J. theor. Biol. (2002) **216,** 209–228
doi:10.1006/jtbi.2002.2545, available online at http://www.idealibrary.com on IDEAL®

The Regulatory Anatomy of Honeybee Lifespan

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(*Received on* 1 *March* 2001, *Accepted in revised form on* 20 *January* 2002)

Honeybee workers (*Apis mellifera*) may be classified as either short-lived summer bees or long-lived winter bees in temperate zones. The protein status appears to be a major determinant of honeybee lifespan, and the lipoprotein vitellogenin seems to play a crucial role. Here, we give a review of the role of the vitellogenin in honeybee workers, and present a data-driven mathematical model describing the dynamics of this representative protein in the individual bee as a function of its task profile under various regimes. The results support the hypothesis that vitellogenin is a *true* storage protein that is utilized for various metabolic purposes including the synthesis of brood food. Except for workers having been foragers for many days, they also suggest that the previous life histories of workers do not constrain them from becoming winter bees as long as they get ample food and time to build up their protein reserves before wintering. The results also indicate that it may not be necessary to introduce the ovary as a storage organ for vitellogenin in order to generate normal winter bees. The insights gained from these results are then discussed in a broader gerontological and life history context. Remarkably similar features concerning regulation of ageing in *Caenorhabditis elegans*, *Drosophila melanogaster* and honeybees are pointed out and discussed. Furthermore, we show that in contrast to the "mutation accumulation" and the "antagonistic pleiotropy" evolutionary theories of ageing, the "disposable soma" theory is capable of explaining the bimodal longevity distribution of honeybees when interpreted in a group selection context. Finally, by showing that depletion of nutrient stores can be actively controlled by pathways connected to regulation of ageing, we strengthen the claim that age-based division of labour, with performance of risky tasks delayed until late in life by workers with depleted nutrient stores, may have evolved as an energy-saving mechanism in insect colonies.

Introduction

Honeybee workers (*Apis mellifera*) show a distinct bimodal longevity distribution in temperate zones, and may be classified as either short-lived summer bees or long-lived winter bees (Maurizio, 1950; Fluri *et al.*, 1982). Bees emerging in spring and mid-summer have a mean lifespan of about 25–35 days, whereas winter bees normally live for 6–8 months (Maurizio, 1950; Free & Spencer-Booth, 1959). The winter bees emerge during a restricted period in late summer and autumn, and differ from summer bees with respect to several physiological characteristics (Maurizio, 1950; Free & Spencer-Booth, 1959; Fluri *et al.*, 1982; Crailsheim, 1990a; Huang & Robinson, 1995).

This bimodal longevity distribution is likely to be the result of a complex dynamics involving extra-colonial biotic and abiotic factors,

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0022-5193/02/$35.00/0

interactions between individuals at the colony level, and a regulatory machinery in the individual bee under the direct influence of intracolonial conditions (Fukuda & Sekiguchi, 1966; Omholt, 1988; Finch, 1990). A colony-level mathematical model based on a hypothesis suggesting that a high nurse activity causes a short lifespan potential, while a low nurse activity causes a long lifespan potential, provided the first theoretical step in understanding this phenomenon (Omholt, 1988). Here, we address the question of honeybee lifespan regulation by focusing on the relevant physiological dynamics within the individual bee.

The protein status appears to be a major determinant of honeybee lifespan (Maurizio, 1950, 1954; Schatton-Gadelmayer & Engels, 1988; Burgess *et al*., 1996). Of the various proteins involved, the very high-density lipoprotein (VHDL) vitellogenin seems to play a crucial role for several reasons. Being the most abundant haemolymph protein found in both workers and queens it strongly reflects the protein status of the bee (Engels & Fahrenhorst, 1974; Cremonez *et al.,* 1998). Vitellogenin is also a potent zinc (Zn) carrier (reviewed by Falchuk, 1998), and the amount of haemolymph zinc is strongly correlated with the vitellogenin level in honeybees [Amdam *et al*. (in prep.)]. Zinc is required as a catalytic, structural and regulatory ion, and zinc deficiency induces oxidative stress and apoptosis in several cell lines in mammals, including nerve and immune cells (reviewed by Mocchegiani *et al.,* 2000).

Oxidative stress may play a major role in ageing of *Caenorhabditis elegans,* and vitellogenin-6 appears to have an anti-oxidant function in this organism (Nakamura *et al*., 1999). In addition, *C. elegans* possesses a facultative age determination system driven by environmental conditions at the larval stage that is remarkably similar to what the honeybees have realized for the adult worker stage. The *C. elegans* dauer larva is a specialized third larval stage adapted for survival in non-optimal environmental conditions. In response to high levels of continuously secreted pheromone and low amounts of food, animals form a dauer larva. The dauer larva is metabolically shifted and stress resistant, and animals arrested at the dauer stage can live up to eight times as long as a non-dauer (reviewed by Riddle, 1988).

Furthermore, it has recently been shown that juvenile hormone deficiency resulting from an insulin receptor signal pathway mutation that affects neurosecretory tissue specialized for secretion of juvenile hormone, is sufficient to extend lifespan in *Drosophila melanogaster* (Tatar *et al*., 2001). As vitellogen in synthesis is under the regulatory control of juvenile hormone in *Drosophila* as well as honeybees (Imboden *et al*., 1976; Pener, 1992; Saunders *et al*., 1990), this may indicate common underlying regulatory features concerning ageing.

The goals of this paper are to:

(i) give an extensive review of the relevant literature concerning the role of the vitellogenin and its dynamics in honeybee workers;

(ii) present and validate a data-driven mathematical model capturing the dynamics of this representative protein in the individual bee as a function of its task profile under various regulatory regimes in order to get a deeper understanding of the proximate mechanisms involved and the underlying evolutionary reasons;

(iii) show how this model provides a theoretical foundation of appropriate resolution to guide the establishment of a research programme aimed at resolving key issues concerning the regulatory anatomy of honeybee lifespan at the individual as well as the colony level;

(iv) discuss the insights gained from the study of the honeybee system in a broader gerontological and life history context, where we address how the ''mutation accumulation'' (Medawar, 1952), the ''antagonistic pleiotropy'' (Williams, 1957), and the ''disposable soma'' (Kirkwood, 1977; Kirkwood, 1996) evolutionary theories of ageing are able to explain honeybee age determination, and point out common features concerning regulation of ageing in *C. elegans*, *D. melanogaster* and honeybees.

We think the paper substantiates the claim that an understanding of the regulatory anatomy of honeybee lifespan in evolutionary as well as proximate terms might be of considerable gerontological interest.

Background

The protein status of a worker bee is mainly given by the amount of protein present in its fat body, haemolymph and hypopharyngeal glands (HPGs).

THE FAT BODY

The fat body consists mainly of thin layers of cells spread against the body wall of the abdomen, where the cells are loosely organized in thin lobes of highly tracheated tissue (Snodgrass, 1956). It builds up during the first days of adult life (Koehler, 1921; Haydak, 1957). In the summer season, the maximum amount of proteins in the fat body of a worker bee is obtained after approximately 12 days (Fluri & Bogdanov, 1987), while it may increase far beyond this level over an extended time period in late autumn worker bees (Maurizio, 1954; Fluri & Bogdanov, 1987). The amount of proteins in the fat body decreases during winter, and spring levels may be even lower than the quantities found in foragers (Maurizio, 1954; Fluri & Bogdanov, 1987), whose fat body cells have a particularly low protein content (Koehler, 1921; Maurizio, 1950; Snodgrass, 1956; Haydak, 1957; Fluri & Bogdanov, 1987).

The insect fat body synthesizes a broad range of storage proteins (Haunerland & Shirk, 1995). They typically build up to high concentrations in the haemolymph and fat body cells of the last larval instar, and disappear during metamorphosis to provide a protein and amino acid source for the construction of adult tissues (Ancsin & Wyatt, 1996). Five storage proteins have been recognized in honeybee worker larvae. These are the (1–3) hexamerins 70a, b and c, (4) a 105–110 kDa polypeptide high in glutamine/glutamic acid and (5) a 160 kDa VHDL (Ryan *et al*., 1984; Shipman *et al*., 1987; Wheeler & Buck, 1995; Danty *et al*., 1998). Storage proteins are also found in adults of the social *Hymenoptera*. Here they appear to serve as an important nutritional source for the maintenance of brood by workers, as well as egg formation by the queen (Martinez & Wheeler, 1993). Three types have been found in ants: (1) hexamerins, (2) proteins high in glutamine/glutamic acid and (3) the VHDLs (Martinez & Wheeler, 1994; Wheeler & Buck, 1995). Only hexamerin 70a (HEX 70a) is found in adult honeybee workers (Danty *et al*., 1998), but information about its synthesis and accumulation pattern in adults is apparently not available (Danty *et al*., 1998).

The VHDL vitellogenin is produced by the fat body of many insect species, and is generally described as a female-specific haemolymph storage protein, a yolk glycolipoprotein that is secreted into the haemolymph and taken up by developing oocytes (Haunerland & Shirk, 1995). However, in honeybees and several ant species, vitellogenin is also synthesized by individuals that do not lay eggs (workers and drones) (Engels, 1968; Trenczek *et al*., 1989).

The rate of vitellogenin synthesis is negligible when the worker emerges, but it increases rapidly within 2–3 days (Engels *et al*., 1990), and may be enhanced when the worker starts nursing (Schabacker, 1973; Engels *et al*., 1990). In a normal colony setting, the rate of vitellogenin synthesis of a worker bee is approximately 1/20 of a laying queen. The rate of synthesis is negatively correlated with haemolymph vitellogenin levels (Engels, 1974; Engels *et al*., 1990), suggesting the existence of a homeostatic regulation mechanism.

In workers, juvenile hormone (JH) is needed for vitellogenin transcription, but a high JH level inhibits synthesis (Imboden *et al*., 1976; Pinto *et al*., 2000). Apart from this, JH does not apparently affect the rate of vitellogenin synthesis in honeybees (Pinto *et al*., 2000). The synthesis in adult queens is not dependent on JH, and it has been suggested that gonadotropic neurohormones or ecdysteroids produced by the ovary support and enhance vitellogenin synthesis in this case (Kaatz, 1988; Kaatz & Dittrich, 1989).

In drones, the rate of vitellogenin synthesis is only 1/100 of that in a laying queen (Engels *et al*., 1990). The temporal pattern of vitellogenin synthesis is similar to that of a summer bee, but the rate of synthesis is reduced to undetectable levels earlier in life (10–15 days, Trenczek *et al*., 1989). This is also the age range when its JH-titre increases, it becomes sexually mature and starts mating flights (Trenczek *et al*., 1989; Oliveira-Tozetto *et al*., 1995).

THE HAEMOLYMPH

The haemolymph of honeybees contains numerous proteins (Engels & Fahrenhorst, 1974). However, vitellogenin is by far the most dominant protein found in both workers and queens, and the temporal and task-related changes of the haemolymph titres in workers are quite well documented (Engels & Fahrenhorst, 1974; Rutz *et al*., 1976; Fluri *et al*., 1982; Engels *et al*., 1990). However, it should be noted that observations are normally based on pooled samples of similarly aged bees with a considerable intra-group variance (Trenczek *et al*., 1989). In worker bees with age $\leqslant$3 days, vitellogenin is almost undetectable in the haemolymph (Rutz & Lüscher, 1974; Pinto *et al*., 2000). In a normal summer colony, the vitellogenin titre increases within the next 7–9 days before it stabilizes. When the worker starts foraging, the titre decreases rapidly towards an undetectable level (Engels & Fahrenhorst, 1974; Rutz *et al*., 1976; Fluri *et al*., 1982). Wintering workers have, in general, a high haemolymph vitellogenin titre, but it is higher in late autumn than at the end of winter (Fluri *et al*., 1982). The titre of broodless worker bees in the summer may be higher than in winter bees (Fluri *et al*., 1982).

Concerning the utilization pattern of vitellogenin, the research efforts have mainly focused on its incorporation into developing oocytes (Hirai *et al*., 1998), but considering that vitellogenin is depleted from the haemolymph of workers and drones (Engels, 1974; Trenczek *et al*., 1989), it is most likely utilized for additional purposes in honeybees.

THE HYPOPHARYNGEAL GLANDS

The HPGs are paired acinous glands located in the head of the worker (Snodgrass, 1956). The glands produce proteinaceous secretions (jelly) that are fed to the larvae, queen, workers and drones (Crailsheim, 1991). In summer bees, acini diameter and HPG protein content increase during the first 5–10 days of adult life (Fluri & Bogdanov, 1987; Hrassnigg & Crailsheim, 1998). Acini size and the amount of protein in the glands then decline in hive bees (non-foraging summer bees) exposed to open brood (Hrassnigg & Crailsheim, 1998). The HPGs are hypertrophied with a particularly high level of proteins in winter bees, but their diameters and protein content tend to decline during winter (Maurizio, 1954; Fluri & Bogdanov, 1987; Crailsheim, 1990a). In broodless periods, summer bees have hypertrophied glands as observed in winter bees (Maurizio, 1954; Fluri *et al*., 1982; Hrassnigg & Crailsheim, 1998). When a worker starts foraging, the glands atrophy quite fast (Rutz *et al*., 1976).

A POSSIBLE STORAGE FUNCTION OF THE OVARY

Worker ovary development is inhibited by the joint effect of queen tergal pheromones and a mix of fatty esters produced by the brood (Arnold *et al*., 1994; Wossler & Crewe, 1999). Over 70% of non-egglaying workers in queenright colonies without brood in summer may have enlarged ovaries due to protein storage (Kropacova & Haslbachova, 1970; Jay, 1972). If the lack of brood in the autumn and the winter induces the same pattern in the wintering bees, the ovaries may play a role as a storage organ for vitellogenin.

Maurizio (1954) found that worker ovaries were enlarged in autumn and atrophied in spring, while Verheijen-Voogd (1959) and Moskalenko (1982) reported that worker oocytes were absorbed during late autumn. Velthuis (1970) reported that the mean percentage of workers with developed ovaries declined during late August, whereas Levin *et al*. (1951) found no seasonal variation in ovary development. The available data are thus somewhat contradictory, but they do not rule out that lack of brood induces a storage function of the ovary and possible secondary effects due to production of ecdysteroids and gonadotropic neurohormones.

FOCUS ON THE VITELLOGENIN DYNAMICS

Although proteins other than vitellogenin may be of importance in connection with honeybee lifespan regulation, we consider vitellogenin to be the most interesting candidate. A clear picture of vitellogenin production, distribution and utilization is likely to contribute to understanding the roles of other possible proteins involved. In the following, we show how the vitellogenin

dynamics may be described by a set of nonlinear differential equations.

Model

MODEL STRUCTURE

The differential equation model may be characterized as a meta-model that intends to predict the vitellogenin dynamics of an individual bee from emergence to a given age that is exposed to various task scenarios (TS) under summer as well as autumn/winter conditions (described below). As the three main vitellogenin compartments are likely to be the fat body, the haemolymph, and the HPGs, they define the three state variables V_F, V_H, and V_G of the model. Based on the assumption that the HPGs have an efficient processing machinery of vitellogenin to make products that end up in the brood food, it is important to note that we consider V_G to include the vitellogenin as well as the amount of brood protein derived from the processing of vitellogenin.

We suggest the time rate of change of the three state variables can be described by the differential equations system

All functions denoted $S(V_x, \theta_i, n_i)$ are sigmoid functions that are given by the Hill function $V^n/\theta^n + V^n$. Through the adjustment of the parameter n it allows a functional relationship to be varied from gently hyperbolic to steeply sigmoidal. As we have no information on the steepness of the regulatory functional relationships, the Hill function is very convenient to use for studying the robustness of the predictions to variation of this steepness [see Omholt *et al.*, 1998; Plahte *et al.*, 1998; Øyehaug *et al.* (in press) for further biological validation of the Hill function].

The model does not pretend to incorporate the various regulatory factors causing a given task scenario. Their effects on the production rate of vitellogenin, the relative rate of transport from the haemolymph to the HPGs, the amount utilized for general metabolic purposes, and the amount used for brood food production, are instead introduced by the positive functions $\alpha(t)$, $\omega(t)$, $\lambda(t)$, and $\psi(t)$ (having units $\mu g\, day^{-1}$). These will be described in more detail below. The positive parameters β_1, β_2 are constants describing maximum relative transport rates (both having unit day^{-1}).

EXPLICATION AND VALIDATION OF PREMISES

Our model is based on two primary hypotheses and four secondary (or auxiliary) ones.

Hypothesis 1: Vitellogenin is a storage protein used for various metabolic purposes in honeybee

1.1: Rate of production in the fat body ($\mu g\, day^{-1}$)

1.2: Rate of transport from the fat body to the haemolymph ($\mu g\, day^{-1}$)

1.3: Rate of transport from the haemolymph to the fat body ($\mu g\, day^{-1}$)

$$\dot{V}_F = \overbrace{\alpha(t)(1 - S(V_F, \theta_1, n_1))} - \overbrace{\beta_1 V_F(1 - S(V_H, \theta_2, n_2))} + \overbrace{\beta_2 V_H S(V_H, \theta_3, n_3)}$$

$$\dot{V}_H = \beta_1 V_F(1 - S(V_H, \theta_2, n_2)) - \beta_2 V_H S(V_H, \theta_3, n_3) -$$

2.3: Rate of transport from the haemolymph to the HPGs ($\mu g\, day^{-1}$)

2.4: Amount used for metabolic purposes ($\mu g\, day^{-1}$)

$$\overbrace{\omega(t) V_H(1 - S(V_G, \theta_4, n_4))} - \overbrace{\lambda(t)}$$

3.2: Amount used for brood food production ($\mu g\, day^{-1}$)

$$\dot{V}_G = \omega(t) V_H(1 - S(V_G, \theta_4, n_4)) - \overbrace{\psi(t)}.$$

workers, and the fat body is the main storage organ [equation term (1.3)].

Validation: Lipophorins are recognized as the major lipid-providing molecules in insects (Babin *et al*., 1999). Babin *et al*. (1999) found that insect apolipophorin II/I, human apolipoprotein B (apoB-100), invertebrate and vertebrate vitellogenin, and the large subunit of mammalian microsomal triglyceride transfer protein (MTP) are members of the same multigene superfamily that have emerged from a single ancestral molecule. Mann *et al*. (1999) demonstrated structural and functional relationships between invertebrate and vertebrate vitellogenin, apoB-100 and MTP. In addition, the crystal structure of lipovitellin (the mature form of vitellogenin) comprises a substantial lipid-binding cavity (Timmins *et al*., 1992).

Fat body accumulation of vitellogenin has not been investigated in honeybee workers. However, protein granules with crystalline nuclei are generally considered to have a storage function, and vitellogenin-containing crystals in the ventral abdominal fat body of *Monomorium pharaons* queens were detected recently (Jensen & Børgesen, 2000). Furthermore, vitellogenin is a major component in the fat body of honeybee queens and queenless *Camponotus festinatus* workers (Lensky & Skolnik, 1980; Martinez & Wheeler, 1991; Rosell & Wheeler, 1995). The fact that vitellogenin is the predominant protein found in the queen as well as worker honeybees (Fluri *et al*., 1982) also lends support to this hypothesis (Fluri *et al*., 1982).

Secretion and accumulation of vitellogenin from fat bodies cultured *in vitro* is dependent on the protein concentration of the medium (Kaatz *et al*.,1985; Hartfelder and Simões (pers. comm.); Amdam and Omholt (in prep.). This is consistent with the notion that the fat body may function as a storage organ for vitellogenin *in vivo* when the haemolymph vitellogenin concentration exceeds a certain threshold.

The first part of the above hypothesis was actually made by Engels *et al*. (1990), as they suggested that the occurrence of vitellogenin in drones, non-laying workers and non-laying queens could be due to metabolic reasons. We suggest that vitellogenin may be a provider of large molecular building blocks and amino acids for vital processes like tissue maintenance, and that it may represent an energy source through catabolism of glucogenic amino acids and lipids.

Hypothesis 2: Vitellogenin is a major amino acid and lipid provider for HPG brood food production [equation terms 2.3(3.1) and 3.2].

Validation: Using SDS-PAGE electrophoresis and anti-sera, Lensky & Rakover (1983) concluded that brood food jelly does not contain vitellogenin. However, this does not rule out that the protein is incorporated and rapidly lysed within the HPG acini. If so, the HPGs would contain only small amounts of vitellogenin, which actually seems to be the case (Rutz & Lüscher, 1974). Crailsheim (1992) demonstrated that proteins stored in fat body tissue of hive bees are converted into brood food when they are exposed to larvae. According to Engels *et al*. (1990), the rate of vitellogenin synthesis of queenright, nursing bees is within the range of 250–750 $\mu g\,day^{-1}$. This is sufficient to produce 30–100 eggs daily, and the rate is about twice that of queenless, laying workers (Schabacker, 1973). As the amount synthesized per day exceeds the total amount of vitellogenin found in the haemolymph (Fluri *et al*., 1982; Engels *et al*., 1990), it also implies a very high turnover rate of vitellogenin in the haemolymph.

In fact, this hypothesis was in principle put forward by Koehler (1921). She observed that the fat body cells of worker bees became densely packed with protein granules as brood rearing ceased in autumn. As these granules were almost absent in summer bees, she suggested that proteins produced by the fat body were elaborated into brood food in summer, and that these proteins accumulated in the fat body cells after the nursing season as food reserve for the winter.

SECONDARY HYPOTHESES

All these premises reflect specific regulatory mechanisms and their functional forms. In all cases, the proposed functional forms are presumed to be hyperbolic or sigmoidal.

(i) the production rate of vitellogenin in the fat body is negatively controlled by a mechanism sensing the vitellogenin content of fat body cells dedicated to storage [in equation term (1.1)]

(alternatively, the negative control may be exerted by a mechanism monitoring the haemolymph vitellogenin titre, but this would not change the behaviour of the model);

(ii) the machinery transporting vitellogenin from the fat body to the haemolymph is negatively controlled by a mechanism sensing the amount of vitellogenin in the haemolymph [in equation term (1.2)];

(iii) the mechanism responsible for the transport of vitellogenin from the haemolymph to the fat body is positively controlled by a mechanism sensing the amount of vitellogenin in the haemolymph [in equation term (1.3)];

(iv) the transport of vitellogenin from the haemolymph to the hypopharyngeal glands is negatively controlled by a mechanism sensing the amount of protein present in the HPGs (in vitellogenin equivalents) [equation term (2.3)].

There is still scarce empirical support for these proposed mechanisms, but they all make sense from the biological point of view, and they have been introduced to ensure that the state variables stay within realistic boundaries for a wide range of parameter values and initial conditions. We have assumed that production and storage are taken care of by two different categories of cells in the fat body [linked to premises (i)–(iii)]. Some empirical support for this is given by the fact that the fat body is made up of different cell types that may have specialized functions (Haunerland & Shirk, 1995; Jensen & Børgesen 2000).

TASK SCENARIOS

The vitellogenin dynamics was studied with regard to various task scenarios (TS). For a worker emerging in summer, we studied two situations:

(TS1) The worker emerges in a normal setting, but functions as a normal nurse for an extended period of 25 days before she becomes a forager;

(TS2) the worker emerges in a manipulated colony situation where the queen is confined, there are no open and sealed brood, and foraging stimuli are almost absent (a ''Maurizio colony''). This condition does not occur naturally, but it reflects the classical experimental setup to generate winter bees in summer (Maurizio, 1954). A model addressing the generation of winter bees must be able to account for this phenomenon.

For a worker emerging in the autumn, we looked at three different situations:

(TS3) The worker experiences no nursing and no foraging before it enters winter cluster conditions (the typical winter bee candidate);

(TS4) the worker experiences the normal nurse load of a summer hive bee before it is exposed to a declining nurse load over a period of two weeks before she experiences winter cluster conditions characterized by no nursing, no foraging and reduced metabolic demand;

(TS5) the worker experiences no nursing before it enters a brief period as a forager prior to being exposed to winter cluster conditions.

PARAMETERIZATION OF THE MODEL

The time-dependent functions $\alpha(t)$, $\omega(t)$, $\lambda(t)$, and $\psi(t)$ are mainly made up of four constant parameter values characterizing a summer hive bee performing the normal set of tasks for its age, a forager exposed to a normal foraging pressure in summer; a forager exposed to a normal foraging pressure in late autumn, and a bee under normal winter cluster conditions. For a given task scenario, a specific temporal sequence of these constant values is glued together by continuous transition functions in order to smooth the change from one state to another. Instead of specifying all the transition functions accounting for the five task scenarios, they will be presented graphically together with the predicted temporal vitellogenin patterns of each scenario. In the following, we just describe and validate our chosen quantifications of these control functions.

$\alpha(t)$: The production rate of vitellogenin is presumed to rise hyperbolically from zero at the time of emergence to $300\,\mu g\,day^{-1}$ within 3 days for all task scenarios (Engels *et al.*, 1990). It is assumed to stay at this level in a summer hive bee being exposed to a normal nurse load. When she enters the transition stage to become a forager the rate of vitellogenin synthesis declines rapidly towards zero (Engels & Fahrenhorst,

1974; Pinto *et al*., 2000). Foragers have normally higher JH titres than summer hive workers and winter bees (Huang & Robinson, 1992; Giray & Robinson, 1994; Huang & Robinson, 1995), but workers observed foraging in late autumn and early spring seem to have a JH titre comparable to that of summer hive bees (Huang *et al*., 1994; Huang & Robinson, 1995). This may prevent the normal cessation of vitellogenin synthesis. On the other hand, foraging increases the metabolic turnover of free amino acids in the haemolymph (Crailsheim, 1986), and this may reduce the availability of amino acids to such an extent that it causes a reduction in vitellogenin synthesis in any case. To account for this possibility, the production rate of an autumn forager was set to be in the interval $[0,300]\,\mu g\,day^{-1}$.

We do not have any conclusive data on the vitellogenin production rate of a bee in the winter cluster. It is not likely to be $>40\%$ of hive bees (Brouwers, 1983; Crailsheim *et al*., 1993). However, it might be much less, and to account for this we made it possible to study the effect of varying the rate from zero to this 40% level ($[0,120]\,\mu g\,day^{-1}$).

$\omega(t)$: The HPGs of a worker having access to a normal supply of food increase in size and start brood food synthesis independent of the brood situation in the hive during the first 4 days after emergence (Huang & Otis, 1989). Presuming vitellogenin is incorporated in the HPGs, this implies that there will be a gradual development of the receptor machinery responsible for this uptake. As the worker approaches 4 days of age, the maximum uptake capacity is assumed to approach an intermediate level balancing the demand associated with a normal nurse load and a normal concentration of vitellogenin in the haemolymph. In accordance with this, we allowed the maximum relative transfer rate of vitellogenin from the haemolymph to the HPGs to increase from 0 to 0.6 in a hyperbolic manner from the time of emergence to day 4 for all task scenarios.

As the HPG incorporation of haemolymph proteins is substantially reduced in foragers (Crailsheim, 1991), we assume that the incorporation of vitellogenin to the HPGs almost ceases as the worker becomes a forager in summer as well as in autumn. This was modelled by letting the input of vitellogenin be a linear function of the brood food export, $\omega(t) = \mu + \sigma\psi(t)$, where $\mu = 0.1$ is the minimum value common for all situations and σ is a scaling factor ensuring the transfer rate to be $\leqslant 1$ in all cases.

$\lambda(t)$: Assuming that the use of vitellogenin for metabolic purposes other than the provision of brood food ingredients is proportional to metabolic rate as is the case for total protein (Crailsheim, 1986), the amounts of vitellogenin used for metabolic purposes in winter bees and foragers are respectively one half and two times that of hive bees (Crailsheim, 1986; Van Nerum & Buelens, 1997). We are not able to make a precise quantification of the metabolic use of vitellogenin in hive bees, but given that the vitellogenin produced by drones is utilized for metabolic purposes, and that the metabolic protein turnover in a hive worker is approximately the same as in a drone $\leqslant$ 10 days, it is not likely to exceed $100\,\mu g\,day^{-1}$ (Engels *et al*., 1990).

$\psi(t)$: In hive bees, the quantity of protein-rich jelly donated by nursing workers varies greatly (Crailsheim, 1990b). Large amounts of jelly are donated to adult members of the colony, and these may exceed the amounts given to the brood (Crailsheim, 1991, 1992). As workers $<$ 3 days distribute negligible quantities of jelly (Seeley, 1982), we presumed the export rate to be zero during the first 3 days after emergence for all task scenarios. Seeley (1982) demonstrated that the probability of feeding brood increases until the worker is 5 days old, whereas the probability of feeding nestmates increases until the age of 10–15 days. Thus, we let the brood food export rate increase linearly during the age interval of 3–10 days for all actual task scenarios. We have no information on the quantity of jelly donated by hive bees $\geqslant$10 days. We have, however, some tentative data on the amount that goes into brood food. One larva needs about 50 mg jelly to complete development (von Rhein, 1956). As the protein content of jelly is about 12% (Osanai, 1960), this amounts to approximately 6 mg crude protein. Assuming that one-fourth of the brood protein building blocks are derived from vitellogenin, and that the nurse load of a hive bee may, somewhat simplified, be seen as equally distributed over the period of 15 days, the drain of vitellogenin from the HPGs is approximately

$100\,\mu g\,day^{-1}$ given a 1:1 open brood:nurse bee ratio. Thus, nurse bees experiencing a 1:2, 1:1 and 2:1 open brood:nurse bee ratio would be exposed to a relative nurse load equivalent to 50, 100 and $200\,\mu g\,day^{-1}$, respectively.

The interadult feeding in the winter cluster condition is likely to be a zero sum game. However, a bee will experience a drain through the HPGs even though the same amount of vitellogenin-derived amino acids is absorbed through the digestive system. The drain in a winter cluster bee relative to a hive bee experiencing a 1:1 open brood:nurse bee ratio is not likely to exceed 20% (Crailsheim, 1986, 1992; Crailsheim *et al.*, 1993).

Quantification of the Amount of Vitellogenin in the Haemolymph

Based on available data, it is possible to make a preliminary quantification of vitellogenin present in the haemolymph. In 10 days old brood rearing workers, the total protein concentration is 40–50 $\mu g\,\mu l^{-1}$, and the vitellogenin fraction is 32–40% (Rutz *et al.*, 1976; Fluri *et al.*, 1982). Letting the total haemolymph volume be 20 µl (Crailsheim, 1985), the total quantity of haemolymph vitellogenin is within the range of 250–400 µg. In broodless summer workers, the total protein concentration is about 80–100 $\mu g\,\mu l^{-1}$ (Fluri *et al.*, 1982), which gives a vitellogenin amount in the range of 500–800 µg. In winter bees 12–165 days of age, the total protein concentration is about 40–90 $\mu g\,\mu l^{-1}$ (Fluri *et al.*, 1977). The vitellogenin fraction is also in this case approximately 32–40% (Fluri *et al.*, 1982), which gives a vitellogenin amount of 250–750 µg.

Results

SUMMER CONDITIONS

The model predicts that in bees exposed to task scenario 1 (TS1), vitellogenin accumulates in the haemolymph from day 3 up to the age of 10 days [Fig 1(a)]. The predicted haemolymph quantity as well as the pattern of accumulation is in accordance with empirical data (Rutz *et al.*, 1976; Fluri *et al.*, 1977, 1982; Engels *et al.*, 1990; Pinto *et al.*, 2000).

During the same period, the quantity of vitellogenin-derived proteins in the HPGs increases, peaking at 7 days. Subsequently, the amounts in the glands stay fairly constant. The fat body vitellogenin builds up over a period of 12 days to a quantity sufficient for upholding brood food production for about one week. This is in agreement with the findings of Haydak (1935) and Crailsheim (1990b), who demonstrated that nurses deprived of pollen will continue to rear brood for approximately one week by converting stored proteins into jelly.

When the worker becomes a forager, the level of vitellogenin in the fat body decreases much faster than the haemolymph level [Fig. 1(a)], which reaches zero 4 days after the transition. These patterns are in accordance with the observations of Engels & Fahrenhorst (1974) and Fluri *et al.* (1982).

For TS2, the model predicts that the vitellogenin levels in the first three compartments will build up and remain at constant levels for a prolonged period [Fig. 1(b)], which is consistent with the observation that bees emerging in Maurizio colonies will develop winter bee characteristics (Fluri *et al.*, 1982). If there is an ovary activation by lack of exposure to brood pheromones that causes the maximum production rate of vitellogenin to increase, and the ovary to accumulate vitellogenin, it follows that bees in Maurizio colonies will have significantly larger stores of vitellogenin in the fat bodies and the ovaries than non-nursing bees being experimentally exposed to brood pheromones. This can be tested experimentally.

In accordance with data provided by Fluri *et al.* (1982), the model predicts the amount of vitellogenin in a bee experiencing no nursing to be about equal to the amount in a winter bee [Figs. 1(b) vs. 2(a)].

Under the given parameterization, nursing workers are not able to provide enough jelly for the larvae when the open brood:nurse bee ratio approaches 2:1 (data not shown), which is in agreement with the results of Eischen *et al.* (1982).

AUTUMN AND WINTER CONDITIONS

For task scenarios 3–5, we have presumed that the focal bees emerge on August 20. As expected,

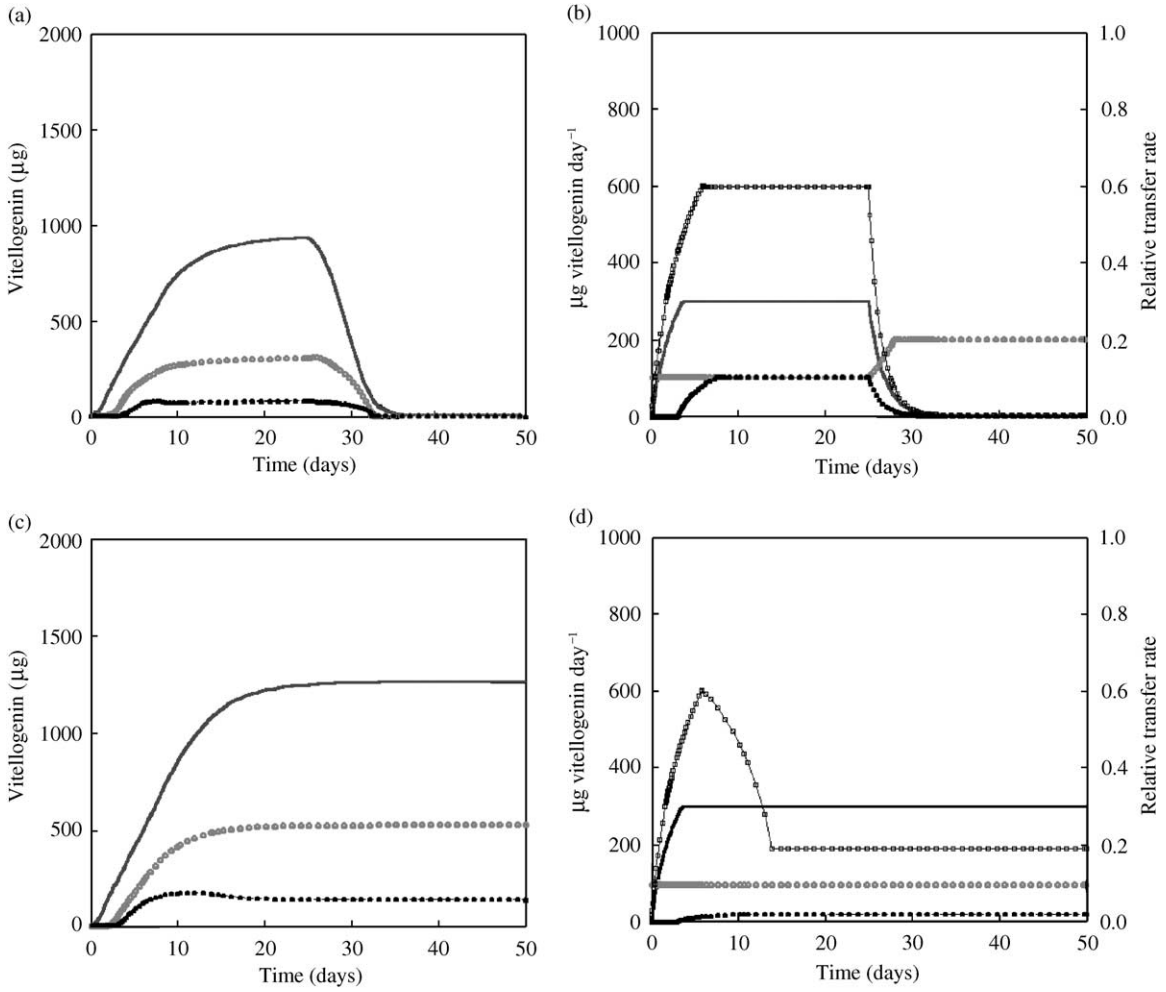

FIG. 1. The total amount of vitellogenin in the fat body, haemolymph and HPGs of summer workers exposed to different task scenarios. (a) TS1. (c) TS2. The worker-state-dependent functions for each situation, $\alpha(t)$, $\omega(t)$, $\lambda(t)$, $\psi(t)$, are given by b–d. The other parameter values are $V_F(0) = V_H(0) = V_G(0) = V_O(0) = 0.01$, $\beta_1 = \beta_2 = 0.8$, $\theta_1 = 1150$, $\theta_2 = 540$, $\theta_3 = 90$, $\theta_4 = 110$, $\theta_5 = 2000$, $\theta_6 = 170$, $n_1 = 4$, $n_2 = \cdots = n_6 = 3$, $\gamma_1 = 0.1$, $\gamma_2 = 0.8$, $\mu = 0.1$. (a): (——)V_F; (●)V_H; (--●--)V_G. (b): (——)$\alpha(t)$; (●)$\lambda(t)$; (--●--)$\psi(t)$; (—□—)$\omega(t)$.

the task scenario of the typical winter bee candidate (TS3) will generate the temporal vitellogenin profile of a typical winter bee (Fluri *et al.*, 1982) [Fig. 2(a)]. Under Norwegian conditions TS3 will reflect the life history of a bee emerging in a colony not given the opportunity to forage on the ling heather in August. In such colonies, there will normally be only small amounts of open brood at the end of August due to lack of other foraging opportunities in the last part of July and in August. A bee emerging later in the autumn under the same task scenario will show the same temporal pattern shifted to the right. If we allow activation of ovaries and a boosting of the vitellogenin production due to lack of brood inhibition, the peak value of fat body vitellogenin will become somewhat higher than depicted in Fig. 2(a), and there will be an additional vitellogenin reserve stored in the ovaries (data not shown).

Under Norwegian conditions TS4 reflects the typical life history of a bee emerging at the end of the heather flow, and which stays as a hive bee until wintering (due to lack of further foraging opportunities). We have assumed that she experiences a normal nurse load the first 15 days, then the amount of open brood (and her nurse load) gradually declines to a minimum level over a period of 2 weeks before the winter conditions set in at ultimo September. Although

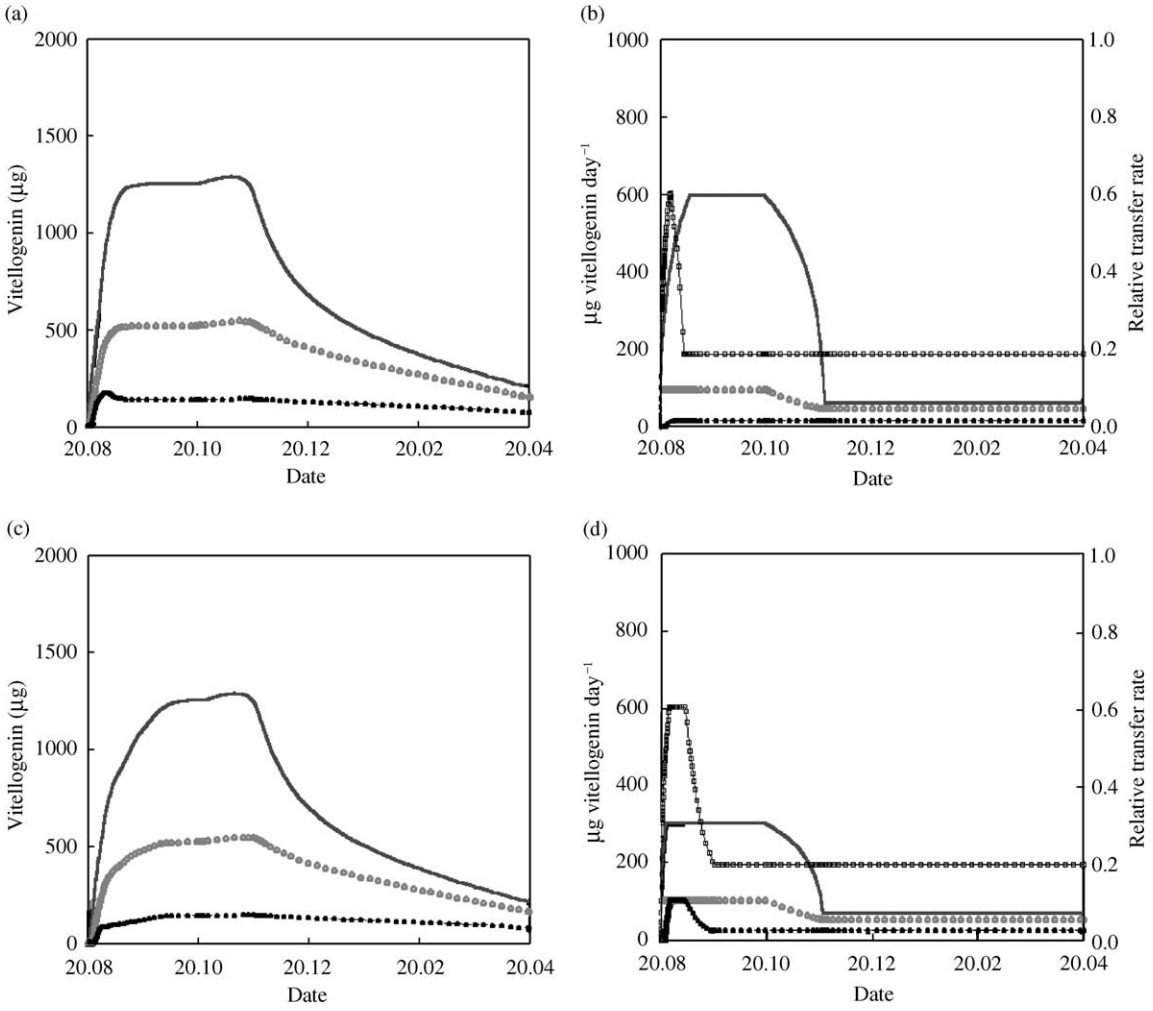

FIG. 2. The total amount of vitellogenin in the fat body, haemolymph and HPGs of bees exposed to different task scenarios in the autumn/winter. (a) TS3. (c) TS4. The worker-state-dependent functions for each situation, $\alpha(t)$, $\omega(t)$, $\lambda(t)$, $\psi(t)$, are given by b–d. The other parameter values are identical to those given in the legend to Fig. 1. (a): (——)V_F; (●)V_H; (--●--)V_G. (b): (——)$\alpha(t)$; (●)$\lambda(t)$; (--●--)$\psi(t)$; (—□—)$\omega(t)$.

this bee is expected to contain less vitellogenin than a bee exposed to TS3 as long as she is nursing, she seems able to catch up during the period of declining brood rearing. At the beginning of the wintering period, she will have a vitellogenin profile comparable to the one exposed to TS3 (Fig. 2). Presuming availability of pollen, and given an ample time period to build up the protein stores before her vitellogenin production rate starts to decline, a worker exposed to a normal nurse load is thus predicted to be able to develop winter bee characteristics.

For TS3 and TS4, the model predicts that the vitellogenin amounts of the haemolymph and the HPGs decline almost linearly throughout the winter, whereas the fat body vitellogenin content decreases in a more exponential-like manner after having reached peak value (Fig. 2). Fluri & Bogdanov (1987) observed a similar temporal total protein fat body profile during winter. It should also be noted that the predicted haemolymph vitellogenin levels for TS3 and TS4 are within the range observed in winter bees (Fluri *et al.*, 1982).

In workers experiencing no nursing before wintering (TS3), the HPGs reach peak values before the fat body. For both task scenarios, the fat body atrophies before the HPGs reach levels typical of summer hive bees.

These results are in accordance with the findings of Maurizio (1954).

Task scenario 5 was introduced to study how foraging affects the build-up of the vitellogenin stores as a function of the forager's capacity for vitellogenin synthesis (see the parameterization section). After emergence on August 20, we let the bee develop as a hive bee without any nurse load for 2 weeks until she became a forager for 10 days from about the second week of September. After cessation of the flow, the bee was assumed to enter wintering conditions. We assumed that there is a connection between the vitellogenin content of the fat body and the mean lifespan of a wintering bee: when the fat body content dropped below 300 μg in late autumn, winter or early spring (which is the expected level of a 3–4 day forager), we let the bee perish [Fig. 3(a)]. As expected, the size of the vitellogenin production rate during the foraging period influences the lifespan potential of late autumn foragers dramatically. The model predicts a steep sigmoidal relationship between rate of vitellogenin synthesis in hive bee units and expected longevity [Fig. 3(b)]. In a narrow range, the forager will be able to build up intermediate quantities of vitellogenin.

It does not seem to be critical for the build-up of winter bee characteristics that the ovary becomes activated in a non-inhibited, non-egglaying worker. However, the mechanism may still be present, and if the amount of vitellogenin consumed for metabolic purposes by hive bees is much higher than the estimated value, it may turn out to be more important than envisaged here.

The predicted haemolymph vitellogenin levels for both types of workers are in the lower part of the estimated range. This is due to the conservative estimate of the rate of vitellogenin synthesis used in the simulations. The predicted levels of vitellogenin in the fat body are somewhat high compared to the total amounts of protein stored in these tissues (Fluri & Bogdanov, 1987). However, we suggest that the quantities found by these authors are minimum estimates as the morphology of the fat body is likely to prevent successful removal of the whole organ.

The predicted levels of vitellogenin in the HPGs of summer hive bees and winter bees suggest that vitellogenin-derived proteins make up 25–60% of the dry weight of the glands (data on gland dry weight in Fluri *et al.*, 1982). These estimates do not seem unreasonable, considering the quantities of protein that may be stored in the HPGs of these worker types (Fluri *et al.*, 1982; Fluri & Bogdanov, 1987; Crailsheim 1990a).

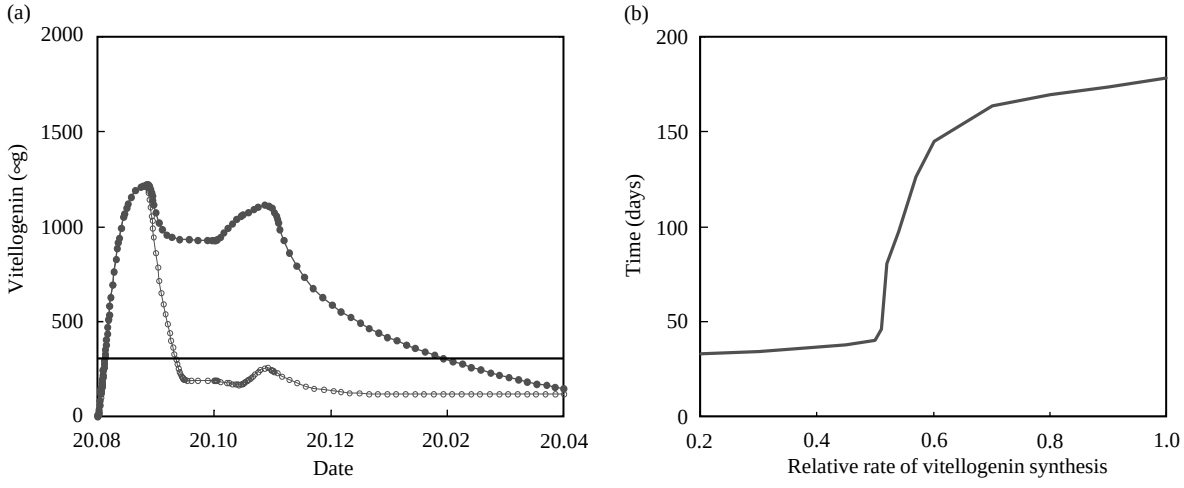

FIG. 3. The effects of a varying vitellogenin synthesis in autumn foragers (task scenario 5). (a) The two temporal patterns illustrate the effects from keeping the vitellogenin synthesis at the hive bee level throughout the foraging period (300 μg day^{-1}) (●), and from letting it stay at a 50% level (○), respectively. The straight line represents the threshold below which the bee is assumed to perish (300 μg). (b) The functional relationship between the relative rate of vitellogenin synthesis in the late autumn forager compared to the summer hive bee, and the subsequent lifespan potential of the worker calculated by recording the first time the vitellogenin level falls below the 300 μg level.

THE EFFECTS OF PATHOGENS ON VITELLOGENIN DYNAMICS

Various pathogens reduce the protein content of honeybee workers (Weinberg & Madel, 1985; Schneider & Drescher, 1987; Schatton-Gadelmayer & Engels, 1988). Infestation by the ectoparasite *Varroa destructor* causes a significant reduction in haemolymph protein levels and the diameter of the HPG acini (Schneider & Drescher, 1987). Spores from the microsporidian pathogen *Nosema apis* and the insecticides BPTI (bovine pancreatic trypsin inhibitor) and SBTI (Kunitz soybean trypsin inhibitor) have similar effects, inhibiting the release of essential amino acids from consumed pollen (Burgess *et al.*, 1996; Malone & Gatehouse, 1998).

Presuming a bee gets infested with one or more *Varroa* mites after emergence, and that she is exposed to the same constant parasitic load for a prolonged period, we studied the effect of a varying parasitic load on the temporal vitellogenin profile. We introduced the parasitic load as a constant term in eqn (2). By using the same scaling procedure as for Fig. 3(a), equating vitellogenin content with lifespan, the model predicts an approximately linear functional relationship between parasitic load and lifespan (not shown). According to Moritz (1981), a *Varroa* mite will consume 0.25 µl haemolymph daily from its adult host, which suggests that the vitellogenin consumption from a winter bee is approximately $8\,\mu g\,day^{-1}$. This implies that the lifespan of a worker in the winter season would be reduced by 30% if it gets exposed to only a single *Varroa* mite. It should be noted that the estimated vitellogenin consumption is probably somewhat high due to the fact that the metabolic rate of the *Varroa* mite will be reduced in the winter cluster.

Furthermore, the *Varroa* mite reduces the total volume of haemolymph in workers (Schneider & Drescher, 1987), and it cannot be ruled out that the sucking of the mite during the pupal stage has a detrimental effect on fat body development, affecting vitellogenin synthesis. As a reduction in the rate of synthesis will have the same effect as the parasitic load term above, we conclude that the model may, at least to some extent, explain increased winter mortality of mite-infested colonies without invoking secondary infection effects.

ROBUSTNESS OF FUNCTIONAL FORMS

The results are robust to changes of the Hill coefficient in all sigmoidal functions of the model except n_1 in eqn (1). Here, we used a value of 4 for all simulations, and if we lowered it to 3, we observed that it was more difficult to obtain consistent results. It would be premature to make any biological interpretation of this. Varying the forms of the transition functions does not change the predicted patterns.

Discussion

THE POTENTIAL WINTER BEE RECRUITMENT POOL

Except in the case where the workers have been foragers for several days, the results suggest that the previous life histories do not constrain workers from becoming winter bees as long as they get ample food and time to build up their protein reserves before they enter winter cluster conditions.

Not taking into consideration the possibility of other preventive mechanisms being induced by nursing or foraging, this implies that the types of bees becoming winter bees may vary as a function of the temperature conditions in the autumn as well as with the size of the colony's pollen store. If the temperature is low, the rate of vitellogenin synthesis may not be high enough to permit previous nurses to develop the characteristics of winter bees (Crailsheim, 1986; Lin & Winston, 1998). If the pollen supply is low, workers are dependent on interadult jelly donations to support vitellogenin synthesis if there are no opportunities for cannibalism of brood. Bees that have enough quantities of stored proteins at this time may engage in trophallaxis as donors more often than workers with low protein levels, such that the colony's protein pool is evenly divided. However, we do not have any data suggesting the existence of such an asymmetric inter-adult feeding pattern in the autumn.

The above considerations indicate the existence of geographical and annual variations in temperate zones such that in some cases the winter bees may be recruited from a pool of

workers defining a wide range of previous life histories, while in other cases they may be recruited from hive bees having been exposed to no, or very little, nurse load.

LIFE HISTORY CONSIDERATIONS

Honeybee colonies in many subtropical areas in Africa annually experience an extended period with no or very few foraging opportunities. This suggests that the mechanism of vitellogenin accumulation promoting longer lifespan has probably been part of the subtropical honeybee's arsenal of life history strategies for a very long time. If it can be shown that European and African colonies share the same regulatory mechanism, it would imply that the dispersal from subtropical to temperate zones demanded only polishing of an already well-established lifespan-promoting trait. As clustering is also part of the African bees' life history repertoire, and considering how the thermoregulation in the winter cluster probably is achieved (Omholt, 1987), the survival of honeybee colonies during winter in temperate zones may not have needed much evolutionary innovation.

From an evolutionary point of view, it also makes sense that vitellogenin has become a central ingredient for brood food production in honeybee workers. Vitellogenin is ubiquitously a key protein for oocyte production among insects. The evolution of the honeybee society from a solitary state has probably included a state where the queen reared her own brood part of the time. In this case, the vitellogenin machinery could be exploited immediately as a combined solution for providing amino acids, lipids and zinc to the brood food. It is hard to see why an alternative receptor and processing machinery based on a separate import of amino acids, lipids and zinc into the brood-producing glands would have been selected. This trait has probably a long evolutionary history culminating in the honeybee society as a specific trait for honeybee workers. If this is correct, the prediction would be that vitellogenin is used for brood food production by queens as well as workers in those *Hymenopteran* species where the queens rear their own brood part of the time.

THE HONEYBEE SYSTEM AS A TEST BED FOR PREVAILING EVOLUTIONARY THEORIES OF AGEING

There are currently three main theories providing complementary explanations for why ageing occurs (reviewed by Kirkwood and Austad, 2000). The "mutation accumulation" theory states that by an age when wild survivorship has declined to very low levels, the force of selection is too weak to oppose the accumulation of germ-line mutations with late-acting deleterious effects (Medawar, 1952). A second theory is that of "antagonistic pleiotropy". It says that because a small beneficial effect early in life can outweigh a late deleterious effect, pleiotropic genes with good early effects would be favoured by selection even if these genes had bad effects at later ages (Williams, 1957). The third is the "disposable soma" theory, which is based on optimal allocation of metabolic resources between somatic maintenance and reproduction (Kirkwood, 1977; Kirkwood, 1996). It says that effective somatic maintenance is required only to keep the organism in sound physiological condition for as long as it has a reasonable chance of survival in the wild.

All three theories address why species have the lifespans they do, and they all predict that the principal determinant in the evolution of longevity is the level of extrinsic mortality. If this level is high, life expectancy in the wild is short, deleterious gene effects accumulate at earlier stages, and there is little selection for a high level of somatic maintenance. Conversely, if the level of extrinsic mortality is low, selection is predicted to postpone deleterious gene effects and to direct greater investment in building and maintaining a durable soma (Kirkwood & Austad, 2000).

The observed longevity patterns apparently confirm these predictions as the summer bee (with its forager stage) and the winter bee are indeed exposed to high and low levels of extrinsic mortality, respectively. However, these two patterns are realized by the same genotype, and none of these theories was designed to address ageing as a facultative trait of a sexually non-reproducing animal. The honeybee worker, thus, provides an unexploited test bed for evolutionary explanations of ageing. It is easy to realize

that the mutation accumulation theory and the antagonistic pleiotropy theory are incapable of dealing with a facultative trait like this because the same genotype can have a short and a long lifespan. However, the disposable soma theory can be adjusted to cope with this problem. The same conclusion seems to be valid for a wide range of organisms displaying facultative age determination, which in turn implies several new testable hypotheses concerning age regulation and evolutionary conservation of ageing pathways [Omholt *et al.* (in prep.)].

The bimodal longevity distribution of honeybees can be considered to be an emergent group trait phenomenon at the colony level. The honeybee worker does not normally reproduce, and at the group level it can be considered to be soma. In this group selection context, it makes sense to talk about optimal allocation of metabolic resources between somatic maintenance and reproduction. Thus, by changing focus from the level of the individual to the level of the colony, the disposable soma theory can be meaningfully applied. In the summer time, the bees need to forage, and as foraging is risky, they are not likely to live long (Visscher & Dukas, 1997). In this case, there will be a group trait selection against investing resources that may increase the potential lifespan of the workers. In the wintertime, however, when there is no risky foraging and the colony has to stay alive for several months before it can continue making reproductives, there will be group trait selection for allocating enough resources to keep the workers alive for a long time.

With two group trait selection pressures working in opposite directions on the same worker population, the disposable soma theory predicts the establishment of a facultative age determination mechanism. It also predicts that the winter bee will have a functioning set of mechanisms ensuring somatic maintenance by preventing intrinsic deterioration of the organism for several months, while the forager will not.

EMPIRICAL SUPPORT FOR THE DISPOSABLE SOMA THEORY IN HONEYBEES

The basic tenet of the proximal oxidative stress explanation of ageing is that senescence-related loss of function is due to the progressive and irreversible accrual of molecular oxidative damage. In light of this, we would predict that foragers would be more exposed to oxidative stress than winter bees. We know that restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum lifespan in mammals (reviewed by Sohal & Weindruck, 1996). The reduced metabolic rate of bees during late autumn and winter is in accordance with this. However, caloric restriction does not provide a complete explanation as nursing bees may also become quite old as long as they are prevented from entering the foraging stage (Haydak, 1963).

The oxidative stress explanation is supported by the fact that overexpression of anti-oxidative enzymes retards the age-related accrual of oxidative damage and extends the maximum lifespan of transgenic *D. melanogaster*. Oxidative stress can play a major role in ageing of *C. elegans,* and vitellogenin-6 in this species appears to have an anti-oxidant function (Nakamura *et al.*, 1999). There are apparently no data connecting the extended lifespan of the dauer stage of *C. elegans* and vitellogenin, but if it can be shown that vitellogenin is one of the main factors causing the enhanced longevity of the dauer stage as well as the winter bee, this would suggest a similarity between these two states from the level of induction all the way down to the basic regulatory machinery.

If honeybee vitellogenin also belongs to the group of anti-oxidant proteins, the prediction would be that there would be less vitellogenin in foragers than in winter bees (which is actually the case). Furthermore, it was recently shown that extended longevity of *Drosophila* in connection with artificial selection experiments is correlated with enhanced anti-oxidant defense system gene expression, accumulation of CuZn-SOD protein, and an increase in ADS enzyme activities (Arking *et al.*, 2000). If these anti-oxidant mechanisms are present in bees too, the prediction would be that they are much less pronounced in foragers than in winter bees. That there is a difference in general detoxification capacity is supported by observations showing

that summer bees appear to be approximately eight-fold more susceptible than winter bees to the synergistic action of prochloraz and deltamethrin (Meled *et al*., 1998).

Furthermore, there are indications that vitellogenin is important for establishment of a normal plasmocyte profile in honeybees [Amdam *et al*. (in prep.)]. According to what would be expected, the proportion of normal plasmocytes in the haemolymph of a hive bee is substantially higher than in a forager (Fluri *et al*., 1977). This implies that the forager may be strongly immunocompromised in addition to being exposed to a high level of oxidative stress.

In the framework of the disposable soma theory, one may ask what is the cost associated with keeping up vitellogenin synthesis in foragers. Pollen is a scarce commodity, and letting foragers take care of their own protein supply while they stay in the hive would probably cause a higher colony consumption of pollen (with a series of additional negative effects) as the foragers would then contain high amounts of protein when they perished in the field. Letting hive bees feed the foragers as a function of the flow conditions (Crailsheim *et al*., 1999), and letting the foragers be incapable of synthesizing vitellogenin to prevent the build-up of a vitellogenin store, enable a much tighter control of the protein consumption of the forager population [Omholt *et al*. (in prep.)].

This explanation suggests that the life history trade-offs involved have to be considered in a group selection context. It also fits with the claim that age-based division of labour, with performance of risky tasks delayed until late in life by workers with depleted nutrient stores, may have evolved as an energy-saving mechanism in insect colonies (Jeanne, 1986; O'Donnell and Jeanne, 1995). However, it also extends this claim by showing that depletion of nutrient stores can be actively controlled by pathways connected to regulation of ageing that can be accounted for by the disposable soma theory.

OUTLINE OF A RESEARCH PROGRAMME MOTIVATED BY THE MODEL

One may argue that by our use of the control functions $\alpha(t)$, $\omega(t)$, $\lambda(t)$ and $\psi(t)$, we are able to force the model to generate temporal patterns in accordance with empirical data even though its key presumptions are wrong. At the present stage of our knowledge of the honeybee society, we think it would have been premature to make the connection between the physiology of the individual worker and its colony environment by other means. As stated previously, varying the forms of the transition functions would not change the predicted patterns, and since several of the worker-state-associated parameter values can be quantified and thereby promote quantification of the remaining ones by indirect means, these time-dependent functions are in fact empirically quite well founded. Furthermore, based on this quantification strategy the model provides several new quantitative predictions to be tested concerning the amounts of vitellogenin in the various compartments as a function of various task scenarios. As these amounts together with various parameters of the model can be measured directly (see below), they will serve as independent test data.

The critical hypotheses behind the model can be tested independently, and if they are refuted the model and the suggested connection between vitellogenin and lifespan regulation would be blatantly wrong. More precisely, if radioactive tracing experiments can conclusively show that vitellogenin is not transported in large amounts to the HPGs during the nurse period and is not used for other metabolic purposes, and antibody staining techniques can conclusively show that the fat body is not a storage organ for vitellogenin, then there would be need to pursue this research programme further.

However, if these predictions are confirmed, we still cannot claim that there is a direct causal link between vitellogenin content and lifespan. Other proteins than vitellogenin may still be responsible, but considering the fractional haemolymph content of vitellogenin, we think this is unlikely.

A more likely alternative would be that juvenile hormone directly or indirectly controls the somatic maintenance machinery similar to what seems to be the case for *Drosophila* (Tatar *et al*., 2001), and that this mechanism alone is sufficient for producing a bimodal longevity distribution. How should we then be able to

distinguish between these three alternatives that may explain honeybee lifespan (vitellogenin only, vitellogenin + JH-mediated regulation of somatic maintenance, and JH-mediated regulation of somatic maintenance only)? An immediate argument against JH-mediated regulation of somatic maintenance only is that we know the vitellogenin content of worker bees starts at a high level in autumn and decreases during winter (Fluri *et al*., 1982). There must have been a strong selection pressure for minimizing the energy expenditure during winter. If the workers had to eat pollen and distribute protein during winter to replenish their protein stores, this would have caused enhanced energy consumption in the winter cluster. Selection for physiological mechanisms promoting accumulation of a storage protein in the autumn large enough to last until spring solves this problem, and given that the essential features of our mathematical model is correct, vitellogenin is the most likely protein candidate to have been used for this purpose. The only thing that can disprove this line of argument is that honeybees do not need vitellogenin for surviving in the winter cluster. A future experiment where a winter cluster is established from bees having a low JH titre and disrupted vitellogenin expression, would resolve this issue. Furthermore, if it can be shown that it is the lack of vitellogenin that causes the dramatic reduction in the proportion of normal plasmocytes in the haemolymph of honeybee foragers, this would imply that there is a direct link between vitellogenin and ageing (this is currently under investigation). Finally, if we in the future can perform experiments with bees that have constitutive expression of vitellogenin as foragers, it will be possible to determine whether vitellogenin can reduce oxidative stress, uphold the cell defence immune system and prolong life in bees with high JH titres.

Based on the available data, we suggest that if JH is responsible for tuning the somatic maintenance machinery up and down in honeybees too, then it represents an additional mechanism ensuring further depletion of, or less demand for, nutrients in foragers. In this connection, the honeybee queen might provide crucial information about the key mechanisms ensuring a long lifespan in honeybee females. The queen may live for several years and has a constant high-vitellogenin titre similar to winter bees. However, in contrast to the worker bee, her vitellogenin titre seems to be insensitive to the JH titre (Kaatz, 1988). This implies that vitellogenin synthesis is not under the negative regulatory control of JH. It is likely that the same is true for other aspects of her somatic maintenance machinery, and as queens normally have very low JH titres, this can be tested by treatment with JH or a JH mimic. We predict that the main distinction between a queen and a worker bee concerning the structure of their somatic maintenance machineries is that JH actively regulates vitellogenin synthesis in workers, and we explain this by the life history argument presented above.

By translating the available empirical knowledge of honeybee lifespan regulation into the language of nonlinear system dynamics, we think we have provided a theoretical foundation that will contribute to a deeper and more quantitative understanding of this interesting and important complex dynamic system.

We thank two anonymous referees, Lisbeth Børgesen, Ingemar Fries, Klaus Hartfelder, Zachary Huang, Tom Kirkwood and Erik Plahte for helpful comments on various versions of this manuscript. Gro Vang Amdam's contribution was financially supported by the Norwegian Research Council, project no. 133680/110.

REFERENCES

Ancsin, J. B. & Waytt, G. R. (1996). Purification and characterization of two storage proteins from *Locustra migratoria* showing distinct developmental and hormonal regulation. *Insect Biochem. Molec. Biol*. **26,** 501–510.

Arking, R., Burde, V., Graves, K., Hari, R., Feldman, E., Zeevi, A., Soliman, S., Saraiya, A., Buck, S., Vattraino, J., Sathrasala, K., Wehr, N. & Levine, R. L. (2000). Forward and reverse selection for longevity in Drosophila is characterized by alternation of antioxidant gene expression and oxidative damage patterns. *Exp. Gerontol.* **35,** 167–185.

Arnold, G., Le Conte, Y., Trouiller, J., Hervet, H., Chappe, B. & Masson, C. (1994). Inhibition of worker ovaries development by mixture of fatty acid esters from larvae. *Life Sci.* **317,** 511–515.

Babin, P. J., Bogerd, J., Koogiman, F. P., Van Marrewijk, W. J. A. & Van der Horst, D. J. (1999). Apolipophorin II/I, apoprotein B, vitellogenin and microsomal triglyseride transfer protein genes are

derived from a common ancestor. *J. Molec. Evol.* **49,** 150–160.

Brouwers, E. V. M. (1983) Activation of the hypopharyngeal glands of honeybees in winter. *J. Apic. Res.* **22,** 137–141.

Burgess, E. P. J., Malone, L. A. & Christeller, J. T. (1996). Effects of two proteinase inhibitors on the digestive enzyme. *J. Insect Physiol.* **42,** 823–828.

Crailsheim, K. (1985). Distribution of haemolymph in the honeybee (*Apis mellifera*) in relation to season, age and temperature. *J. Insect Physiol.* **31,** 707–713.

Crailsheim, K. (1986). Dependence of protein metabolism on age and season in the honeybee (*Apis mellifera carnica*). *J. Insect Physiol.* **32,** 629–634.

Crailsheim, K. (1990a). The protein balance of the honey bee worker. *Apidologie* **21,** 417–429.

Crailsheim, K. (1990b). Protein synthesis in the honeybee (*Apis mellifera*) and trophallactic distribution of jelly among imagoes in laboratory experiments. *Zool. J. Physiol.* **94,** 303–312.

Crailsheim, K. (1991). Interadult feeding of jelly in honeybee (*Apis mellifera*) colonies. *J. Comp. Physiol. B* **161,** 55–60.

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

Crailsheim, K., Hrassnigg, N., Gmeinbauer, R., Szolderits, M. J., Schneider L. H. W. & Brosch, U. (1993). Pollen utilization in non-breeding honeybees in winter. *J. Insect Physiol.* **39,** 369–373.

Crailsheim, K., Riessberger, U., Blaschon, B., Nowogrodizki, R. & Hrassnigg, N. (1999). Short-term effects of simulated bad weather conditions upon the behaviour of foodstorers honeybees during day and night. *Apidologie* **30,** 299–310.

Cremonez, T. A., De Jong, D. & Bitondi, M. M. (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, J. C., Huet, D., Pernollet, J. C. & Masson, C. (1998). Identification and developmental profiles of hexamerins in the antenna and haemolymph of the honeybee, *Apis mellifera*. *Insect Biochem. Molec. Biol.* **28,** 387–397.

Eischen, F. A., Rothenbuhler, W. C. & Kulincevic, J. M. (1982). Length of life and dry weight of worker honeybees rears in colonies with different worker–larva ratios. *J. Apic. Res.* **21,** 19–25.

Engels, W. (1968). Extraoocytäre Komponenten des Eiwachstums bei *Apis mellifera*. *Insectes Soc.* **15,** 271–288.

Engels, W. (1974). Occurrence and significance of vitellogenins in female castes of social Hymenoptera. *Am. Zool.* **14,** 1229–1237.

Engels, W. & Fahrenhorst, H. (1974). Alters- und kastenspezifische Veeränderungen ser Haemolymph-Protein-Spectren bei *Apis mellifera*. *Wilhelm Roux Archiv.* **174,** 285–296.

Engels, W., Kaatz, H., Zillikens, A., Simões, Z. L. P., Trube, A., Braun, R. & Dittrich, F. (1990). Honey bee reproduction: vitellogenin and caste-specific regulation of fertility. In: *Advances in Invertebrate Reproduction* 5 (Hashi, M. & Yamashita, O., eds), pp. 495–452. Amsterdam: Elsevier Science Publishers B. V.

Falchuk, K. H. (1998). The molecular basis for the role of zinc in developmental biology. *Molec. Cell. Biochem.* **188,** 41–48.

Finch, C. E. (1990). *Longevity, Senescence and the Genome*. Chicago: University of Chicago Press.

Fluri, P. & Bogdanov, S. (1987). Age dependence of fat body protein in summer and winter bees (*Apis mellifera*). In: *Chemistry and Biology of Social Insects* (Eder, K. & Rembold, H. eds) pp. 170–171. München: Verlag J. Peperny.

Fluri, P., Wille, H., Gerig, L. & Lüscher, M. (1977). Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees (*Apis mellifera*). *Experimentia* **33,** 1240--1241.

Fluri, P., Lüscher, M., Wille, H. & Gerig, L. (1982). Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone and vitellogenin in worker honeybees. *J. Insect Physiol.* **28,** 61–68.

Free, J. B. & Spencer-Booth, Y. (1959). The longevity of worker honey bees (*Apis mellifera*). *Proc. R. Entomol. Soc. London* **34A,** 141–150.

Fukuda, H. & Sekiguchi, K. (1966). Seasonal change of the honeybee worker longevity in Sapporo, North Japan, with notes on some factors affecting life-span. *Japan J. Ecol.* **16,** 206–212.

Giray, T. & Robinson, G. E. (1994). Effects of intracolony variability in behavioral development on plasticity of division of labor in honey bee colonies. *Behav. Ecol. Sociobiol.* **35,** 13–20.

Haunerland, N. H. & Shirk, P. D. (1995). Regional and functional differentiation in the insect fat body. *Annu. Rev. Entomol.* **40,** 121–145.

Haydak, M. H. (1935). Brood rearing by honeybees confined on a pure carbohydrate diet. *J. Agric. Res.* **49,** 21–28.

Haydak, M. H. (1957). Changes with age in the appearance of some internal organs of the honeybee. *Bee World* **38,** 197–206.

Haydak, M. H. (1963). Age of nurse bees and brood rearing. Paper No. 5122 *Sient. J. Series*, Minnesota Agricultural Experiment Station.

Hirai, M., Watanabe, D., Kiyota, A. & Chinzei, Y. (1998). Nucleotide sequence of vitellogenin mRNA in the bean bug, *Riptorus clavatus*: analysis of processing in the fat body and ovary. *Insect Biochem. Molec. Biol.* **28,** 537–547.

Hrassnigg, N. & Crailsheim, K. (1998). Adaptation of hypopharyngeal gland development to the brood status of the honeybee (*Apis mellifera*) colonies. *J. Insect Physiol.* **44,** 929–939.

Huang, Z.-Y. & Otis, G. W. (1989). Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). *Insectes Soc.* **36,** 264–276.

Huang, Z.-Y. & Robinson, G. E. (1992). Honeybee colony integration: worker–worker interactions mediate hormonally regulated plasticity in division of labor. *Proc. Natl Acad. Sci. USA* **89,** 11726–11729.

Huang, Z.-Y. & Robinson, G. E. (1995). Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bee. *J. Comp. Physiol. B* **165,** 18–28.

Huang, Z.-Y. , Robinson, G. E. & Borst, D. W. (1994). Physiological correlates of division of labor among similarly aged honey bees. *J. Comp. Physiol. A* **174,** 731–739.

Imboden, H., Wille, H., Gerig. L. & Lücher, M. (1976). Die Vitellogeninsynthese bei der Bienen-Arbeiterin (*Apis mellifera*) und ihre Abhängigkeit von Juvenilhormon (JH). *Rev. Suisse Zool.* **83,** 928–933.

Jay, S. C. (1972). Ovary development of worker honeybees when separated from worker brood by various methods. *Can. J. Zool.* **51,** 629–632.

Jeanne, R. L. (1986). The evolution of the organization of work in social insects. *Monitore. Zool. Ital*. **20,** 119–133.

Jensen, P. V. & Børgesen, L. W. (2000). Regional and functional differentiation in the fat body of pharaoh's ant queens, *Monomorium pharaonis* (L.). *Arth. Struc. Dev.* **29,** 171–184.

Kaatz, H. (1988). Juvenilhormon-unabhängige Initiation und Regulation der Vitellogenin Synthese bei der Bienenkönigin (*Apis mellifera*). *Verh. Dtsch. Zool.* **81,** 272–273.

Kaatz, H. & Dittrich, F. (1989). Hormonal and pheromonal regulation of vitellogenin synthesis in honeybees. In: *Molecular Insect Science* (Hagedorn, H. H., ed.), pp. 325–326. New York: Plenum press.

Kaatz, H., Hagedorn, H. & Engels, W. (1985). Culture of honeybee organs: development of a new medium and the importance of tracheation. *In vitro Cell Dev. Biol.* **21,** 347–352.

Kirkwood, T. B. L. (1977). Evolution of ageing. *Nature* **270,** 301–304.

Kirkwood, T. B. L. (1996). Human senescence? *BioEssays* **18,** 1009–1016.

Kirkwood, T. B. L. & Austad, S. N. (2000). Why do we age? *Nature* **408,** 233–238.

Koehler, A. (1921). Beobachtungen über Veranderungen am Fettkörper der Biene. *Schweiz. Bienen-Ztg.* **44,** 424–428.

Kropacova, S. & Haslbachova, H. (1970). The development of ovaries in worker honeybees in queenright colonies examined before and after swarming. *J. Apic. Res*. **9,** 65–70.

Lensky, Y. & Rakover, Y. (1983). Separate protein body compartments of the worker honeybee (*Apis mellifera*). *Comp. Biochem. Physiol.* **75B,** 607–615.

Lensky, Y. & Skolnik, H. (1980). Immunological and electrophoretic identification of the vitellogenin proteins of the queen bee (*Apis mellifera*). *Comp. Biochem. Physiol.* **66B,** 185–193.

Levin, M. D., Mykola, H. & Haydak, H. (1951). Seasonal variation in weight and ovarian development in the worker honeybee. *J. Econ. Entomol.* **44,** 54–57.

Lin, H. & Winston, M. L. (1998). The role of temperature in the ovarian development of the worker honeybee (*Apis mellifera*). *Can. Entomol.* **130,** 883–891.

Malone, L. A. & Gatehouse, H. S. (1998). Effects of *Nosema apis* infection on honey bee (*Apis mellifera*) digestive proteolytic enzyme activity. *J. Invert. Path.* **71,** 169–174.

Mann, C. J., Anderson, T. A., Read, J., Chester, S. A., Harrison, G. B., Köchl, S., Amey, J., Vanloo, B., Rosseneu, M., Infante, R., Hancock, J. M., Levitt, D. G., Banaszak, L. J., Scott, J., Shoulders, C. (1999). The structure of vitellogenin provides a molecular model for the assembly and secretion of atherogenic lipoproteins. *J. Mol. Biol.* **285,** 391–408.

Martinez, T. & Wheeler, D. E. (1991). Identification of vitellogenin in the ant *Camponotus festinatus*: changes in hemolymph proteins and fat body development in workers. *Arch. Insect Biochem. Physiol.* **17,** 143–155.

Martinez, T. & Wheeler, D. (1993). Identification of 2 storage hexamers in the ant, *Camponotus festinatus* — accumulation in adult queenless workers. *Ins. Biochem. Molec. Biol.* **23,** 309–317.

Martinez, T. & Wheeler, D. E. (1994). Storage proteins in adult ants (*Camponotus festinatus*), roles of colony founding by queens and in larval rearing by workers. *J. Insect Physiol.* **40,** 723–729.

Maurizio, A. (1950). The influence of pollen feeding and brood rearing on the length of life and physiological conditions of the honeybee. *Bee World* **31,** 9–12.

Maurizio, A. (1954). Pollenernährung und Lebensvorgänge bei der Honigbiene (*Apis mellifera* L.). *Landwirtsch. Jahr. Schweiz* **245,** 115–182.

Medawar, P. B. (1952). *An Unsolved Problem of Biology*. London: Lewis.

Meled, M., Thrasyvoulou, A. & Belzunces, L. P. (1998). Seasonal variations in susceptibility of *Apis mellifera* to the synergic action pf prochloraz and deltamethrin. *Environ. Toxicol. Chem.* **17,** 2517–2520.

Mocchegiani, E., Muzzioli, M. & Gicconi, R. (2000). Zinc, metallothioneins, immune responses, survival and ageing. *Biogerontology* **1,** 133–143.

Moritz, R. F. A. (1981). Altersabhängige Empfindlichkeit von Varroa jacobsoni, Diagnose und Therapie der Varroatose, In *Apimondia*, pp.62–68. Bukarest.

Moskalenko, C. T. (1982). Some physiological characteristics of wintering honeybees. *Pchelovodstvo* **12,** 18–19.

Nakamura, A., Yasuda, K., Adachi, H. & Sakurai, Y. (1999). Vitellogenin-6 is a major carbonylated protein in aged nematode, Caenorhabditis elegans. *Biochem. Biophys. Res. Commun.* **264,** 580–583.

O'Donnell, S. & Jeanne, R. L. (1995). Worker lipid stores decrease with outside-nest task performance in wasp: implications for the evolution of age polyethism. *Experientia* **51,** 749–752.

Oliveira-Tozetto, S., Rachinsky, A. & Engels, W. (1995). Reactivation of juvenile hormone synthesis in adult drones of the honey bee. *Experientia*, **51,** 945–946.

Omholt, S. W. (1987). Thermoregulation in the winter cluster of the honeybee, *Apis mellifera*. *J. theor. Biol*. **128,** 219–231.

Omholt, S. W. (1988). Relationships between worker longevity and the intracolonial population dynamics of the honeybee. *J. theor. Biol*. **130,** 275–284.

Omholt, S. W., Kefang, X., Andersen, Ø. & Plahte, E. (1998). Description and analysis of switchlike regulatory networks exemplified by a model of cellular iron homeostasis. *J. theor. Biol*. **195,** 339–350.

Osanai, M. (1960). Zinc in the royal jelly of the honey bee, *Apis mellifera*. *Zoology* **9,** 1–8.

Pener, M. P. (1992). Environmental cues, endocrine factors, and reproductive diapause in male insects. *Chronobiol. Int.* **9,** 102.

Pinto, L. Z., Bitondi, M. M. G. & Zilá, L. P. S. (2000). Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *J. Insect Physiol.* **46,** 153–160.

Plahte, E., Mestl, T. & Omholt, S. W. (1998). A methodological basis for description and analysis of

systems with complex switch-like interactions. *J. Math. Biol.* **36,** 321–348.

Riddle, D. L. (1988). The dauer larva. In *The nematode Caenorhabditis elegans* (Wood, W. B., ed.), pp. 393–414. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Rosell, R C. & Wheeler, D. E. (1995). Storage function and ultrastructure of the adult fat body in workers of the ant *Camponotus festinatus*. *Int. J. Insect Morphol. Embryol.* **24,** 413–426.

Rutz, W. & Lüscher, M. (1974). The occurrence of vitellogenin in workers and queens of *Apis mellifera* and the possibility of its transmission to the queen. *J. Insect Physiol.* **20,** 897–909.

Rutz, W., Gerig, L., Wille, H. & Lüscher, M. (1976). The function of juvenile hormone in adult worker honeybees, *Apis mellifera*. *J. Insect Physiol.* **22,** 1485–1491.

Ryan, R. O., Schmidt, J. O. & Law, J. H. (1984). Arylphorin from the haemolymph of the larval honeybee, *Apis mellifera*. *Insect Biochem*. **14,** 515–520.

Saunders, D. S., Richard, D. S. & Applebaum, S. W. (1990). Photoperiodic diapause in *Drosophila-melanogaster* involves a block to the juvenile-hormone regulation of ovarian maturation. *Gen. Comp. Endocrinol.* **79,** 174.

Schabacker, H. (1973). Haemolymph-Proteine und Oogenese-Activität der 4 rezenten Honigbienen-Arten der Gattug Apis. Staatsexamenarbeit: Zool Inst Univ Münster.

Schatton-Gadelmayer, K. & Engels, W. (1988). Hemolymph proteins and body weight in newly emerged worker honeybees according to the different rates of parasitation by brood mites. *Entomol. Gener.* **14,** 93–101.

Schneider, P. & Drescher, W. (1987). The influence of *Varroa jacobsoni* oud on weight, development of weight and hypopharyngeal glands, and longevity of *Apis mellifera* L. *Apidologie* **18,** 101–109.

Seeley, T. D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav. Ecol. Sociobiol*. **11,** 287–293.

Shipman, B. A., Ryan, R. O., Schmidt, J. O. & Lae, J. H. (1987). Purification and properties of a very high density lipoprotein from the hemolymph of the honeybee *Apis mellifera*. *Biochemistry* **26,** 1885–1889.

Snodgrass, R. E. (1956). *Anatomy of the Honeybee.* London: Cornell University Press.

Sohal, R. S. & Weindruch R. (1996). Oxidative stress, caloric restriction, and aging. *Science* **273,** 59–63.

Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, M. C. & Garofalo R. S. (2001). A mutant Drosophila insulin reseptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292,** 107–109.

Timmins, P. A., Poliks, B. & Banaszek, L. (1992). The location of bound lipid in the lipovitellin complex. *Science* **257,** 652–655.

Trenczek, T., Zillikens, A. & Engels, W. (1989). Developmental patterns of vitellogenin haemolymph titre and rate of synthesis in adult drone honey bees (*Apis mellifera*). *J. Insect Physiol.* **35,** 475–481.

Van Nerum, K. & Buelens, H. (1997). Hypoxia-controlled winter metabolism in honeybees (*Apis mellifera*). *Comp. Biochem. Physiol.* **117A,** 445–455.

Velthuis, H. H. W. (1970). Ovarian development in *Apis mellifera* worker bees. *Entomol. Exp. Appl*. **13,** 377–394.

Verheijen-Voogd, C. (1959). How worker bees perceive the presence of their queen. *Z. Vgl. Physiol.* **41,** 527–582.

Visscher, P. K. & Dukas, R. (1997) Survivorship of foraging honeybees. *Insectes Soc.* **44,** 1–5.

Von Rhein, W. (1956). Über die Ernärung der Arbeitermade von *Apis mellifera* L insbesondere in der Altersperiode. *Insectes Soc.* **3,** 203–212.

Weinberg, K. P. & Madel, G. (1985). The influence of the mite *Varroa jacobsoni* on the protein concentration and the haemolymph volume of the brood of workers and drones of the honeybee *Apis mellifera*. *Apidologie* **16,** 421–436.

Wheeler, D. E. & Buck, N. A. (1995). Storage proteins in ants during development and colony founding. *J. Insect Physiol*. **41,** 885–894.

Williams, G. C. (1957). Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11,** 398–411.

Wossler, T. C. & Crewe, R. M. (1999). Honeybee queen tergal gland secretion affects ovarian development in caged workers. *Apidologie* **30,** 311–320.