

Protein content and pattern during mucus gland maturation and its ecdysteroid control in honey bee drones

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Abstract – We analyzed mucus gland protein content and pattern for drones of Africanized honey bees. The effect of exogenous ecdysteroids on mucus gland maturation was judged against the endogenous ecdysteroid titer. During the first 5 days of adult life, the mucus protein content increases steeply, whereas the protein pattern becomes reduced in complexity. Subsequently, the protein content decreases, reaching a plateau level at day 8. The protein pattern of mature glands is characterized by three dominant polypeptides. Injection of 20-hydroxyecdysone into newly emerged drones abolished the normal increase in protein content and prolonged the persistence of the protein pattern typical for immature glands. Ecdysteroids thus appear to act as negative regulators in the maturation process of drone mucus glands. This hypothesis received support from analyses of the hemolymph ecdysteroid titer, which was found to rapidly decline soon after emergence.

male accessory gland / ecdysone / radioimmunoassay / *Apis mellifera* / mucus protein

1. INTRODUCTION

Male accessory gland products are potent regulators of female reproduction in insects. The highly viscous secretions of this gland are transmitted to females either via spermatophore uptake or by deposition into the *bursa copulatrix* during copulation. After transmission they may form a mating plug whose primary function is to ensure paternity by mechanically preventing females from mating with other males. In addition, the mainly proteinaceous secretion serves as an energy source and aids in sperm capacitation and storage (for review see: Chen, 1984; Gillott, 1996). A most remarkable property of the male accessory gland secretions,

however, is their long-term effect on female reproductive physiology (Wolfner, 1997). Some of the proteins, including the 36 amino acid-residue sex peptide of *Drosophila melanogaster* (Chen et al., 1988; Schmidt et al., 1993), are transported across the vaginal membrane (Lung and Wolfner, 1999) and reach their target sites via hemolymph transport. The *Drosophila* sex peptide targets the female's neuroendocrine axis, stimulating juvenile hormone biosynthesis by the corpus allatum (Moshitzky et al., 1996; Fan et al., 1999), which then controls the oogenic cycle in the ovaries (Soller et al., 1997). Sex peptide binding sites detected in the mushroom bodies appear to be the anatomical correlates for the postcopulatory behavioral

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changes, such as the decrease in female receptivity (Fleischmann et al., 2001).

More than 16 accessory gland proteins of *Drosophila melanogaster* males have been identified, and functionally and/or structurally similar proteins have been isolated from mucus and spermatophores of other insect species, as well. These proteins cover a wide range of molecular mass. For example, a 4 kDa ovulation stimulating substance and a sex peptide homolog of 5.1 kDa have been identified in *Drosophila sukuzii* (Ohashi et al., 1991; Schmidt et al., 1993). In *Locusta migratoria*, a 13 kDa peptide was shown to increase oviposition rates in females with mature oocytes (Lange and Loughton, 1985). A much larger protein, consisting of two 30 kDa subunits, acts as an oviposition stimulating protein in the stick insect, *Melanoplus sanguinipes* (Yi and Gillott, 1999), and in the army worm, *Helicoverpa armigera*, oogenesis- and oviposition-stimulating proteins are even larger, ranging between 55 and 66 kDa (Jin and Gong, 2001).

Postcopulatory stimulation of oogenesis and oviposition is also a constitutive element in the reproductive biology of the highly eusocial bees. *Apis mellifera* queens usually take their mating flights five to seven days after emergence. During the mating flight(s), a queen copulates with ten to twenty, or even more males (Adams et al., 1977; Estoup et al., 1994). A few days later she starts to lay eggs. Queens that are prevented from mating delay the onset of oviposition for several weeks (Koeniger, 1986a). Two qualitatively different modalities of oogenesis/oviposition stimuli can be envisaged, first, a mechanical stimulation of the queens's bursa copulatrix by the everted endophallus of the drone, and second, bioactive mucus gland factors transmitted during copulation (Koeniger, 1976, 1981). The two modalities of stimulation need not be mutually exclusive, and, in fact, may act synergistically.

Amazingly little attention has been paid to the potentially pleiotropic biological functions of mucus factors of honey bee drones. Despite the plethora of data for postcopulatory stimulation by male accessory gland products in other insects (for review see: Chen, 1984; Gillott 1996; Heifetz et al., 2001), only one product, a hyaluronidase, has been

characterized from drone mucus glands (Allalouf et al., 1974). The present study, therefore, aimed at a biochemical characterization of proteins secreted by the mucus gland of honey bee drones. Since drones are not capable of mating immediately after emergence, but rather pass through an 8–10-day period of sexual maturation before they start flying to drone congregation areas, we focused on developmental changes in protein content and pattern of the mucus gland secretions during this maturation period. The maturation process appears to be controlled and synchronized by the endocrine system, with juvenile hormone as one of the major players. Corpora allata activity in drones, and consequently also the juvenile hormone titer, increase within the first days of adult life, and exhibit a peak during this period of sexual maturation (Tozetto et al., 1995; Giray and Robinson, 1996), one of the consequences being promotion of flight activity (Tozetto et al., 1997). Ecdysteroids, which play important roles in the reproductive physiology of males in many insect species, and have been studied extensively with regard to their effects on male accessory gland products (Ismail and Gillott, 1995, 1997; Herndon et al., 1997), have not been investigated in this context in honey bees. In the present study, the hemolymph ecdysteroid titer was determined for sexually immature and mature drones, and these titer analyses were then compared to the effects on protein concentration and pattern in mucus gland secretion observed after 20-hydroxyecdysone (20E) injection.

2. MATERIALS AND METHODS

2.1. Collection and treatment of *Apis mellifera* L. drones

Drones were collected from hives of Africanized honey bees kept at the Experimental Apiary of the Department of Genetics, Faculty of Medicine in Ribeirão Preto, University of São Paulo, Brazil. To obtain drones of known age, frames with drone brood were transferred into bee-tight collector cages that were kept in an incubator (34 °C, 80% r.h.) for up to 8 hours. Drones emerging from brood cells during this time interval were sampled and received a paint mark or an Opalith plate on the thorax.

Drones were divided into three groups: untreated drones ($n = 78$) and drones treated with 20-hydroxyecdysone ($n = 56$) or with saline ($n = 62$), the latter being the transport vehicle for 20E. Newly emerged drones received an injection of 1 μL 20-hydroxyecdysone (Simes, Milan, diluted to 5 $\mu\text{g}/\mu\text{L}$ in saline solution), whereas solvent controls received 1 μL saline (NaCl 0.9%). The injection into the hemocele was performed with a Hamilton syringe, through the intersegmental membrane of one of the posterior abdominal segments. The injected and also the untreated drones were returned to 3-frame nuclei or single-story hives. To prevent aggression from workers, the drones were first kept under a queen excluder cage (20 \times 10 cm) fixed on a pollen comb, and were then released the next day. After introduction, drones were sampled at different ages for mucus gland dissection or hemolymph collection.

2.2. Mucus extraction and quantification of total protein content

For each drone, the paired accessory glands were dissected, the mucus was extruded from the glands by piercing with forceps, and stored individually at $-20\text{ }^{\circ}\text{C}$, for protein quantification and polyacrylamide gel electrophoresis. The mucus of each pair of glands was dissolved in 25–400 μL distilled water, depending on protein concentration of the sample. Aliquots of these extracts were used for total protein quantification by the method of Bradford (1976) using bovine serum albumin as standard.

2.3. SDS-PAGE

Separation of proteins secreted by accessory glands was routinely performed on 7–15% gradient polyacrylamide slab gels run under denaturing conditions (SDS-PAGE). Samples from accessory gland extracts containing 1 μg protein diluted in SDS-PAGE sample buffer were boiled for 5 min, spun at $10\,000 \times g$ for 5 min at $4\text{ }^{\circ}\text{C}$ and loaded onto the polyacrylamide gels. After electrophoresis, the gels were silver-stained. Molecular weight markers (a high MW standard mixture, Sigma, and a globin fragment marker for low MW proteins, Amersham Biosciences) were used as standards to determine the relative molecular mass of the secreted proteins.

2.4. Collection of hemolymph and ecdysone radioimmunoassay

Hemolymph of untreated, paint-marked drones was obtained from a small incision in the dorsal abdominal cuticle, or from the base of cut wings. Phenylthiourea-rinsed microcapillaries were used

to collect hemolymph. The hemolymph was stored at $-20\text{ }^{\circ}\text{C}$ until ecdysteroid extraction. After reading its volume in the microcapillaries to nearest 0.1 μL , the hemolymph was delivered into a 100-fold volume of methanol ($-20\text{ }^{\circ}\text{C}$) to precipitate hemolymph proteins and extract ecdysteroids. The precipitate forming at $4\text{ }^{\circ}\text{C}$ within at least 1 h was removed by centrifugation ($14\,000 \times g$, $4\text{ }^{\circ}\text{C}$, 10 min). Aliquots of the supernatant were transferred to 1 mL glass vials, and the solvent was evaporated by vacuum centrifugation. The ecdysteroids were quantified by radioimmunoassay (RIA), as previously described (Feldlaufer and Hartfelder, 1997; Pinto et al., 2002). [$^{3}\text{H}(\text{N})$]ecdysone (NEN, spec. act. 102 Ci/mmol) served as labeled ligand. Standard curves were established using 20-hydroxyecdysone (Simes, Milan) as nonradioactive ligand. Results are, therefore, expressed as 20E equivalents ($\text{pg}/\mu\text{L}$ hemolymph). Serum cross reactivity coefficients with other ecdysteroids were previously determined in displacement tests for our assay conditions (Pinto et al., 2002).

2.5. Data analysis

One way ANOVA tests (Tukey or Kruskal-Wallis), followed by appropriate post hoc tests (Tukey or Dunn) were performed to detect statistically significant differences ($P < 0.05$) in total protein content of mucus collected from control, 20E- and saline-injected drones of corresponding ages. Ecdysteroid titers of unknown samples were calculated by log-linear regression analysis of standard curve doses (25–2000 pg 20E) on logit binding values.

3. RESULTS

3.1. Content and pattern of mucus gland proteins during sexual maturation of honey bee drones

The quantity of proteins secreted by the glandular epithelium into the lumen of the mucus gland increases almost fifteenfold within the first five days of the adult life cycle of a honey bee drone (Fig. 1). After reaching peak levels around 1800 μg of total protein per gland at day 5, it slowly declines until day 8, settling at a plateau level between 1300 and 1400 μg total protein. This level remained stable during the rest of the period (up to day 14) monitored in this study. Thus, the temporal profile in mucus gland protein content can be broken down into two periods: a period of

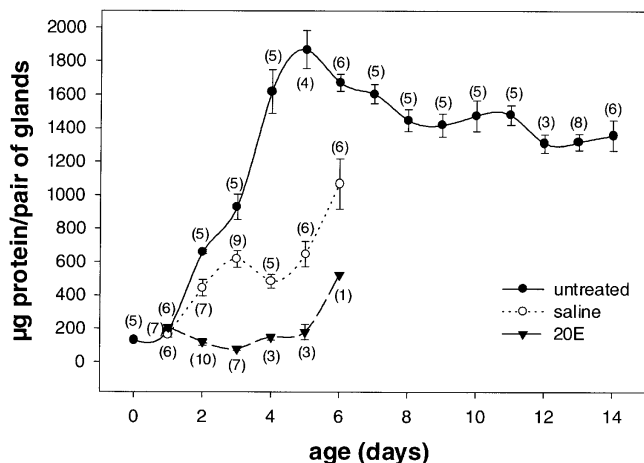


Figure 1. Protein content in the mucus gland of adult honey bee drones. The paired mucus glands were dissected from the reproductive tract, the secretion in the lumen of the glands was extracted and the protein content was quantified spectrophotometrically. Glands from untreated drones (closed circles) showed a steep increase in protein content during the first five days after emergence from the brood cell. The mucus protein content for drones that had received an injection of 20-hydroxyecdysone (closed triangles) shortly after emergence was strongly reduced (ANOVA, $P < 0.05$). Saline-injected drones (open circles), which are the control group for the experimental manipulation, showed an increase in protein content similar to untreated drones until day 3. The sham injected drones, however, exhibited transiently reduced protein levels during days 4 and 5 in comparison to the untreated drones (ANOVA, $P < 0.05$). Means \pm sem, and, in parentheses, sample size.

sexual maturation until day 8, and a period of stable protein amount in sexually mature drones.

The pattern of water-soluble proteins secreted by the mucus gland was analyzed by SDS-PAGE for the first 14 days of the adult life span of drones (Fig. 2). The molecular mass of mucus proteins covers the range between 174 and 25 kDa. In this pattern, a set of three proteins is dominating, characterized by molecular masses between 43 and 47.5 kDa. Since they show a persistent appearance during the life cycle stages analyzed in this study they can be considered as a major class of mucus proteins. Three further proteins, a high MW (174 000) and two low MW (25 000 and 26 000) proteins also make a stable contribution to the mucus proteins, yet in lesser relative amounts.

When comparing the mucus gland proteins of a newly emerged drone to a sexually mature drone, we observed a striking reduction in protein pattern complexity. This seems to occur in two steps, first, by a strong reduction or even disappearance of at least 10 protein

bands up to day 2 after emergence, and second, by another marked quantitative reduction in 6 further protein bands (94, 71.5, 28 kDa, and 3 proteins with molecular mass around 55 kDa) occurring between days 5 and 6. The first reduction step coincides with the increase in protein content in the mucus glands, and the second one happens concomitantly with the gradual decline in protein content observed in mucus glands of drones which are older than 5 days. Thereafter, only the proteins of 174, 47.5, 45, 43, 26 and 25 kDa persist, characterizing the mucus protein pattern of sexually mature drones.

3.2. Effect of 20E on the amount and pattern of proteins secreted by the mucus gland

To address the question of endocrine regulation in the adult life cycle of honey bees, we investigated in this study whether and how ecdysteroids may be involved in sexual maturation of drones, particularly with respect to the mucus glands. In an experimental

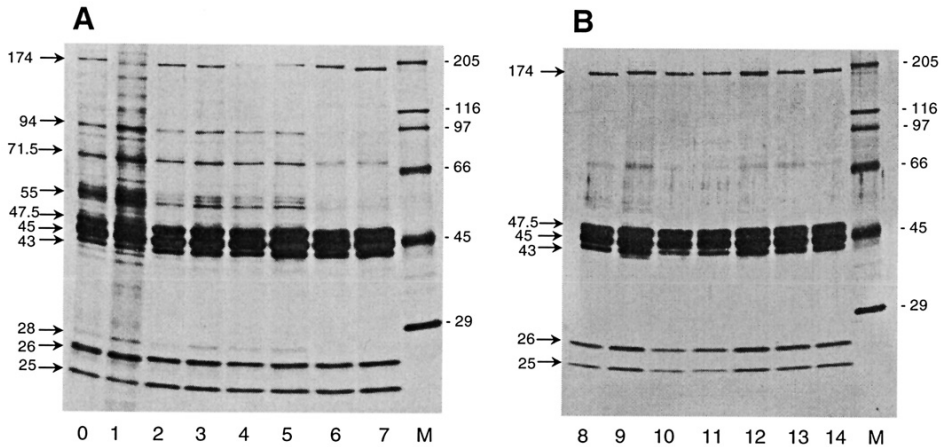


Figure 2. Protein pattern of mucus gland secretion of newly emerged to 14 day-old honey bee drones (A: days 0 to 7; B: days 8 to 14). Equal amounts of total protein (1 μg) were separated by SDS-PAGE. Molecular weight of the mucus gland proteins was determined by regression analysis on molecular weight standards (M). The highly complex protein pattern detected in newly emerged (0) and 1-day-old drones becomes reduced to a simpler pattern at day 2 and again at day 5, where three proteins with molecular mass between 43 and 47.5 kDa become highly dominant.

approach, we injected 5 μg of 20E diluted in saline into the hemocoel of newly emerged drones and monitored the profile of mucus gland proteins. Saline-injected drones served as controls for the injection procedure. The treatment with 20E had a pronounced effect on glandular activity as it essentially abolished the increase in mucus-gland protein content, normally observed during the first days of adult life (Fig. 1). Mucus glands of 20E-injected drones had a significantly lower protein content during the entire observation period (one-way ANOVA, $P < 0.05$), and, except for day 4, their glandular protein content was also lower than that of saline-injected drones. Monitoring of the mucus gland protein concentration in response to an ecdysteroid application was terminated at day 6 due to the high mortality of 20E injected drones. Protein content in the mucus glands of saline-injected controls was not statistically different from that of untreated controls, except for days 4 and 5 (one-way ANOVA, $P < 0.05$). At present we have no explanation for this transient difference in mucus gland protein levels of untreated versus sham-injected drones. The fact that the transient depression in protein levels in the sham-injected group became manifest after three days only suggests that it may be a delayed

wound effect. Furthermore, it demonstrates the necessity of including such sham injections in the experimental protocol when injecting bioactive compounds into the hemocoel of adult bees.

Subsequently we investigated whether and how this remarkable suppressive effect of 20E on protein accumulation in the mucus gland was reflected in the protein spectrum, i.e. whether it was due to a reduced production of specific proteins, or whether it is more general, affecting the secretion of all or most of the mucus gland proteins. As in the previously established age profile, we applied to the gels equal amounts of total protein (1 μg per sample) obtained from mucus gland extracts of 20E-treated drones and compared them directly to saline-treated and untreated (control) drones. 20E injection had a prolonged effect on the protein pattern of mucus glands dissected from drones that were 3 days of age or older (Fig. 3). Protein patterns in 1- and 2-day-old drones were similar in the respective experimental groups (data not shown). 3 to 5 days-old saline-treated drones exhibited a similarly reduced protein pattern as control drones, whereas the 20E-treated drones conserved the complex protein pattern typical of newly emerged drones. This

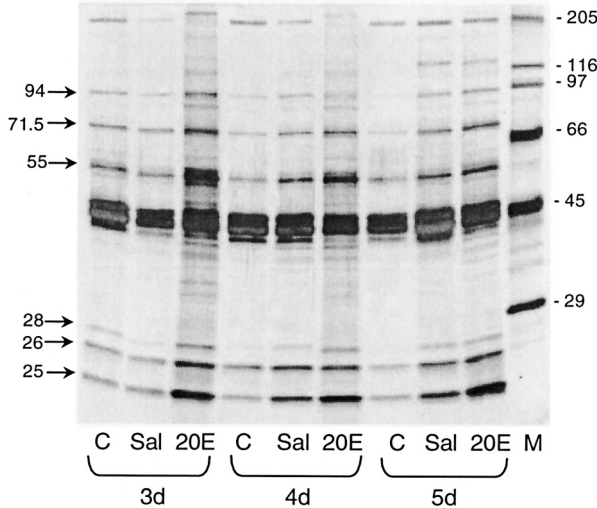


Figure 3. Protein pattern of mucus gland secretion of 3 to 5-day old drones treated at emergence with 20-hydroxyecdysone (20E) or saline (Sal), in comparison to mucus glands of untreated drones (C). Equal amounts of total protein (1 μ g) were separated by SDS-PAGE. Molecular weight of the mucus gland proteins was determined by regression analysis on molecular weight standards (M). 20E-treated drones maintain for a prolonged period the highly complex protein pattern characteristic for newly emerged drones, suggesting that an elevated 20E titer leads to a retardation of sexual maturation in mucus gland activity.

analysis also shows that sham-injected drones differ in their protein pattern from 20E-injected drones, even at stages when the two groups were similar in their mucus gland protein content. The 174 kDa band was virtually absent in 3-day old 20E-injected drones, which instead continued to express the 205 kDa protein typical of 1-day old control drones (see Fig. 2). Also, the expression of the 43 kDa protein appeared to be significantly reduced in 20E-injected drones, when compared to the control and sham-injected groups. This permits us to conclude that an experimentally elevated ecdysteroid titer may retard the maturation of the mucus gland by (a) maintaining the expression of most mucus gland proteins at the low levels that are characteristic for newly emerged drones, and (b) by specifically regulating the expression of at least three prominent mucus proteins.

3.3. The edysteroid titer of adult drones

Exploring the physiological meaning of the strong repressive effects on protein content and pattern detected after 20E injection required determination of endogenous ecdysteroid titers. The age samples analyzed in this study cover the most important part of the adult life cycle of honey bee drones, that is the maturation phase and the period of mating flights, which are usually initiated between days 8 and 10 after emergence (Drescher,

1969; Fukuda and Ohtani, 1977; Tozetto et al., 1997). The titer profile exhibits two noteworthy characteristics (Fig. 4): first, a marked decrease in ecdysteroid levels within the first day after emergence, and second, a small peak around day 8. During the rest of the adult life cycle, the ecdysteroid titer fluctuates around basal levels.

The drastic drop in the hormone titer during the first 24–32 hours of adult life was only detectable because the drones were sampled within a few hours after emergence from the brood cell. The declining ecdysteroid titer precedes the increase in mucus gland protein content during this time interval (Fig. 1), and coincides with the disappearance of many proteins from the initially complex pattern of proteins secreted by the mucus gland (Fig. 2). The results of the hormone titer analysis, thus, provide an endocrine explanation for the suppression of mucus gland activity – both in protein synthesis and pattern maturation – observed after 20E-injection into newly emerged drones.

4. DISCUSSION

The quantitative and qualitative analyses of proteins secreted by the mucus gland of honey bee drones revealed a striking age-related developmental profile with marked cut-off points at days 2 and 5 of the adult life cycle.

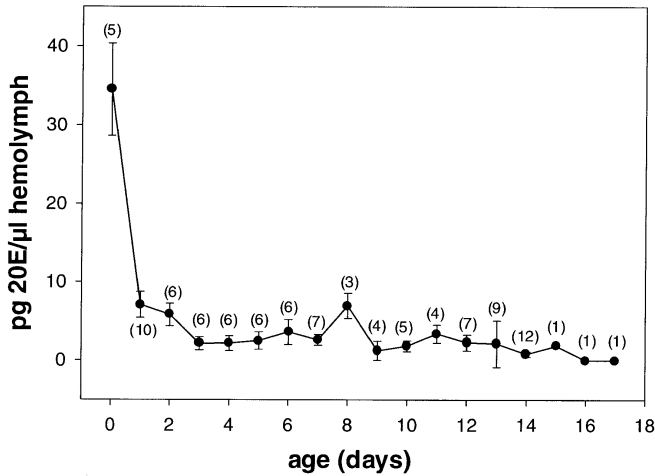


Figure 4. Hemolymph ecdysteroid titer of newly emerged to 17-day-old drones. The titer was determined by radioimmunoassay using 20E as unlabeled ligand for standard curve preparation. Titer values (means \pm sem; sample size in parentheses) are thus presented as pg 20E equivalents per μ l hemolymph. The ecdysteroid titer strongly decreases after emergence from the brood cell and exhibits a small peak at day 8.

From day 2 on, the protein content increases steadily until reaching peak levels at day 5, and the protein pattern exhibits a first reduction in complexity. As of day 5, the protein content in the mucus secreted per gland gradually decreases until reaching a stable plateau level at around day 8. This is accompanied by a further reduction in protein pattern complexity. In an attempt to come to an understanding of why these changes occur and how they may relate to life cycle aspects of honey bee drones, we analyzed the endogenous ecdysteroid titer by RIA, and also manipulated the titer by injections of 5 μ g 20E into newly emerged drones. 20E-injections caused a drastic reduction in protein content of mucus and delayed the normal protein pattern changes. The developmental profile of the endogenous ecdysteroid titer, characterized by a steep decline during the first 24–32 hours after emergence from the brood cell, may explain the changes in protein content and pattern observed in the mucus gland of very young adult drones. In combination, the age profile of the endogenous hormone titer and the effects of its experimental manipulation provide strong evidence for an inhibitory role of ecdysteroids in the maturation of this accessory gland of the drones' reproductive system. The fact that sham-injected drones also exhibited a transient reduction in mucus gland protein content – though distinct from that of 20E-injected drones – is an observation that we currently cannot satisfactorily explain. It clearly deserves more detailed attention,

especially in the context of possible wound reactions in *in vivo* experiments involving bees.

As to why the ecdysteroid titer levels are elevated at emergence and rapidly decrease thereafter, is a subject that still needs investigation. As it is also observed in female honey bees, it appears to represent a general developmental phenomenon of the pupal-adult transition. The pupal and adult ecdysteroid titers for honey bee queens and workers have recently been determined (Hartfelder et al., 2001; Pinto et al., 2002), showing a marked drop from levels around 50 pg 20E-equivalents/ μ L hemolymph, detected in worker pupae shortly before eclosion, to levels around 5 pg 20E-equivalents/ μ L hemolymph in newly emerged workers. This decline to five to tenfold lower levels around emergence of bees from the brood cell may be a result of enzymatic degradation or conversion of biologically active ecdysteroids to biologically and also RIA-inactive metabolites. It is interesting to note in this context that the minor ecdysteroid peak in adult bees, which was detected between days 3 and 5 in workers, appears to be shifted to a much later age in drones, where it occurs around day 8. We have yet little information as to the functional significance of this small ecdysteroid peak, but it is notable that it coincides with a behavioral change, since workers and drones are known to initiate first orientation flight activities at the respective ages (Ruttner, 1966; Capaldi et al., 2000).

Taken together, the results of this study provide evidence that ecdysteroids play a role as negative regulators in this aspect of sexual maturation of drone honey bees, apparently inhibiting a precocious mucus gland activity before or shortly after emergence. With respect to ecdysteroid-dependent changes in male accessory gland activity, honey bee drones are not exceptional, and, both, inhibitory and stimulatory effects of ecdysteroids on protein synthesis in male accessory glands have been reported for *Spodoptera litura* (Sridevi et al., 1988), *Chilo partellus* (Ismail and Dutta-Gupta, 1990), *Melanoplus sanguinipes* (Ismail and Gillott, 1995) and *Locusta migratoria* (Ismail and Gillott, 1997). In most of these cases, male accessory gland activity appears to be regulated antagonistically by juvenile hormone and ecdysteroids, not only at general levels, but also individual proteins may be regulated differentially by these hormones.

Considering the changes in mucus proteins and the profiles of corpora allata activity and juvenile hormone titer of *Apis mellifera* drones (Tozetto et al., 1995; Giray and Robinson, 1996), a role for juvenile hormone in the context of sexual maturation in general, not only in mating flight initiation, is also strongly suggested. The juvenile hormone titer exhibits a marked increase around day 5, coinciding with peak values in mucus protein content and with the second reduction in protein pattern complexity. Ecdysteroids and juvenile hormone, thus, may be regulating in a coordinate manner the maturation process of the mucus gland of drones. But while such a (inhibitory) role has now been experimentally evidenced for ecdysteroids, an experimental analysis on the putative role of juvenile hormone in mucus gland maturation is still lacking.

The present study aimed at a description of proteinaceous male accessory gland products during sexual maturation of honey bee drones. We see this investigation as a contribution to elucidate functional aspects of such male-produced compounds in the regulation (manipulation) of female reproductive biology. The honey bee is an interesting system because queens exhibit clear postcopulatory changes in physiology and behavior. In distinction to most other insects, however, honey bee queens are extremely polyandrous, and the mating

sign of a single drone – and the possibly bioactive compounds contained therein – are, thus, unable or insufficient to ensure exclusive paternity to a drone. The mating sign, in fact, appears to play multiple roles (Koeniger, 1986b, 1990) and its original function to hinder additional copulations, seems to have been converted to a mating stimulus in the polyandry repertoire of the honey bee queen (Tapy and Page, 2000). Water-soluble mucus proteins or peptides certainly are candidates in the search for bioactive compounds that trigger postcopulatory changes in the queen, and, for this reason, have been investigated in this study. Other compounds, however, may also be of importance since postmating behavioral changes in the bumble bee, *Bombus terrestris*, have been shown to be elicited by linoleic acid (Baer et al., 2001).

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Résumé – Teneur en protéines et spectre des protéines de la glande à mucus et leur contrôle par l'ecdystéroïde au cours de la maturation sexuelle des mâles d'abeilles domestiques. Chez de nombreux insectes les produits sécrétés par la glande accessoire du système reproducteur mâle joue un rôle important dans la biologie de la reproduction des femelles. Les composantes des sécrétions protéiniques, qui peuvent être utilisées pour former un spermatophore ou être transférées avec le sperme durant la copulation, sont susceptibles de modifier le comportement post-copulatoire des femelles et d'accroître leur taux de ponte. Les reines d'abeilles (*Apis mellifera* L.) présentent de telles modifications dans le comportement et la physiologie de la reproduction après le vol nuptial. Afin d'obtenir des informations sur le cycle de fonctionnement de la glande à mucus des mâles, et d'en tirer d'éventuelles conclusions concernant les processus de maturation sexuelle, nous avons étudié la teneur en protéines et le spectre des polypeptides des sécrétions de ces glandes. Afin d'étudier la régulation hormonale sous-jacente aux

processus de maturation, nous avons injecté à des mâles naissants 5 µg de 20-hydroxyecdysone (20E) et suivi les effets sur la teneur en protéines du mucus et sur leur spectre. Nous avons analysé en parallèle par méthode radioimmunologique (RIA) le titre d'ecdystéroïde de mâles non traités.

La teneur en protéines de la sécrétion de la glande à mucus est passée, par paire de glandes, de 125 µg juste après l'émergence à plus de 1 800 µg cinq jours plus tard (Fig. 1). Pendant la même période, la complexité du spectre des protéines, déterminé par électrophorèse sur gel de polyacrylamide en présence de SDS, s'est réduit. A partir du 5^e jour, la teneur en protéines a diminué légèrement pour atteindre un niveau stable aux alentours de 1 400 µg par paire de glandes au 8^e jour. Durant cette phase, on a observé une nouvelle réduction de la complexité du spectre, de sorte que trois polypeptides de 43 à 47,5 kDa prédominaient, suivis par une protéine de 174 kDa et de deux protéines plus petites de 25 et 26 kDa (Fig. 2). Une injection de 20E à des mâles naissant a supprimé l'augmentation de la teneur en protéines et retardé la réduction de la complexité du spectre (Fig. 3). Les analyses par RIA du titre d'ecdystéroïde ont confirmé ces résultats, suggérant un rôle inhibiteur des ecdystéroïdes sur la maturation de la glande à mucus. Le titre d'ecdystéroïde des mâles adultes a chuté de façon drastique dans les 24–32 heures après l'émergence, passant de 35 pg/µL d'hémolymphe au niveau de base de 5–8 pg/µL (Fig. 4).

Les changements observés dans la teneur en protéines de la sécrétion de la glande à mucus et dans son spectre surviennent en liaison avec les processus généraux de maturation sexuelle. Le déclin du titre d'ecdystéroïde de sa valeur au stade nymphal avancé jusqu'au niveau juste après l'émergence semble être un élément essentiel dans le démarrage des processus de maturation. Puisque le titre d'hormone juvénile présente un accroissement qui coïncide avec l'augmentation de la teneur en protéines au 5^e jour, on peut penser à un rôle antagoniste de ces deux hormones dans le contrôle de la maturation de la glande à mucus. Dans ce contexte, les ecdystéroïdes semblent agir comme des régulateurs négatifs empêchant l'activité précoce de la glande à mucus. Il est intéressant d'observer un pic mineur supplémentaire dans le titre d'ecdystéroïde chez les mâles âgés de 8 jours, peu de temps avant le début de l'activité de vol qui est sous le contrôle de l'hormone juvénile.

***Apis mellifera* / glande accessoire mâle / ecdysone / radioimmunologie / protéine du mucus**

Zusammenfassung – Gehalt und Muster von Mucusdrüsenproteinen und ihre Kontrolle durch Ecdysteroide während der sexuellen Reifung der Drohnen der Honigbienen. Produkte

der akzessorischen Drüsen des männlichen Reproduktionsapparats spielen bei vielen Insekten eine grosse Rolle in der Reproduktionsbiologie der Weibchen. Komponenten der proteinhaltigen Sekrete, die entweder eine Spermatophore bilden oder bei der Kopulation direkt mit dem Sperma übertragen werden, können das postkopulatorische Verhalten der Weibchen modifizieren und ihre Eilegerate erheblich steigern. Entsprechende Änderungen in Verhaltensweisen und Reproduktionsphysiologie sind auch bei Bienenköniginnen nach dem Hochzeitsflug klar auszumachen. Um einen Einblick in den Funktionszyklus der Mucusdrüsen der Drohnen zu erhalten und mögliche Schlussfolgerungen in Hinblick auf sexuelle Reifungsprozesse ziehen zu können, wurden in der vorliegenden Studie die Proteingehalte und Polypeptidmuster der Sekrete dieser Drüsen untersucht. Zur Beantwortung der Frage der hormonellen Regulation dieser Reifungsprozesse wurde frischgeschlüpften Drohnen 5 µg 20-Hydroxyecdysone (20E) injiziert, und die Auswirkungen dieser experimentellen Manipulation des Ecdysteroid-Titers auf Proteingehalt und -Muster des Drüsensekrets untersucht. Parallel hierzu wurde der Ecdysteroid-Titer unbehandelter Drohnen mittels Radioimmunologie (RIA) bestimmt.

Der Proteingehalt im Sekret der Mucusdrüsen stieg innerhalb der ersten 5 Tage nach dem Schlupf von etwa 125 µg auf über 1800 µg pro Drüsenpaar an (Abb. 1). Gleichzeitig verringerte sich die Komplexität des Proteinmusters (SDS-PAGE). Ab Tag 5 fiel der Proteingehalt leicht ab und pendelte sich ab Tag 8 auf einem Niveau von etwa 1400 µg pro Drüsenpaar ein. In dieser Phase war ein zweiter Schritt in der Verringerung der Komplexität des Proteinmusters zu verzeichnen, so dass im Sekret der Mucusdrüsen geschlechtsreifer Drohnen drei Polypeptide von 43 bis 47,5 kDa dominierten, gefolgt von einem Protein um 174 kDa und zwei kleineren Proteinen um 25 und 26 kDa (Abb. 2). Eine 20E-Injektion in frischgeschlüpfte Drohnen verhinderte den Anstieg im Proteingehalt und verzögerte die Reduzierung der Komplexität des Proteinmusters im Drüsensekret (Abb. 3). Eine inhibitorische Kontrolle des Reifungsprozesses der Mucusdrüsen durch Ecdysteroide wird auch durch die Befunde der RIA-Analysen gestützt. Der Ecdysteroid-Titer adulter Drohnen sinkt innerhalb der ersten 24–32 h nach dem Schlupf drastisch ab, von ca. 35 pg/µL Hämolymphe auf ein Grundniveau von 5–8 pg/µL (Abb. 4).

Die Veränderungen im Proteingehalt und Proteinmuster der Sekrete der Mucusdrüsen adulter Drohnen erfolgen damit im Zusammenhang allgemeiner sexueller Reifungsprozesse. Das Absinken des Ecdysteroid-Titers, von spätpupalen Titerwerten auf ein Grundniveau kurz nach dem Schlüpfen aus der Brutzelle, stellt eine wesentliche Voraussetzung für den Beginn dieser Reifungsprozesse dar. Da der Juvenilhormon-Titer

einen nahezu parallelen Anstieg mit dem Proteingehalt der Mucusdrüsen aufweist, liegt es nahe, eine antagonistische Kontrolle der Reifung der Mucusdrüse durch diese beiden Hormone zu postulieren. Ecdysteroiden spielen in diesem Kontext offensichtlich eine Rolle als negative Regulatoren, die eine verfrühte Aktivität der Mucusdrüsen verhindern. Interessanterweise war im Ecdysteroid-Titer von acht Tage alten Drohnen ein kleiner Peak zu verzeichnen, also kurz vor dem Beginn der Flugaktivität, die ebenfalls wesentlich von Juvenilhormon beeinflusst wird.

Männliche Anhangdrüsen / Ecdyson / Radioimmunoassay / *Apis mellifera* / Mucus Proteine

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