n = 12; untreated pupae, n = 14) were also dissected and homogenized in the same Tris-HCl-SDS buffer. After 3 hours of incubation at 25 °C, the tissue extracts were centrifuged at 12 000 *g* for 5 min at 4 °C, and supernatants were stored at –20 °C for protein quantification and SDS-PAGE.

2.6. Total protein quantification

Aliquots were taken from the extracts prepared with pairs of testes, seminal vesicles or accessory glands, and subsequently assayed using bicinchoninic acid (Smith et al., 1985), which permits to quantify proteins in the presence of SDS. Bovine serum albumin was used for construction of standard curves. Concentration of proteins in the samples was represented as μg protein per 50 μL tissue extract, meaning the total protein content of one testis, seminal vesicle or accessory gland.

2.7. SDS-PAGE

Separation of proteins from testes, seminal vesicles and accessory glands was routinely carried out on denaturing 7–15% or 10–18% polyacrylamide slab gels. Tissue extract samples containing 1 μg protein were added to sample buffer (Laemmli, 1970), boiled for 2 min, centrifuged at 12 000 *g* for 5 min at 4 °C, and loaded onto polyacrylamide gels. Electrophoresis was conducted at 15–20 mA and the gels were silver nitrate-stained (Caetano-Anollés and Gresshoff, 1994). Commercial molecular mass markers were used to estimate the relative molecular mass of the respective polypeptides.

3. RESULTS

3.1. Staging drone pupae

The pupal development of drones was subdivided into 13 phases (P1 to P13) according to the progressive changes observed in the color of compound eyes and of the thoracic cuticle. A time scale for drone pupal development was also constructed to correlate pupal phases to age in hours (Tab. I).

Table I. Phases of drone pupae development distinguished according to the color of compound eyes, intensity of thoracic cuticle melanization and time (hours) to adult emergence. Colour-coded staging system was based on Rembold et al. (1980) and Michelette and Soares (1993) descriptions for female castes. In parenthesis is the color number according to Ral (1976) classification.

Pupal phases	Characteristics of pupal development					
	Eye color	Thoracic cuticle pigmentation	Hours to adult emergence			Pupal phase duration (hours)
			X	SD	n	
P1	Pearl (1013)	Pearl (1013)	263.60	6.00	4	55.00
P2	Beige (1001)	Pearl	208.60	5.04	43	15.72
P3	Rosy (3012)	Pearl	192.88	7.58	42	31.43
P4	Violet (4002)	Pearl	161.45	5.17	64	22.37
P5	DarkViolet (4004)	Pearl	139.08	3.63	30	14.93
P6	Dark Violet	Mesoscutum pigmentation	124.15	2.82	56	7.14
P7	Dark Violet	Ivory (1015)	117.01	3.51	46	6.52
P8	Light Violet (4001)	Ivory (1014)	110.49	3.66	57	12.21
P9	Light Violet	Beige (1001)	98.28	4.92	38	19.79
P10	Light Violet	Dark Beige (1011)	78.49	7.81	59	24.41
P11	Light Violet	Light Brown (7008)	54.08	6.85	17	23.06
P12	Brown (8017)	Brown (8014)	31.02	4.08	28	12.71
P13	Brown	Dark Brown	18.31	7.92	19	18.31

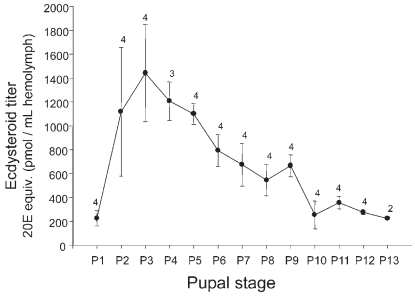

Figure 1. Means and standard deviations of ecdysteroid measurements in hemolymph of developing *Apis mellifera* drone pupae. P1 to P13 are the successive phases of the pupal stage classified according to criteria showed in Table I. Numbers above the curve represent hemolymph pools from each pupal phase used in radioimmunoassays.

3.2. Ecdysteroid titers in drones

Ecdysteroid levels were measured at each phase of drone pupal development (Fig. 1). An increase in the levels of ecdysteroids was characterized during the beginning of the pupal stage. In P2, P3, P4, and P5 pupal phases, hormone levels were 4.8 to 6.4 times (1098.66 ± 89.40 to 1441.04 ± 407.17 pmol 20E/mL hemolymph) the value determined for P1 drones. Subsequent to the P5 pupal phase, the ecdysteroid titer decreased gradually, reaching a value at the end of the pupal stage (223.24 ± 0.62 pmol 20E/mL hemolymph) that did not differ from the one observed immediately after pupal ecdysis, at the P1 pupal phase (226.45 ± 64.91 pmol 20E/mL hemolymph).

3.3. Development of reproductive organs evaluated by protein measurement

Figure 2A shows the relative amount of proteins quantified in testes during drone pupae development. The protein content increased approximately threefold from P1 to P10 pupal phases and then decreased at the end of the pupal stage.

In the seminal vesicles, there was a progressive increase in protein content during the pupal stage. At the end of this stage, protein content was more than eightfold greater than at the P1 pupal phase (Fig. 2B). Accessory glands also showed increased protein content during pupal development. Glands from P13 pupae showed approximately sevenfold more protein than glands from P1 pupae (Fig. 2C).

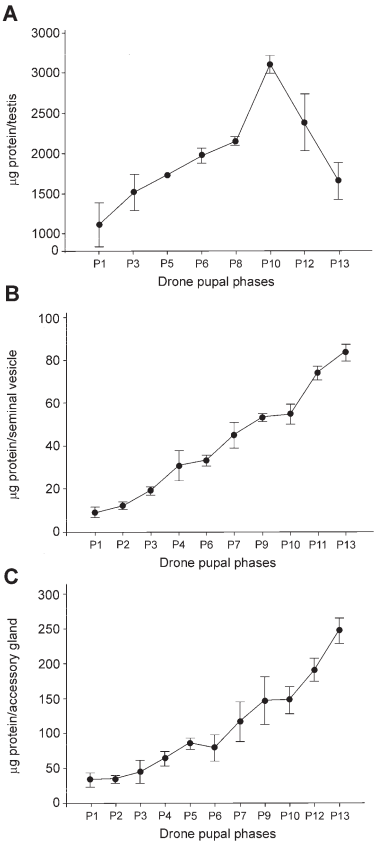

Figure 2. Protein content in (A) testes, (B) seminal vesicles, and (C) accessory glands of developing *Apis mellifera* drone pupae. Each point of the graph corresponds to a mean of 2 or 3 individual measures. P1 to P13 are the successive phases of the pupal stage classified according to criteria showed in Table I.

3.4. Development of reproductive organs evaluated by SDS-PAGE

SDS-PAGE analysis of testes, seminal vesicles and accessory glands during pupal development showed a conserved protein pattern. The same polypeptide bands detected in testes were equally present in seminal vesicles and accessory glands. Thus, there were no relevant qualitative differences in protein patterns in these compartments of the reproductive system. The only differences were quantitative, as deduced from polypeptide band intensity. In Figures 3, 4 and 5 the main polypeptide bands that are common to developing testes, seminal vesicles and accessory glands are indicated. To simplify, we excluded from these figures the pupal phases in which no changes in protein pattern were observed. The changes in band intensities, which are indicative of quantitative differences, are mentioned below. Only consistent (i.e., reproducible) differences were considered.

Testes

The SDS-PAGE pattern of polypeptides, or groups of polypeptides, was studied throughout testes development (Fig. 3). In the high molecular weight region (group a), the one-band pattern typical of P3 was substituted by a double band in P6-P7 that disappeared in P12. The region b was very poor in polypeptides throughout pupal development. In this region, the subunits of lower molecular mass were hardly visualized at the end of the pupal stage. Polypeptide c, which was very prominent in accessory glands (see Fig. 5, pupal phases P8 and P12) and in seminal vesicles (see Fig. 4, pupal phases P7 and P13) was underrepresented in testes. The groups of polypeptide bands in the regions d to i consistently decreased in intensity as pupal development progressed. The most prominent band in the group i (arrow in Fig. 3B, phase P7) increased in intensity from P3 to P7, and then subsequently diminished in intensity in the P12 phase. Polypeptide j showed increased intensity as pupal development advanced.

Seminal Vesicles

We noted that all polypeptides found in testes were also observed in seminal vesicles (Fig. 4). However, in contrast to testes where a striking reduction in polypeptide pattern intensity was observed as development progresses (an exception was polypeptide j), a gain in intensity was noticed for the majority of seminal vesicle polypeptide bands. Thus, bands in the regions a, c, d, f, g, h, i and j showed increased intensity from the pupal phase P3 to P13. Polypeptide e, however, decreased in intensity. The changes observed in the b region were not consistent. As observed for testes, the

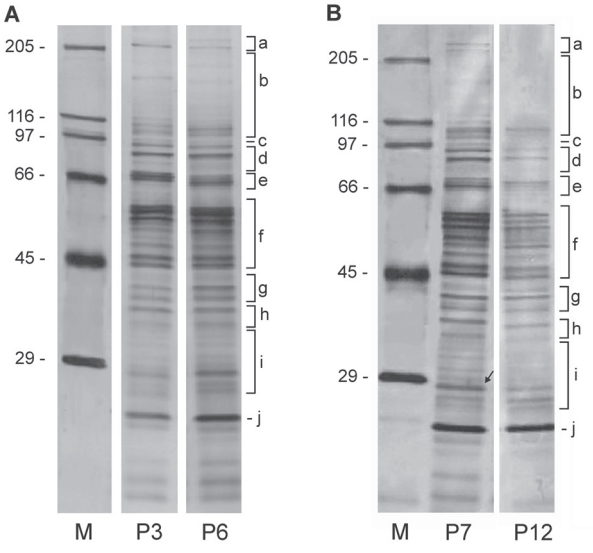

Figure 3. Silver nitrate-stained SDS-PAGE showing polypeptide patterns from testes of *Apis mellifera* drone during pupal stage. Polypeptide bands that were also found in seminal vesicles and accessory glands are indicated at the right of the gels. See Results for comments on changes in band pattern intensities. (A) P3, P6, and (B) P7 and P12 are pupal phases of drone development. Molecular mass markers (M) are indicated at the left of the gels.

polypeptide pattern of the seminal vesicle did not show consistent qualitative changes in developing pupae.

Accessory glands

Polypeptide bands identified in testes and seminal vesicles were also detected in developing accessory glands (Fig. 5A, B). As observed in seminal vesicles, the majority of polypeptides or groups of polypeptides (a, c, d, f, g, h, i and j) showed increased intensity during pupal development whereas the polypeptide e showed decreased intensity from P3 to P12. In the group b, pattern complexity was reduced as development advanced.

Of interest was the onset of two new polypeptides (k and l) in accessory glands of late pupae. They were detected after ecdysteroid titer had decayed to a low level (see Fig. 1), suggesting a negative regulation by this hormone. To test the assumption that high levels of ecdysteroids could impair the advent of polypeptides k and l, they were investigated in accessory glands of 20E-injected pupae. This experimental manipulation maintained ecdysteroid titers at high levels for a prolonged period of time. In 20E-treated pupae, both polypeptides were absent at the time when they were clearly visible in control-pupae (Fig. 5C), thus confirming the inhibitory effect of high levels of ecdysteroids on their expression. 20E-treatment, however, did not specifically inhibit polypeptides k and l, but also partially inhibited polypeptide j (Fig. 5C).

Polypeptides k and l are markers of the final steps of accessory gland differentiation (Fig. 5B, lane P12). The observed inhibition of these polypeptides by 20E-treatment provided strong evidence that ecdysteroids negatively regulate the last events of accessory gland differentiation.

Note that polypeptide l is weakly represented in Figure 5C (untreated and ethanol/Ringer-treated samples). Unfortunately, we could not reproduce in this figure, an l band with the intensity of that observed in Figure 5B, although it is clear that it was

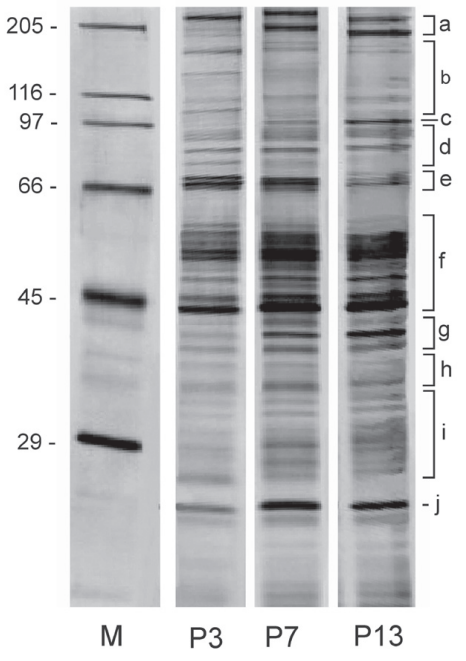

Figure 4. Silver nitrate-stained SDS-PAGE showing polypeptide patterns from seminal vesicles of *Apis mellifera* drone during pupal stage. At the right of the gel, polypeptide bands that were also found in testes and accessory glands are indicated. See Results for comments on changes in band pattern intensities. P3, P7 and P13 are pupal phases of drone development. Molecular mass markers (M) are indicated at the left.

present in the controls and was not detected in 20E-injected pupae.

4. DISCUSSION

Our analysis showed a progressive increase in protein content in testes from the beginning to the P10 phase of the pupal stage. This pupal phase corresponds to 78.5 hours before adult emergence. At this pupal period spermatogenesis is already terminated (Bishop, 1920), and testes attain their greatest development (Bishop, 1920; Louveaux, 1977). Apparently, the increasing protein concentration in testes of developing pupae reflects growth of these organs and the intense process of sperm production. The observed decrease in the concentration of testis proteins at the end of the pupal stage coincided with the time reported for spermatozoa migration for storage in the seminal vesicles and onset of testes degeneration (Bishop, 1920).

The increasing protein titer in developing seminal vesicles apparently reflects the increase in volume of these organs in preparation for sperm storage. There is no register of secretory activity in seminal vesicles which could explain the observed increase in protein titer. The seminal vesicles of most insects do not appear to produce a secretion. Evidence suggests that in seminal vesicles, the compounds necessary for maintenance of spermatozoa enter by diffusion from the hemolymph (Davey, 1985). In fact, seminal vesicles of newly emerged males of a stingless bee, *Melipona bicolor*, did not show consistent morphological signals of secretory activity (Dallacqua and Cruz Landim, 2003). In addition, epithelial cell structures involved in secretory activity were not found. Although seminal vesicles of sexually mature *M. bicolor* males were observed to be filled with an amorphous material where spermatozoa were immersed, the origin of this material is unknown, and could be produced in other regions of the reproductive system (Dallacqua and Cruz-Landim, 2003). Cell structures related to protein secretion have also not been found in seminal vesicles of honey bee drones (Cruz-Landim and Cruz-Höfling, 1969). Like seminal vesicles, accessory glands also presented increased protein content throughout the pupal stage, reflecting the growth of these organs in developing pupae.

Protein patterns in testes from successive pupal stages, as determined by SDS-PAGE, were practically invariable in qualitative terms. Except for polypeptide j, which showed increased expression as pupal development advanced, all the other polypeptide bands in the testes showed decreased intensity or even disappeared at the end of the pupal stage. These quantitative differences may be related to the termination of spermatogenesis, followed by initiation of testes degeneration, and is consistent with the marked decay in the titer of proteins in these organs in late pupae.

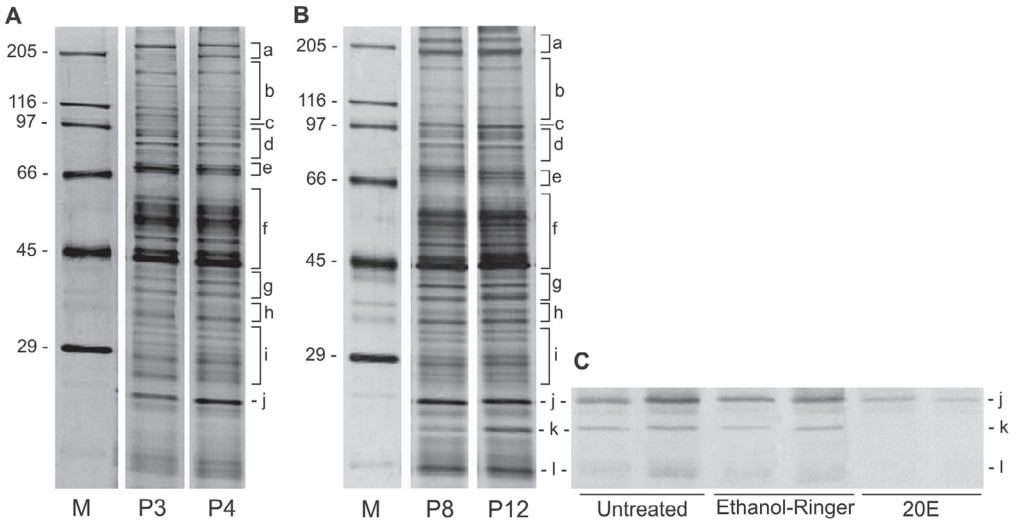

Figure 5. (A, B) Silver nitrate-stained SDS-PAGE showing polypeptide patterns from accessory glands of *Apis mellifera* drone during pupal stage. Polypeptide bands are named at the right of the gels. See Results for comments on changes in band pattern intensities. (A) P3, P4 and (B) P8 and P12 are pupal phases of drone development. Molecular mass markers (M) are indicated at the left of the gels. (C) Part of a silver-stained SDS-polyacrylamide gel showing total inhibition of polypeptides k and l, and partial inhibition of polypeptide j in accessory glands of 20-hydroxyecdysone (20E)-injected pupae in comparison to untreated and ethanol/Ringer-treated controls.

The distinct compartments of the drone reproductive system showed a very similar polypeptide pattern throughout the pupal stage. All polypeptides identified in testes by SDS-PAGE were also observed in seminal vesicles and accessory glands. Exceptions were polypeptides k and l, which appeared only in the accessory gland of late pupae. Polypeptide k migrated at the same position of the polypeptide of 25 kDa detected in the mucus isolated from accessory gland of adult drones (Colonello and Hartfelder, 2003), suggesting that it is the same product of secretory activity. The presence of this polypeptide may signalize the onset of gland activation and of secretory activity at the end of the pupal stage. This assumption is in accord with data recently obtained by Moors et al. (2005) in a study on accessory gland ultrastructure. These authors considered that the thick ephitelium of the pupal accessory glands and characteristics of their cells, such as the presence of RER and intracellular secretory droplets, suggested secretory activity at this stage. It is implausible, however, that this incipient secretory activity significantly contributed to the observed increase in total protein content of accessory glands at the end of the pupal stage (see Fig. 2c).

Accumulation of secretion in the lumen of accessory glands occurs after adult emergence, reaching its maximum by the 6th day of adult life (Moors et al., 2005). From the 1st to the 5th days of adult life, protein content in the mucus increases steeply, almost fifteen-fold, thus accompanying the period of sexual maturation (Colonello and Hartfelder, 2003). Therefore, as mentioned above, the observed increase in total protein content in pupal accessory glands reflects growth of these structures and not secretion accumulation.

Polypeptides k and l were only clearly identified in accessory glands after ecdysteroid titers have decayed to a low level, suggesting a negative regulation by these hormones. In fact, the maintenance of a high level of ecdysteroids in 20E-injected drone pupae resulted in inhibition of the onset of both polypeptides,

denoting that accessory gland differentiation was delayed. Together, the age profile of the ecdysteroid titer (Fig. 1) and the effects of its experimental manipulation (Fig. 5) provide strong evidence for an inhibitory role of ecdysteroids in the final steps of gland differentiation. Colonello and Hartfelder (2003) described a similar effect in adult drones. They showed that the protein pattern of gland secretion, typical for newly emerged drones, persisted for a prolonged period of time in 20E-treated drones. In addition, the protein content in the mucus did not increase as usual. Therefore, in developing pupae and in adults, ecdysteroids act as negative regulators of accessory gland differentiation and maturation, respectively.

The last events of drone pupae maturation occur under a physiological condition characterized by a low level of ecdysteroids in the hemolymph. By examining the external aspect of 20E-treated drone pupae, it was evident that they were less pigmented than their respective controls, indicating that the injected hormone retarded the progression of pupal development. Thus, the response to 20E-treatment may reflect a generalized effect that results in retardation of pupal maturation and ultimately in accessory gland differentiation, rather than a direct effect on the expression of specific polypeptides. This consideration clearly deserves attention and a detailed analysis that includes a search for and identification and characterization of structural elements responsive to ecdysteroids in accessory gland genes.

In conclusion, we observed that the high titer of ecdysteroids at the P3 stage, coincided with the maturation divisions of germ cells at the second day of pupal stage, as reported by Louveaux (1977), suggesting a function of these hormones in meiosis of honey bee male gamete. Declining levels of ecdysteroids at the subsequent pupal stages coincided with the termination of spermatogenesis, which was set at the fifth to sixth day of the pupal period by Zander (1916, in Snodgrass, 1956). Declining levels of ecdysteroids was also related to increased protein levels in seminal vesicles and accessory glands and to the onset of specific polypeptides in glands, suggesting that differentiation and growth of these components of the reproductive system require low ecdysteroid titer.

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Relations entre les profils protéiniques des testicules, des vésicules séminales et des glandes accessoires des nymphes d'abeilles (*Apis mellifera*) et la teneur en ecdystéroïdes.

***Apis mellifera* / système reproducteur mâle / ecdystéroïde / testicule / vésicule séminale / glande accessoire**

Zusammenfassung – Proteinmuster der Hoden, Samenblasen und akzessorischen Drüsen und ihr Bezug zum Ecdysteroidtiter von Honigbienenpuppen. Um der Frage der Rolle von Hormonen in der Differenzierung des Reproduktionssystems nachzugehen, untersuchten wir in dieser Studie die Proteingehalte und Proteinmuster der Hoden, Samenblasen und der akzessorischen Drüsen in der pupalen Entwicklung von Drohnen und betrachten diese in Bezug zum jeweiligen Ecdysteroidtiter in der Hämolymphe. Dafür legten wir zuerst Kriterien fest, die die pupale Entwicklungsphase in 13 Stadien unterteilen (P1–P13) (Tab. I). Dann bestimmten wir die Proteingehalte dieser Organe und erstellten mittels SDS-PAGE ihre Proteinmuster. Der Ecdysteroidtiter der jeweiligen Puppenphasen wurde mittels eines Radioimmunassays bestimmt. Ein starker Anstieg des Ecdysteroidtiters liess sich zu Beginn der Puppenhphase feststellen. In Phasen P2, P3, P4 und P5 der Drohnen erreichte der Ecdysteroidtiter einen 4,8 bis 6,4 fach höheren Wert als in der P1 Phase. Danach fiel der Hormontiter langsam ab, bis er zum Ende der Puppenphase sich wieder auf basale Werte einstellte (Abb. 1). Für die sich entwickelnden Hoden zeigte der Gesamtproteingehalt einen graduellen Anstieg in den Puppenphasen P1 bis P10, bevor er dann rapide abnahm (Abb. 2A). Ein maximaler Proteingehalt in der P10-Phase fiel zeitlich mit einem abnehmenden Ecdysteroidtiter zusammen. Wie bereits früher festgestellt, ist zu diesem Zeitpunkt die Spermatogenese bereits beendet, so dass die beobachtete rapide Abnahme im Proteingehalt mit der Wanderung der Spermien aus den Hoden in die Samenblasen und der Degeneration der Hoden zusammenfällt. Samenblasen und die akzessorischen Drüsen zeigten von P1 bis P13 eine kontinuierliche Zunahme im Proteingehalt (Abb. 2B, C), der das Wachstum dieser Strukturen widerspiegelt.

Die verschiedenen Organe des Reproduktionstraktes, die wir in der Drohnenentwicklung untersuchten, zeigten durchwegs vergleichbare Proteinmuster in den SDS-PAGE Auftrennungen, abgesehen von quantitativen Schwankungen in einzelnen Banden (Abb. 3, 4 und 5). Ausnahmen bildeten die Polypeptide k und l, die sich als spezifisch für die akzessorischen Drüsen später Puppenstadien erwiesen (Abb. 5B) und die erstmals zu finden waren, als der Ecdysteroidtiter bereits auf niedrige Werte gefallen war. Eine Injektion von 20-Hydroxyecdyson (20E) in Drohnenpuppen, und die damit einhergehenden über eine längere Zeitspanne erhöhten Hormonwerte, verhinderten das Erscheinen dieser beiden Polypeptide. Auch das Polypeptid j erwies sich nach der Hormonbehandlung als partiell reduziert (Abb. 5C). Die Polypeptide k und l können somit als Marker für die späten Stadien der pupalen Differenzierung der akzessorischen Drüsen angesehen werden und die Blockierung ihres Auftretens durch einen hohen 20E-Titer weist darauf hin, dass Ecdysteroide eine negative Kontrolle in der späten Differenzierungsphase dieser Drüsen spielen. Insgesamt weisen diese Daten darauf hin, dass ein abnehmender Ecdysteroidtiter eine wichtige Bedingung für die Normalentwicklung der akzesorischen Drüsen darstellt.

***Apis mellifera* / Drohnenreproduktionsorgane / Hoden / Samenblase / akzessorische Drüsen / Ecdysteroide**

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