STORAGE PROTEINS IN WINTER HONEY BEES

G. W. Otis[1]; D. E. Wheeler[2]; N. Buck[2]; H. R. Mattila[1]

[1] University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] University of Arizona, Tucson, Arizona, USA 85718

ABSTRACT

Honey bee colonies in temperate climates begin brood rearing in late winter before floral resources are available. Protein required for brood rearing comes from pollen stored in combs as well as proteins stored internally in the bees' bodies. We studied the changes in amounts of several proteins that could serve as internal storage compounds in fall-emerging bees that contributed to the wintering population.

Vitellogenin, a known storage protein, increased from none in newly emerged bees to ~60 (range: 10-200)ug/abdomen in winter bees (60-day-old bees sampled in late November). Probable jelly proteins from bee heads were present in minor amounts. A third previously unreported protein from adult honey bee abdomens was a six-unit storage protein, probably arylphorin. Like vitellogenin, it was nonexistant in newly emerged bees and reached an average of 75 ug/bee by late November.

Although the two hives from which we sampled bees were randomly selected, there were highly significant colony-related differences in amounts of protein in the bees. Bees from one colony consistently had higher levels of all proteins studied.

We have demonstrated the existence of a previously unknown storage protein in wintering bees that is probably arlyphorin. Although wintering bees have significantly elevated amounts of vitellogenin and arylphorin, the amounts of protein present in the bodies of wintering bees are not sufficient to rear large amounts of brood. We suggest that their primary function may be to allow colonies to continue brood rearing when extremely cold periods prevent bees from leaving the cluster to feed on pollen. Other possible functions are suggested.

Keywords: storage proteins, vitellogenin, winter bees, arylphorin

INTRODUCTION

A key adaptation that enabled tropical *Apis* to colonize temperate regions was the ability to survive cold winters. Several traits must co-occur to make this possible: regulation of the cessation of brood rearing in fall and its initiation in the late winter prior to the availability of floral food resources; the ability of individual worker bees to live for many months; storage of large amounts of honey for thermoregulation; and storage of sufficient protein for late winter and early spring brood-rearing (Seeley 1985).

All protein in a colony of bees is ultimately derived from floral pollen. Some pollen is fed directly to older larvae. Additional pollen is ingested by adult worker bees, converted to jelly, and fed to larvae of all castes as well as to older adult workers,

drones, and queens (Crailsheim 1992). Protein from pollen is required for the development of glands (e.g., hypopharyngeal glands, wax glands) during the behavioural ontogeny of worker bees. Finally, some protein is stored internally within worker bees, primarily in the fat body, haemolymph, and hypopharyngeal glands (Amdan and Omholt 2002).

In late summer and fall in temperate regions, bees store pollen along with honey. They also consume pollen and develop hypertrophied hypopharyngeal glands and large fat bodies containing globules of protein, traits related to the development of long-lived winter bees (Ribbands, 1953). Vitellogenin, a known internal storage protein, increases in amount in wintering bees along with several other proteins (Amdan and Omholt 2002). When brood rearing commences in mid-winter, bees must obtain protein for larvae from stored pollen and internal protein reserves.

A wide variety of insects synthesize special storage proteins during periods of resource abundance. These are retained until the time and setting required for egg production or other functions including cuticle formation, transport of organic compounds, and humoral immune defense (Burmester 1999). For example, queen ants of several species have large amounts of large hexameric (e.g., comprised of six subunits) proteins that are digested to produce food for their first larvae during claustral colony founding (Wheeler and Martinez 1994, 1995, Wheeler and Buck 1995). Adult worker honey bees have been documented as containing Hex70a in relatively large amounts (Danty et al. 1998), but details about the amounts and its function are lacking. In addition to hexamerins, there are additional proteins that may serve as protein storage molecules, including vitellogenin which is a very high density lipoproteins (VHDLs) (Wheeler and Buck 1995, Amdan and Omholt 2002). There has been little research into internal sources of protein in honey bees with the recent exception of Amdan and Omholt (2002). Our study investigated the amounts of stored protein in developing winter bees, and discusses their likely usage within the colony.

MATERIALS AND METHODS

For this study two colonies (#77 and #91) were selected at random in late summer at the University of Guelph, Ontario. Frames of emerging bees were removed, incubated overnight, and large cohorts of bees <1 day old were paint marked and returned to their colonies of origin on 4 September and 30 September, 2000. Previous studies indicated that these would become “summer” and “winter” bees, respectively. Samples of ten bees from each cohort were collected at various ages (e.g., 0, 2, 5, 7, 11, 14 days of age) and frozen at –80 C. On November 30, when all bees in the colonies were >60 days of age, an additional sample of “winter bees” was obtained. The bees were shipped on dry ice to the University of Arizona where the methods previously reported by Wheeler and Martinez (1994) were used to analyze proteins present in the bees. In summary, the method involved removal of the gut and sting, after which the head, thorax and abdomen were homogenized individually in tris-buffered saline in the presence of several protease inhibitors. After centrifugation, the supernatent was analyzed with SDS-PAGE (polyacrylamide gel electrophoresis). After electrophoresis, a numerous bands were evident on the gels. By their size and location in the bee, several of these were identified and quantified: lipophorin, vitellogenin, a putative storage protein (probably arylphorin), an abundant thorax protein (probably muscle-related), and a putative jelly protein.

RESULTS

LIPOPHORIN

Lipophorin is a lipid-carrying protein that is generally a good indicator of the physical condition of an insect/colony. There was a significant colony effect ($p=0.02$), indicating that bees from Colony 77 had significantly more lipophorin than bees from Colony 91. There was no effect of age ($p=0.59$) or age*colony effect ($P=0.86$). Although we selected colonies at random within the home apiary, this analysis and all others (below) indicate that Colony 77 was nutritionally better off.

VITELLOGENIN

Vitellogenin (VG) is a VHDL that acts as a storage protein that appears to be involved in various metabolic functions including the production of hypopharyngeal gland secretions (Amdan and Omholt, 2002). We quantified a significant increase in the amount of total VG as bees aged in the fall ($p<0.0001$), from very small amounts upon emergence to an overall mean of ~60 цg/bee. There was also a significant colony effect ($p<0.0001$), with the winter bees in late November in Colony 77 having much more VG (~75 цg) than bees in Colony 91 (~30 цg). The age*colony interaction was also highly significant ($p<0.0001$). There were very large differences in the amounts of VG per bee. For example, in bees sampled at the end of November amounts ranged from 0-220 цg/bee.

PUTATIVE STORAGE PROTEIN

This protein was found only in abdomens and is likely stored in the fat body. The molecular size was indicative of other hexameric storage proteins in insects, and is probably an arylphorin protein as that is the only class of storage protein that has been identified in adult *Apis* (Danty et al., 1998). It will be referred to as arylphorin subsequently.

The patterns in the amounts of arylphorin are almost identical to those discussed for VG. There were highly significant effects of age ($p<0.0001$), colony ($p<0.0001$), and age*colony interactions ($p=0.015$). Amounts of arylphorin were generally low at emergence (~20 цg/bee), and increased gradually with age to wintering levels of ~75 цg/bee (range from 0-195 цg/bee).

THORAX PROTEIN (MUSCLE)

We are unsure what this protein is, but its abundance in the thorax suggests it is related to thoracic musculature. This putative muscle protein increased quickly with age to a maximum at around age 35 days, after which it appeared to decline slightly with further increases in age.

PUTATIVE JELLY PROTEIN

This protein was detected in bee heads, suggesting it may have been a jelly protein. Amounts were relatively small, with the amounts in bees from Colony 77 (41.0 цg/bee) and Colony 91 (25.7 цg/bee) being significantly different ($p=0.003$). There was no significant effect of bee age on the amounts of this protein, although it appeared that the amount of this protein in bees in colony 91 gradually increased until they matched the levels found in bees from colony 77.

DISCUSSION

It has been shown recently that adult worker honey bees contain a hexameric storage protein, Hex70a, but details relating to its rate of accumulation were lacking (Danty et al. 1998). In the absence of more detailed information about hexameric storage proteins, research attention has focussed on vitellogenin (VG) which is the most abundant haemolymph protein and a good indicator of the protein status of the bee (Cremonez et al. 1998; Amdan and Omholt 2002). In our study of wintering bees, we found a putative storage protein (probably an arylphorin) that increased gradually over the fall, had the same pattern of accumulation as VG, and exceeded the total amount of VG by approximately 25%. If VG is a true storage protein, as concluded my Amdan and Omholt (2002), its amount in bees in early winter is exceeded by quantities of arylphorin. The other proteins quantified in this study (lipophorin, putative muscle protein, putative jelly protein) did not increase with bee age, suggesting that they are not involved in protein storage to the same extent.

Both VG and arylphorin exhibited very strong colony effects, indicating that the nutritive state of colonies within the same apiary can differ tremendously. In addition to the colony effects on amounts of VG and arylphorin, additional colony effects were detected in the amounts of lipophorin and putative jelly protein. Despite these strong differences between bees from the two colonies, there were no outward signs during bee collection to suggest the colonies differed in their protein nutrition. Poor protein nutrition cannot be observed directly except by assessing amounts of stored pollen and even that is problematic as it depends on many additional factors. Protein nutrition undoubtedly affects colony health quite considerably, but goes largely undiagnosed by beekeepers and bee scientists alike. The very large variability in amounts of VG and arylphorin within the bees of a colony also raises questions as to the past and current behavioural roles of bees by early winter that differ so greatly in protein status.

Even summing the amounts of VG, arylphorin, and putative jelly protein in “well-endowed” winter bees at the end of November, the amount of stored protein is still relatively small (e.g., ~400 µg). It seems that proteins stored within the bodies of wintering bees are insufficient to maintain brood rearing for long. To put this into perspective, Alfonsus (1933; cited by Ribbands 1953) calculated that the amount of protein required to rear a single bee was ~29 mg. From our study, the amount stored within an individual bee’s tissues that can be devoted to feeding larvae is nearly an order of magnitude smaller. Our initial idea was that internally stored protein would enable colonies to continue brood rearing in spring when pollen stores were depleted and ambient conditions prohibited the collection of pollen from flowers, but the amount of protein stored within a bee’s tissues is clearly insufficient to achieve that purpose.

Why then do worker bees synthesize large storage proteins when stored pollen is available within their nest? There are several possible explanations. First, evidence is accumulating that storage proteins are a regular feature of insect biology, in many cases as a way to store proteins when amino acids are in abundance temporally and/or spatially. The continued synthesis of storage proteins in an insect that does not require them may reflect the evolutionary history of social honey bees from solitary ancestors for which protein storage may have been more important. A second possibility may relate to the fact that over time the quality of pollen stored in

honey bee nests decreases. Converting the amino acids in stored pollen into high-quality, easily accessible storage proteins may enable the worker bee to always have a ready source of high quality protein for the production of jelly fed to colony members (Crailsheim, 1992). A third possibility may relate to the early brood rearing by workers while it is still winter. During cold snaps, contraction of the bee cluster may prevent individual bees from accessing the pollen required for feeding larvae. Collectively, workers with good internal supplies of stored proteins could continue to feed some larvae during such periods of time, thereby buffering the effects of the cold temperatures. For colonies infested with parasitic mites, good quantities of storage proteins may enable bees to tolerate the negative effects of haemolymph loss to mites. Finally, good protein nutrition may enable good functioning of the bee's immune system, enabling it to resist microbial infections both in the absence and presence of parasitic mites. These explanations are mutually compatible and demand attention from the research community.

ACKNOWLEDGMENTS

Paul Kelly maintains the bee hives at the University of Guelph. At the time of this research, G. Otis was receiving substantial infrastructure support from the Ontario Ministry of Agriculture and Food.

REFERENCES

Alfonsus A. (1933) Zum Pollenverbrauch des Bienenvolkes, Arch. Bienenk. 14, 220-223.

Amdan AG, Omholt SW (2002) The regulatory anatomy of honeybee lifespan, J. Theor. Boil. 216, 209-228.

Burmester T (1999) Evolution and function of the insect hexamerins. Eur. J. Entomol. 96, 213-225.

Crailsheim K (1992) The flow of jelly within a honeybee colony. J. Comp. Physiology B. 162, 681-689.

Cremonez TA, de Jong D, Bitondi MM (1998) Quantification of hemolymph proteins as a fast method for testing protein diets for honeybees. J. Econ. Entomol. 91, 1284-1289.

Danty E, Arnold G, Burmester T, Huet JC, Huet D, Pernollet JC, Masson C (1998) Identification and developmental profiles of hexamerins in the antenna and hemolymph of the honeybee, *Apis mellifera*. Insect Biochem. Molec. Biol. 28, 387-397.

RibbandsR (1953) The Behaviour and Social Life of Honeybees, Dover, New York

Seeley TD (1985) The Ecology of the Honey Bee, Princeton University, Princeton.

Wheeler DE, Buck NA (1995) Storage proteins in ants during development and colony founding, J. Insect Physiology 41, 885-894.

Wheeler DE, Martinez T (1994) Storage proteins in adult ants (*Camponotus festinates*) – roles in colony founding by queens and in larval rearing by workers. J. Insect Physiol. 40, 723-729.

Wheeler DE, Martinez T (1995) Storage proteins in ants (Hymenoptera, Formicidae). Comp. Biochem. and Physiol. B. 112, 15-19.