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Original article

Concentration of hemolymph proteins during postembryonic worker development of Africanized honey bees in Brazil and Carniolans in Europe

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Summary — The profiles of hemolymph protein content during postembryonic development of honey bees *(Apis mellifera)* were determined for the first time. Larval and pupal worker stages of Africanized honey bees in Brazil and Carniolans in Germany were studied. A rapid increase in protein concentration was found in the late larval stages, followed by a decrease in prepupae and a minimum amount near the pupal moult period. In the early pupal stages, the protein concentration increased again, and then decreased until adult eclosion. Only the first protein peak in spinning L5 was more pronounced in the Africanized honey bees. No differences between the hemolymph protein profiles of Africanized bees and Carniolans could be detected during pupal development. The duration of several larval and pupal stages was shorter in Africanized than in Carniolan workers. The data reflect the general characteristics of the Africanized biotype reared in tropical conditions exhibiting accelerated patterns of development and lower body weight at emergence in comparison to the European *Apis* subspecies reared in temperate conditions. The ontogenetic patterns described for hemolymph protein concentration in preimaginal honey bee development are also discussed, specifically in regard to the regulation of metamorphosis in holometabolous insects.

hemolymph / protein concentration / postembryonic development / *Apis mellifera carnica* / Africanized honey bee

INTRODUCTION

During the postembryonic stages of insects, the protein concentration in the hemolymph undergoes considerable changes related to developmental events (Levenbook, 1985). In the holometabolous groups, the most pronounced alterations occur in the course of metamorphosis (Kanost *et al,* 1990) when storage proteins accumulate in the

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hemolymph (Telfer and Kunkel, 1991). The storage hexamer arylphorin has been described from the hemolymph of larval honey bees (Ryan *et al,* 1984), but so far the total protein content has not been analyzed.

Since the duration from oviposition until emergence of a worker is about 1 d shorter in Africanized than in European honey bees (Kerr *et al,* 1970; Rosenkranz, 1990; Michelette and Soares, 1993; Rosenkranz and Engels, 1995), the question arises whether this is reflected in the level of the hemolymph protein concentration. The Africanized honey bee is generally characterized by rapid colony growth and frequent swarming (Needham *et al,* 1988; Spivak *et al,* 1991; Winston, 1992). Much less is known about individual characters, especially the development of this biotype of bees which still retains genetic similarities to its African ancestor *Apis mellifera scutellata* (Lobo *et al,* 1989). The body weight of newly emerged Africanized worker bees is only about 85% of Carniolans (Engels *et al,* 1986).

In addition to possibly genetic differences, climate, nutrition, colony management and other conditions may also influence the postembryonic development of the bees. In consideration of all these factors, the hemolymph protein concentration in the postembryonic worker stages was studied comparatively under natural biotope conditions in tropical Brazil using Africanized honey bees and in Germany with a temperate climate using *A m carnica.*

MATERIALS AND METHODS

Experimental honey bee colonies

Colonies of Africanized honey bees (*A m scutellata* Lepeletier hybrids), kept in Langstroth hives, and colonies of Carniolans (*A m carnica* Pollmann), housed in Deutsch-Normalmaß (DN) magazine hives, were maintained at the experimental apiaries of the University of São Paulo and the University of Tübingen, respectively. If necessary, sugar solution was fed to obtain strong colonies with ample brood. The queens were confined on brood combs for 6 h periods to obtain larvae and pupae of known ages. Brood samples were collected from the center of the brood nest every 6 h from 6 Africanized colonies in Ribeirão Preto (21° southern latitude, 48° western longitude) and from 4 Carniolan colonies in Tübingen (48° northern latitude, 9° eastern longitude). Brood frames were removed in rotation from different hives. The staging of postembryonic instars was done according to Rachinsky *et al* (1990) as previously described by Michelette and Soares (1993).

Hemolymph analyses

After making a small dorsal incision in the abdominal integument, hemolymph was collected from bees in capillaries and stored on ice. Because it was difficult to sample pure hemolymph from newly hatched and second instar larvae, only the stages from the early 3rd instar until adult eclosion were used. Per age group, 2–6 hemolymph pools of 20–100 individuals were sampled from different experimental colonies. A few crystals of phenylthiourea were added to each pool to prevent melanization. Centrifugation to remove the hemocytes was done at 4°C for 10 min with 5 000 *g.* Because of the high number of cells floating in the hemolymph of pupal stages, these samples were centrifuged twice. The hemolymph pools were stored at –20°C. No protein degradation could be observed prior to sample analysis. The determination of hemolymph protein concentration (Bradford, 1976) was carried out in Germany and Brazil using identical equipment. Calibration curves were established using bovine serum albumin (BSA) as a standard protein. Aliquots of the hemolymph pools were diluted for the measurements which were repeated 3 times.

RESULTS

A steady increase in hemolymph protein concentration occurs during the late larval worker development period. A decrease

occurs during the prepupal stages. After pupation, the protein content again increases during the first 3 d, and then decreases during late pupal development of pharate adults until the occurrence of the imaginal moult. This general pattern is the same in the Africanized (fig 1) and Carniolan (fig 2) worker developmental period. However, there was more variation of the pooled samples in Africanized honey bees, especially near the peaks in protein concentration. To improve the resolution for these periods, up to 6 samples per age group were taken from different Africanized honey bee colonies. In the Africanized worker bees, the first late larval peak in hemolymph protein concentration was 10–20 μg/μl higher than the second peak during early pupal stages (fig 1). In contrast, the second peak was even a little higher in the Carniolans along with some more variation than observed in the first peak (fig 2).

The mean duration of several stages was shorter in the Africanized workers compared with the Carniolan worker bees (fig 1 and 2), resulting in a difference of about 1 d for the preimaginal development period. To compare the ontogenetic profiles in the protein content of the hemolymph, common stage averages were computed by using all the mean values within the same instar. Using this procedure, instead of a time scale, an *x*-axis of developmental stages was constructed (fig 3). The resulting stage-specific patterns of postembryonic hemolymph protein concentration, in fact, allow a better discrimination of similarities and discrepancies between Africanized and Carniolan worker honey bee development. The rapid increase in concentration from L3

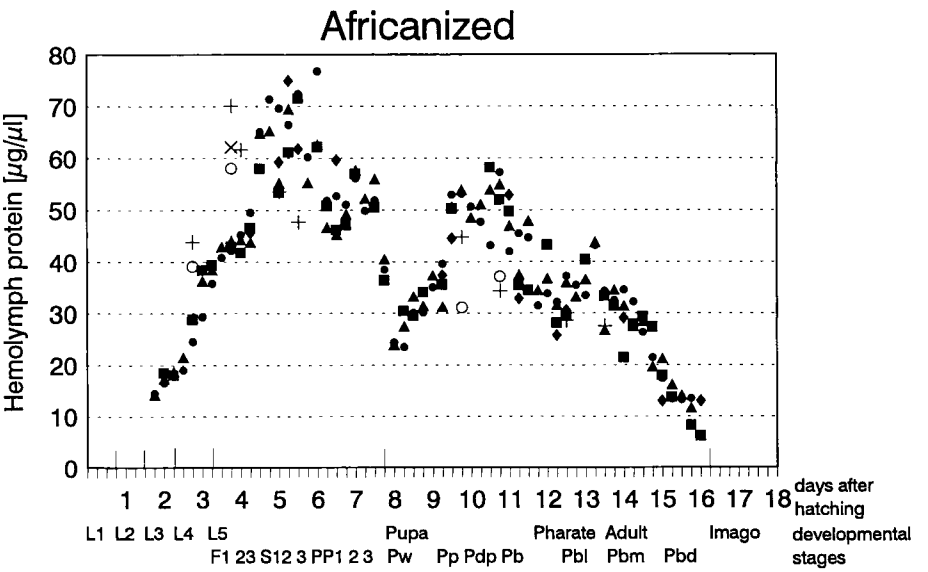

Fig 1. Protein concentration in the hemolymph of postembryonic worker stages of Africanized honey bees (*A m scutellata* hybrids). Every 6 h, pooled samples were taken from 2–6 colonies at the tropical study site in Brazil. Symbols indicate different samples from various colonies. Means of 3 measurements per sample. L1–L5 = larval instars, F = feeding, S = spinning, PP = prepupae, Pw = white-eyed pupae, Pp = pink-eyed pupae, Pdp = dark-pink-eyed pupae, Pb = brown-eyed pupae, Pbl = pupae with light-brown dorsal mesothorax, Pbm = pupae with medium-brown dorsal mesothorax, Pbd = pupae with dark-brown dorsal mesothorax.

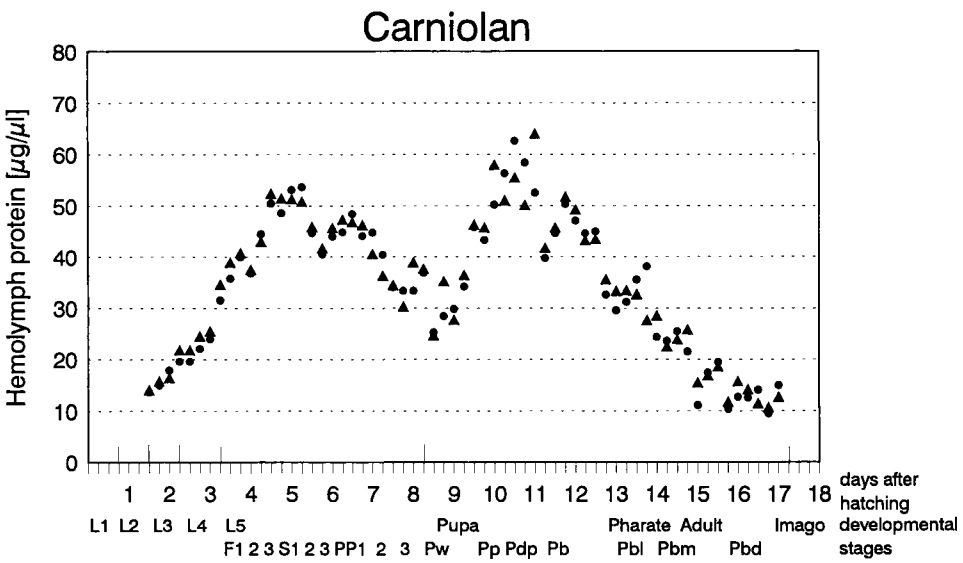

Fig 2. Protein concentration in the hemolymph of postembryonic worker stages of Carniolan honey bees (*A m carnica).* Every 6 h, pooled samples were taken from 2 colonies at the temperate study site in Germany. Abbreviations in figure 1.

to mid-L5 began a little earlier in the Africanized honey bee larvae, and the subsequent peak was significantly higher ($p < 0.05$, Friedman test). In addition, the minimum concentration around the pupal moult was somewhat lower in Africanized than in Carniolan honey bees. However, during the pupal stages the hemolymph protein concentration did not differ between Africanized and Carniolan honey bee workers until adult eclosion. The range of the average hemolymph protein content in the postembryonic stages was 9.8–63.5 µg/µl in the Africanized and 12.2–56.3 µg/µl in the Carniolan samples (fig 3).

DISCUSSION

The developmental patterns of protein concentration in the hemolymph of postembryonic stages of worker bees have not been studied previously (Ryan *et al,* 1984). Comparable data from other insects are also sparse in the literature (Kanost *et al,* 1990). The general hemolymph protein profiles with peaks occurring during late larval and around the transition from pupal to pharate adult stages are in good agreement with results obtained for other holometabolous insects (Chen and Levenbook, 1966). The high level of protein concentration in the hemolymph of mid-pupal stages is apparently related to the metamorphic dismantling of larval structures whereas the late larval peak corresponds to the arylphorin discharge (Ryan *et al,* 1984).

Since the fat body is the major source of hemolymph proteins, a corresponding activity in its mRNA synthesis can be assumed (Bosquet *et al,* 1989). Juvenile hormone is not only the main factor in stimulating protein synthesis (Keeley, 1978), but also in enhancing the export of hemolymph proteins by the fat body (Kanost *et al,* 1990). The hemolymph titers of juve-

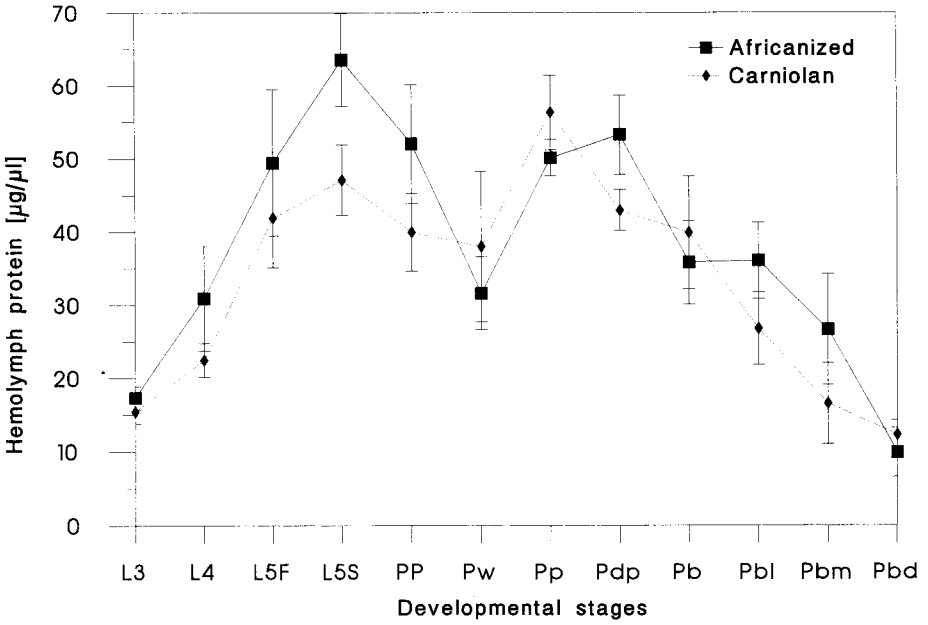

Fig 3. Comparison of the average protein concentration in the hemolymph of postembryonic worker stages from L3 until adult eclosion in Africanized and Carniolan honey bees. Per stage the mean values as shown in figures 1 and 2 were taken to calculate these average data, altogether based on a total of over 30 000 individuals. Abbreviations as in figure 1.

nile hormone in honey bees have been analysed during larval and pupal stages in queens and workers (Rachinsky *et al*, 1990). A peak was found during the L5 feeding phase in queens, but only an increase in the hormone level of workers. A few hours later this is followed by the first peak in hemolymph protein concentration. This peak coincides with the maximum larval weight during the preimaginal developmental period of worker bees (Michelette and Soares, 1993).

In tropical Brazil, the well-known peculiarities in behavior and population dynamics of the Africanized biotype can be observed (Gonçalves and Stort, 1978; Dietz, 1992; Winston, 1992). Additionally, some special traits in ontogenetic parameters such as duration of preimaginal developmental stages are also of interest (Michelette and Soares, 1993). These differences between Africanized and European honey bees are less pronounced if the latter are kept under tropical conditions (Rosenkranz and Engels, 1995).

Therefore, such characteristics are assumed to depend on both genetic and environmental factors. The differences found in the developmental profiles of postembryonic hemolymph protein concentration in Africanized and Carniolan worker bees reared in Brazil and Germany, respectively, were only relatively small. These differences are restricted to the late larval phase (fig 3). The larger variation in hemolymph protein content in the *A m scutellata* hybrids (fig 1) may reflect the more heterogeneous genome status of an only recently established biotype in comparison to the subspecies *A m carnica* (fig

2). The faster increase in protein content and the higher peak during the spinning period of the last larval instar in Africanized worker bees may reflect a more rapid food intake and increased growth rate. These activities are presumably under genetic control.

The Africanized biotype is generally characterized by less nutritional investment in the individual bee (Winston, 1992). This results in adult workers which are smaller (Engels *et al,* 1986) than most of the European races (Ruttner, 1988). On the other hand, Africanized honey bees require less time for preimaginal development (Michelette and Soares, 1993; Rosenkranz and Engels, 1995). Together with an earlier onset of foraging tasks (Winston and Katz, 1982; Sommer, 1986; Robinson *et al,* 1987), our findings contribute to the well-known fact of explosive population dynamics of the Africanized honey bees and their successful occupation of much of the American continent.

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Résumé — Teneur de l'hémolymphe en protéines au cours du développement postembryonnaire de l'ouvrière d'abeilles africanisées au Brésil et d'abeilles carnoliennes en Europe. La concentration de l'hémolymphe en protéines a été pour la première fois mesurée au cours du développement postembryonnaire de l'abeille mellifère. On a étudié chez des larves et des nymphes d'ouvrières issues de colonies d'abeilles africanisées (hybrides *d'Apis mellifera scutellata)* à Ribeirão Preto, SP, Brésil (fig 1), et de colonies d'abeilles carnoliennes *(Apis mellifera carnica)* à Tübingen, Allemagne (fig 2) tous les stades depuis L3 juqu'à la mue imaginale. On a divisé le stade L5 en 9 sous-stades et celui de nymphe, y compris les nymphes juste avant l'émergence, en 7 sous-stades. Pour chacun d'entre eux on a prélevé toutes les 6 h des échantillons d'hémolymphe sur 20 individus au minimum, de sorte que les moyennes représentées sur les figures 1 et 2 sont celles d'échantillons de grande taille. La teneur en protéines a été évaluée d'après Bradford (1976) avec une courbe étalon établie avec de l'albumine de sérum de bovin (BSA). Puisque la durée de développement pré-imaginal de l'abeille africanisée est plus courte d'un jour que celle de l'abeille *carnica* nous avons calculé aussi les moyennes non pas en fonction de l'âge mais du stade de développement, de façon à pouvoir comparer entre elles des phases physiologiques semblables (fig 3). L'allure générale de la courbe de la teneur en protéines de l'hémolymphe est la même chez les abeilles africanisées et chez *carnica.* Les maximums se situent à la fin de la période larvaire et au début de la phase nymphale. Chez les abeilles africanisées l'augmentation de la concentration pendant la période larvaire est plus rapide et atteint au stade de filage du cocon des valeurs significativement plus élevées que chez *carnica.* Nous en concluons que la teneur en protéines est plus influencée par les facteurs génétiques que par les facteurs du milieu. Il n'y a en revanche aucune différence entre les 2 groupes durant le développement nymphal. Ces résultats sont discutés par rapport aux particularités du biotype africanisé et aux caractéristiques générales de la biologie du développement des insectes holométaboles.

abeille africanisée / *Apis mellifera carnica* / hémolymphe / teneur en protéines / développement postembryonnaire

Zusammenfassung — Proteingehalt der Haemolymphe während der präimaginalen Entwicklung von Afrikanisierten Honigbienen in Brasilien und von *Apis mellifera carnica* in Europa. Die Konzentration der Haemolymph-Proteine in der postembryonalen Entwicklung wurde für Honigbienen erstmals ermittelt. Bei Larven und Puppen der Arbeiterinnen von Afrikanisierten in Ribeirão Preto / Brasilien (Abb 1) und von *Carnica*-Völkern in Tübingen / Deutschland (Abb 2) wurden alle Stadien vom L3 bis zur Imaginalhäutung untersucht, wobei das L5 in 9 und die Puppe, einschließlich der Pharat-Adulten, in 7 Unterstadien eingeteilt wurden. Von jedem wurden im 6-Std-Abstand einige Haemolymph-Sammelproben von 20 oder mehr Individuen präpariert, so daß die in Abbildungen 1 und 2 dargestellten Mittelwerte große Versuchstier-Stichproben repräsentieren. Der Proteingehalt wurde nach Bradford (1976) mit einer BSA-Eichkurve bestimmt. Da die präimaginale Entwicklungsdauer bei Afrikanisierten etwa einen Tag kürzer ist als bei Carnica-Bienen, wurden außerdem Durchschnittswerte berechnet, die nicht auf das Alter, sondern auf die Entwicklungs-Stadien bezogen sind, um so einander entsprechende physiologische Phasen vergleichen zu können (Abb 3). Der allgemeine Kurvenverlauf des Proteingehalts der Haemolymphe bei Arbeiterinnen-Larven und Puppen ist bei Afrikanisierten und Carnica-Bienen gleich. Gegen Ende der Larvenperiode und zu Anfang der Puppenphase treten Maxima auf. Bei Afrikanisierten erfolgt der larvale Konzentrationsanstieg rascher und erreicht in der Spinnphase des L5 signifikant höhere Werte als bei Carnica. Deswegen wird geschlossen, daß dies mehr auf genetischen als auf Umwelteinflüssen beruht. Während der Puppenentwicklung gibt es dagegen keine Unterschiede zwischen beiden Gruppen. Diese Ergebnisse werden unter Berücksichtigung von Besonderheiten des Biotyps Afrikanisierte und allgemeiner entwicklungsbiologischer Eigenschaften holometaboler Insekten diskutiert.

Konzentration der Haemolymph-Proteine / präimaginale Entwicklung / *Apis mellifera carnica* / Afrikanisierte Honigbiene

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