FEBS Letters 584 (2010) 2496–2503

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

The curious case of aging plasticity in honey bees

Daniel Münch [a,*], Gro V. Amdam [a,b]

[a] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Aas, Norway
[b] School of Life Sciences, Arizona State University, P.O. Box 874501, AZ 85287-4501 Tempe, United States of America

ARTICLE INFO

Article history:
Received 1 March 2010
Revised 29 March 2010
Accepted 7 April 2010
Available online 10 April 2010

Edited by Jan Rydström

Keywords:
Senescence
Social behavior
Care-giving
Sibling
Vitellogenin
Insulin-like signaling

ABSTRACT

As in all advanced insect societies, colony-organization in honey bees emerges through a structured division of labor between essentially sterile helpers called workers. Worker bees are sisters that conduct all social tasks except for egg-laying, for example nursing brood and foraging for food. Curiously, aging progresses slowly in workers that engage in nursing and even slower when bees postpone nursing during unfavorable periods. We, therefore, seek to understand how senescence can emerge as a function of social task performance. The alternative utilization of a common yolk precursor protein (vitellogenin) in nursing and somatic maintenance can link behavior and aging plasticity in worker bees. Beneficial effects of vitellogenin may also be mediated by inhibitory action on juvenile hormone and insulin-like signaling.

1. Introduction

A human that lives 30% longer than average is a curiosity, a singular case. Yet, in advanced social bees, ants or termites, extraordinary longevity is not a curious anomaly. Life spans of genetically similar individuals (usually siblings) can vary from weeks to decades in these group-living insects [1], providing unique opportunities to study effects of social factors on aging. Here, we use honey bees (*Apis mellifera*) to exemplify how variation in onset of senescence can develop through well-regulated and highly plastic processes that emerge as functions of social relationships.

Apart from studying effects of allelic variation on fitness traits, life-history research seeks to understand how a single genotype can produce different phenotypes through exposure to environmental variation (phenotypic plasticity, [2]). A primary motivation to study phenotypic plasticity is to assess to what extent an individual's development, physiology, health and aging progression can be changed in response to environmental factors, and what the consequences are for fitness. In many animals, the social environment has important influences on life-history that sometimes are poorly understood. Social insects, such as honey bees, provide systems for exploring these relationships – particularly those where complex interactions between siblings have alternative phenotypic outcomes (sibling effects, [3]).

Sibling effects can be uniquely positive or antagonistic. Social interactions between young mice and rats can shape adaptive aspects of the adult phenotype, including sexual and exploratory behavior [4]. Competition between hatched birds, conversely, may result in siblicide [5]. While most sibling studies focus on development, the organization of honey bee societies is built from social relationships that are not limited to a certain developmental period. Instead, individual life-histories change in response to interactions between siblings that occur throughout adulthood. The resulting phenotypic plasticity is believed to be beneficial for colony-level fitness (i.e., the production of reproductives and swarms [6]).

In honey bees, plasticity of adult physiology, behavioral ontogeny and longevity are connected to social resource transfers – primarily patterns of nourishment and food provisioning between sisters. Recent research elucidates how these factors influence the progression of senescence, to show how sibling effects translate into changes at different levels of biological complexity: from gene expression and biochemistry to variation in physiology and behavior that has life-shortening and life-promoting effects. These results provide a comprehensive dataset for understanding how social environment affects aging, and therefore can be of general interest to life-history research.

Abbreviations: HPG, hypopharyngeal glands; IIS, insulin/insulin-like signaling; ILP, insulin-like peptide; IRS, insulin receptor substrate; QTL, quantitative trait loci

* Corresponding author. Fax: +47 64 96 59 01.
E-mail address: daniel.munch@umb.no (D. Münch).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.04.007

2. Honey bee social caste organization and life span variation

The nests of honey bees are sophisticated social architectures where females engage in brood rearing, food storage and defense, in debris removal and in maintaining a nest environmental homeostasis of about 33 °C with 80% relative humidity. The vast majority of the colony's population are facultatively-sterile helpers called workers, which are usually sister bees. Each society is headed by a single queen [7], the mother to the colony. A queen lays up to 1000–2000 eggs daily, but shows no subsequent care-behavior toward the offspring. Consequently, the helper cast of workers conducts all aspects of care-giving for the young brood, such as feeding, cleaning and warming [8].

The nurse bees are a temporal worker sub-caste that specializes in brood rearing. They have physiological adaptations to this social role, such as protein and lipid stores in abdominal fat body (the functional homologue to vertebrate liver and white fat) and hypertrophied hypopharyngeal head glands (HPG). The HPG take up nutrients and constituents produced by the fat body and use these to produce a proteinaceous secretion. This substance is mixed with other elements like sugar before the resulting jelly is fed to the brood (for review see [9]). To fuel fat body protein synthesis and the production of jelly, nurse bees eat pollen – the major amino acid source of honey bees. Such details of honey bee brood care might seem rather trivial, but provide a surprising link to plasticity of aging. When brood is absent and not much nursing or provisioning activity is required, workers called *diutinus* or 'winter bees' develop excessive fat body protein stores. These *diutinus* bees exhibit the longest reported life span of worker bees. While nurse bees may senesce after 30–50 days (see below, [10]), winter bees survive for about 10 months without measurable deficits related to aging.

After nursing, however, bees will usually transition to foraging tasks outside the colony. This temporal task-schedule shortens life span, as most foragers perish within 7–10 days. Foragers, thereby, are the shortest-lived phenotype among the sister bees. In contrast, retaining characteristics of the nurse phenotype and delaying onset of foraging drastically increases life expectancy [7]. Workers also have the capacity to revert from the short-lived (forager) to a long-lived phenotype (nurse). The driving force for such reversal of the normal task-schedule is an absence of nurse bees in the colony, a social context that can be induced by experimental manipulation of colony demography [11].

In summary, ontogenetic transitions from nest tasks to foraging results in a temporal and spatial division of labor between worker bees: new care givers emerge in the central nest to replace those that shift to foraging at the periphery and eventually die. Diversification of social tasks, moreover, is associated with different patterns of survival that are sensitive to social-environmental changes conveyed by the amount of brood, nurse bees, and foragers in the colony.

3. Not so curious – bees show common characteristics of aging

In the field, foragers experience sources of extrinsic mortality, such as predation and adverse environmental conditions that nurse bees do not typically encounter. That task performance within a well-protected nest environment can extend life span through reduced extrinsic mortality, however, is not surprising and hardly appealing to aging researchers. Consequently, an initial question to ask is, whether intrinsic functional decline (senescence) can be detected in the shadow of the numerous external hazards of foraging.

That senescence contributes to overall forager mortality is, indeed, supported by a recent study that shows an exponential mortality increase in bees after several days of natural foraging activity. While all forager age groups experience constant, age-independent mortality rates, mortality was more or less constantly low until day 10, but thereafter increased steeply to almost 100% already after 18 days of foraging [12].

A growing number of functional studies have also detected senesced phenotypes, which show common characteristics transferable to other models of aging. Aging often is accompanied by a decreased immune response and increased frailty when exposed to pathogens [13]. While insects do not exhibit the vertebrate's acquired T-cell response, they rely on innate immune mechanisms that include specialized blood cells (hemocytes) as a defense against invading microorganisms. Counts of functional hemocytes are high in nurse bees, while the transitions to foraging tasks is associated with a change to deformed, apoptotic immune cells [14]. Remarkably, the number of functional hemocytes is restored along with hemolymph clearance-ability, if foragers revert to nurse tasks in the nest [15]. This finding shows that senesced phenotypes can recover through changes of social cues related to sibling interactions.

Senescence is not exclusive to foragers. Age-associated frailty is detected in over-aged nurse bees (30–50 days old), which are characterized by increased sensitivity to starvation, thermal and oxidative stress [10]. Yet, nurse bees are usually chronologically younger than foragers, and the association between behavior and aging is best tested if nurse and forager bees with the same chronological age are compared. Such experiments demonstrate that starvation resistance is reduced in foragers compared to nurse bees (Speth, M., Amdam, G.V.A., unpublished), and that flight capacity declines in old foragers [16].

Research on honey bee learning and memory was begun decades ago and established a large number of laboratory test paradigms [17]. Two such studies controlled for the chronological age of workers to test whether learning senesces as a function of social role [18,19]. Indeed, age-associated deficits in olfactory and tactile learning were specific to foragers, appearing 15 days after foraging onset. This functional decline also emerges in post-winter bees, implying that bees show a general progression of aging when *diutinus* workers are induced to segregate into nurse bees and foragers (Münch, D., Kreibich, C.D., Amdam, G.V., unpublished). Although post-*diutinus* bees are many months old, deficits in learning performance still requires 15 days of foraging to develop. This might suggest that the *diutinus* life-stage (survival through winter) does not confer a cost on post-*diutinus* function or survival (performance in spring).

Taken together, studies on mortality, stress resilience, and brain function corroborate that rates of aging accelerate about 15 days after workers begin foraging. This pattern is seemingly independent of substantial chronological age differences between foragers, which spent a variable amount of time on nest activities before starting to forage – from a couple of weeks (nurse bees) to many months (*diutinus* bees).

4. Unresolved aspects of worker aging

While all the afore mentioned studies draw a fairly coherent picture that points to foraging as the major determinant of aging, there are unsettled aspects of honey bee senescence that should be addressed further. Among worker bees, the highest levels of protein carbonylation, a marker of oxidative stress damage, are found in forager brains during summer and not in brains from chronologically much older winter bees [20]. However, the more exceptional longevity of honey bee queens (several years) is not associated with higher expression of genes for antioxidant enzymes [21]. This finding seemingly conflicts with predictions of the free radical theory of aging (e.g., [22]), or, alternatively, might imply that other mechanisms of oxidative stress defense are in play in the bee.

Further, in comparison to foragers, nurse bees survive longer under starvation stress, have larger nutrient stores, and genes that are central to energy metabolism are up-regulated in nurse bee brains [23]. Yet, high nutrient availability in animals is typically associated with higher activity of nutrient-sensing genes that are part of insulin/insulin-like growth factor signaling (IIS) [24]. IIS, however, appears to be up-regulated in foragers – perhaps particularly in their brains [23] – despite having the lowest nutrient state of honey bee workers. The latter is communicated also by their increased sensitivity to starvation stress (see above). Associations between intrinsic stores of proteins and lipids, nutrient sensing/IIS and survival in honey bees, therefore, appear to be partly at odds with findings in other laboratory animals (see Section 5).

5. Evolution of honey bee aging and rejuvenation

While symptoms of age-associated functional decline in honey bee immunity, stress resistance, flight- and learning ability are not unlike what is observed in other animal systems, the plasticity of worker aging and its link to social environment can elucidate unique questions of senescence. For example, how can social environment cause very similar genotypes (sisters) to display distinct and dissimilar aging phenotypes? One mechanism, clearly, is the behavioral schedule of task performance that emerges through the interaction of siblings. Yet, this answer is not complete: it does not address explicitly how social interactions mediate physiological changes that are life-shortening or life-promoting, nor does it explain how honey bee life-history evolution shaped the link between sibling interaction and aging patterns.

Established evolutionary theories have provided complementary explanations for why aging causes deterioration and mortality increase, despite no apparent benefit on the level of a single individual. The "mutation accumulation" theory [25] posits that deleterious mutations during late life (post reproduction) are opposed only by weak selection force, and therefore can accumulate. Late-life genetic deterioration is not selected against because successful reproduction has already been achieved and extrinsic mortality risk may also cause most animals to die before adverse mutations can take effect. Another theory, "antagonistic pleiotropy", considers that many genes affect multiple traits. Some traits may be beneficial during early life and thus selectively advantageous, but the same trait or another trait influenced by the same gene may be detrimental during old age when selection for survival is weaker. Fitness benefits in early life, consequently, may largely outweigh negative selection forces caused by deleterious effects of the same gene network later in life [26]. While both theories provide reasoning for deterioration in post-reproductive solitary individuals, they were not originally conceptualized to incorporate a fundamental characteristic of honey bee biology, which is resource transfer between non-reproductive relatives.

Another evolutionary aging theory on "disposable soma" [27] assumes an optimal resource allocation between reproduction and somatic maintenance, and was proposed as a better explanatory framework for honey bee aging [6]. Arguing largely on the level of colony reproduction, the authors modeled the dynamics of changing protein levels as "resource differences" between worker bees, and suggested that protein stores could shape senescence. Nurses, and particularly *diutinus* bees, have larger intrinsic protein stores and, thus, hold (embody) more resources to aid colony-level fitness than foragers [28]. The more intrinsic resources that are associated with a behavior, accordingly, the less disposable will the respective individual be, and the stronger is selection for survival during that life-stage. This approach was improved by the "social transfer" theory of Lee [29] that explained how selection for survival can act on the level of resources embodied by the individual. Social transfer theory demonstrates how resource levels and extrinsic mortality can determine the remaining lifetime transfers each individual can make to others in a social group [29]. When much lifetime resource transfers remain, selection on longevity is strong. Selection for survival decreases as resources are expended over the life-course, and when extrinsic mortality risk increases with age – because less lifetime resource transfers remain as individuals grow older. These considerations can be particularly relevant for understanding aging patterns in social species with elaborate care-behavior toward offspring [30].

How did associations between lifetime resource transfers and aging evolve in worker bees? Three insights allow us to suggest how selection acted on worker survival to improve colony fitness [31]. First, the largest amounts of resources, in the form of nutrient stores, are found in the longest-lived phenotype among all colony members, the reproductive queen [28]. Nutrient-associated benefits to queen fecundity and survival sustain colony integrity, and increase fitness by facilitating repeated production of reproductives and swarms [7]. Second, only one worker phenotype, the *diutinus* bees, can accumulate queen-like nutrient levels [28,32]. *Diutinus* bees may also achieve almost queen-like lifespans (1 year) but are specific to honey bee subspecies that live in temperate climates [33]. Third, honey bee colony organization, likely including queen longevity, did not originate in temperate climates but in subtropical Africa – where worker bees are still short-lived [34]. These insights lead to the hypothesis that survival-promoting traits associated with nutrient storage in queens evolved to become conditionally expressed also in workers when honey bees migrated north to temperate climates [33]. During temperate winter, replacement of dead workers through brood rearing is restricted or unfeasible. At the same time, colonies can only sustain a limited drop in the worker population. Extension of worker lifespan in the absence of brood, thereby, was a required adaptation for the bees' colonization of Europe. The associated colony context (no brood) provided a conditional cue to release expression of extended lifespans in workers, but also, this social condition requires workers to postpone caregiving. In consequence, their lifetime resource transfers to others remain largely unchanged until the next favorable period – providing the link between transfers and aging.

6. Vitellogenin – the key in our hands?

Evolutionary theory on resource transfers, as outlined in the previous paragraph, became an excellent example for how theory can guide molecular studies on mechanisms that could confer the association between care-behavior and survival in honey bees. One of the best-studied candidates for regulation of honey bee aging is vitellogenin. This phosphoglycolipo-protein is a female-specific egg yolk precursor that is common to most oviparous animals. Vitellogenin is synthesized in the liver of vertebrates and in the fat body of insects prior to egg-formation [35]. For honey bees, however, this generalization is at odds with the presence of vitellogenin in workers that do not lay eggs. Behavioral-specific expression of vitellogenin in worker bees first lead to the hypothesis that this protein was co-opted by natural selection to serve alternative purposes in division of labor [36]. Later, vitellogenin was also proven to be a promising candidate for linking resource transfer during sib care with aging disparities.

To provide this link, the utilization of vitellogenin must meet the following requirements:

- *Social transfers:* Vitellogenin constituents are transferred during sibling interaction.

– *Resource allocation:* Vitellogenin is a signal of general adiposity and nutrient storage.
– *Survival:* Vitellogenin has a positive influence on longevity.

6.1. Social transfers

The role of vitellogenin in worker bees was long ambiguous. Making up 30–50% of the hemolymph protein fraction, vitellogenin is the most abundant blood protein in nurse bees [37]. To test if vitellogenin or vitellogenin constituents are transferred between siblings, ^{14}C labeled vitellogenin was injected into worker blood [38]. After 12 h, ^{14}C activity was found in jelly and also was traced to larvae and other bees. These data suggest that jelly is the major vehicle through which amino acids derived from vitellogenin are allocated to foster siblings.

6.2. Resource allocation

The use of vitellogenin in jelly but also in somatic maintenance results in a colony-level tradeoff between the production larvae and new adults versus the retention of life-promoting intrinsic resources in mature nurse bees [6,30].

6.3. Survival

Retention of vitellogenin has a positive influence on worker survival by inhibiting the behavioral transition to the life-shortening foraging stage, as well as by promoting immunity and stress resilience (for review see [39], and further details below).

Fig. 1 illustrates how vitellogenin, via social transfers and resource allocation can shape the survival capacity of worker bees: during the summer, both nurse bees (high survival capacity, slow aging) and foragers (low survival capacity, rapid aging) engage in provisioning. Nurse bees require the pollen resource from foragers for the synthesis of vitellogenin (green lightning bolt next to bee icons in Fig. 1), and jelly for transfer to larvae, callow nest bees, and mature foragers. The amino acids in pollen are only accessible to nurse bees because all the recipient groups lack digestive enzymes to extract nutrients from pollen grains [40]. Consequently, nurses are net donors of "protein active" food jelly (bold arrows in Fig. 1). In parallel, they have the most capacious internal protein reservoirs of summer bees with hypertrophied fat bodies and HPG (filled red circles superimposed on bee icons) conferring their higher survival capacity. With foraging, vitellogenin synthesis ceases, the HPG as well as fat body atrophy, body stores of protein and lipids are drastically reduced (shown as small symbols in Fig. 1) and bees become frail net receivers of jelly (for review see [9]). The worker phenotype with the highest survival capacity is the *diutinus* stage that develops in the absence of brood [6]. According to the theory of Lee [29], these workers postpone their remaining lifetime transfers to the next favorable period – and aging is similarly delayed (see above).

6.4. Vitellogenin functional versatility and aging

Vitellogenin appears to be the major zinc-carrier of honey bee hemolymph, where lack of zinc triggers hemocyte pycnosis and reduced immunity. Reduced vitellogenin levels, thereby, may cause immune senescence in foragers [14,15]. Three lines of evidence established the role of honey bee vitellogenin also as an antioxidant [31]. Vitellogenin levels vary naturally between individuals, and high hemolymph titers are positively correlated with survival

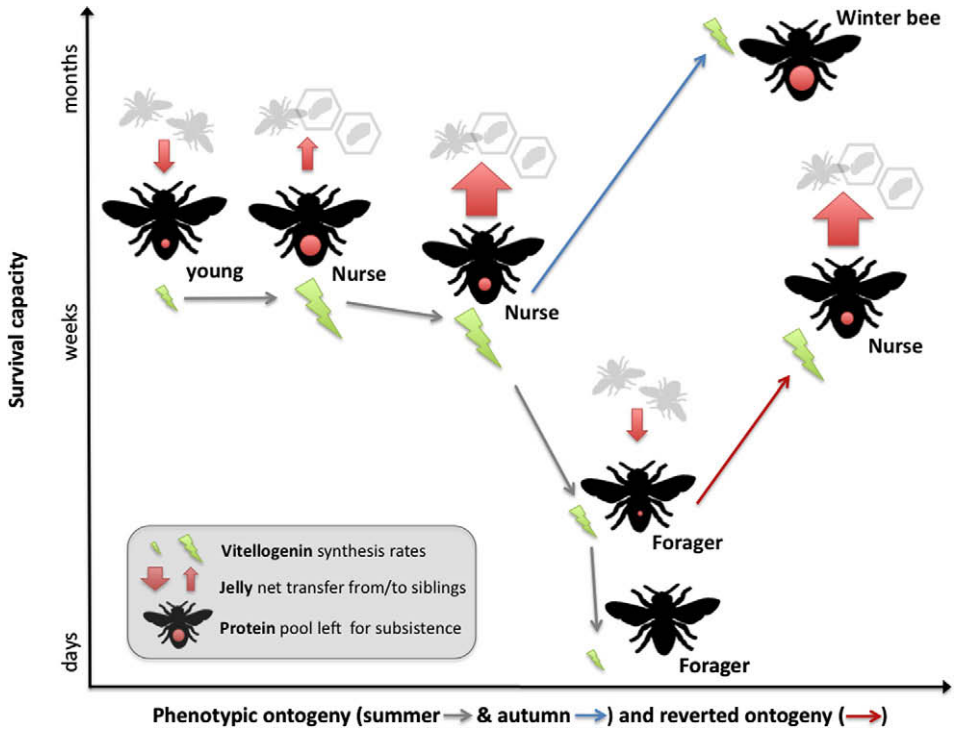

Fig. 1. Phenotypes with different life expectancies emerge through social transfers and resource allocation between siblings (schematic depiction). Protein currency is transferred as jelly, where constituents of the yolk precursor vitellogenin are included. Larvae, callow nest bees ("young") and mature foragers are among the net receivers of jelly (bold red arrows). Nurse bees, with an intermediate average life expectancy ("survival capacity", *y*-axis) constantly produce large amounts of vitellogenin (indicated by the larger green lightning bolts) in their abdominal fat bodies and transfer jelly to the net receivers. In contrast, the net receivers have low nutrient stores (indicated by smaller filled red circles) and show low levels of vitellogenin production (smaller green lightning bolts). Accordingly, foragers also represent the shortest-lived phenotype that usually dies after some days (*y*-axis). However, if reverting to nurse tasks (slim red arrow), foragers can recover characteristics of the phenotype with an intermediate survival capacity. When brood load declines in autumn, bees transition to the long-lived winter state (slim blue arrow). While both, nurse and winter bees, have considerable vitellogenin storages (larger filled red circles), winter bees display greatly reduced sib care transfers and outlive nurse bees in summer by several months. These dynamics suggests that vitellogenin is the major currency of honey bee survival.

after inducing oxidative stress. Likewise, RNAi knockdown of the *vitellogenin* gene results in poorer survival after oxidative stress insult. Lastly, vitellogenin is strongly carbonylated by paraquat, an oxidative stress agent, suggesting that vitellogenin scavenges oxidants. These positive effects are perhaps not limited to honey bees. Studies in nematode (*Caenorhabditis elegans*) [41] and fish (*Anguilla japonica*) [42] support antioxidant activity of vitellogenin. Concerning host immune defense too, vitellogenin from different fish species was recently shown to be involved in recognition and also in the killing of bacterial pathogens (e.g., [43]).

Vitellogenin, furthermore, may modulate longevity as a signaling molecule. *Vitellogenin* knockdown reduces life span in part by inducing an earlier shift from nursing to the short-lived forager stage [44]. Although not being a classical endocrine factor, vitellogenin suppresses juvenile hormone [45], a life-shortening and aging-promoting hormone in insects [46]. Reciprocally, juvenile hormone application suppresses vitellogenin and induces precocious foraging in worker bees, an effect similar to that observed when *vitellogenin* expression is directly inhibited [47]. Worker division of labor and resulting longevity variation, thereby, is influenced by a mutual repressor feedback between vitellogenin and juvenile hormone [48]. A study on queens [49] as well as gene mapping of worker behavior [50] also suggests that the vitellogenin – juvenile hormone system interacts with another well-known lifespan regulating pathway, IIS (see also Section 7).

As of yet, however, the functional versatility of vitellogenin poses two challenging questions, which provide the road map for forthcoming research:

- What are the structural aspects of vitellogenin's functional diversity?
- How is vitellogenin function associated with localization in different tissues?

6.5. Structural aspects

A causal link between honey bee vitellogenin levels and immunity is implicated by its zinc binding capacity (see above, [51]), while in other systems, destabilized zinc bindings also can cause dysfunction of vital enzymes, such as Cu, Zn superoxide dismutase [52]. Comparative studies on insect vitellogenins show that yolk precursors undergo substantial structural modifications, such as glycosylation, phosphorylation, proteolytic cleavage and lipidation, to facilitate carbohydrate and lipid transport [53]. Vitellogenins are part of a multigene family that includes insect apolipophorins and human apolipoprotein B. These phospholipoglycoproteins affect lipid profiles, which – in humans – play a key role in emergence of age related cardiovascular disease [54]. Aiming to explain the functional diversity from lipid transport to signaling, we recently began to study how vitellogenin can fragment into two different domains that host the lipid cavity versus the binding site for the vitellogenin receptor, respectively (Havukainen, H., Halskau, Ø., Martinez, A., Amdam, G.V., unpublished).

6.6. Localization

To date, honey bee vitellogenin has been confirmed to be present in compartments that more or less directly serve reproduction, brood care, and transport purposes. These include the ovary, fat body, HPG and hemolymph [49]. Yet, due to the inherently random nature of age deterioration, one would argue that all survival-critical organs should have access to a potentially vital, anti-aging molecule. Within the body there are various obstacles to metabolite and body fluid exchange, with the blood-brain barrier being the most prominent. A direct role for vitellogenin in protecting brain function would, thus, be difficult to envision. New anatomical data, however, supports vitellogenin to be present also in the brain (Fig. 2b; Münch, D., Kreibich, C.D., Amdam, G.V.; unpublished). Transcript profiling, furthermore, suggests a compensatory effect in brain tissue, such that vitellogenin is up-regulated in the brain compartment when levels are low in other parts of the body (Fig. 2c; Ihle, K., Amdam, G.V.; unpublished). The interface between abdominal, head and in particular brain compartments (Fig. 2c), therefore, deserves more attention in research on vitellogenin. First, interdependent metabolite (vitellogenin) dynamics in different tissues including the brain may improve our understanding of social behavior. Second, tissue-specific studies may allow us to model main routes of mammalian age related dysfunction. These include changing brain lipid profiles, the emergence of vascular diseases, as well as the well-established aging modulation by IIS and growth factor signaling [55].

7. Care related factors and the building blocks of functional pleiotropy – a quite common theme

Not unlike sib care in honey bees, many species express facets of maternal care behavior during specific phases of the reproductive process, which affects the life-history traits of a care giver in various ways. A level of pleiotropic regulation between reproductive physiology and behavior, therefore, is likely a common feature in animals. Pleiotropy can be seen, e.g., in solitary insects, where ovarian signals (such as ecdysteroid hormones) act together with the systemic juvenile hormone to modulate yolk precursor synthesis and egg-formation, but also affect behavioral and sensory states when individuals shift between different stages of reproductive development [56]. For example, the major endocrine factors are sensitive to changes in environment and nutrition, and influence life-history transitions such as the switch from nectar to blood-host foraging that is required for egg-formation in female *Culex nigripalpus* mosquitoes [57], as well as the shift from feeding and sexual behavior to fasting and parental activity in the earwig *Labidura riparia* [58]. In honey bee workers, similar life-history traits co-vary with ovary size that is quantified as the number of ovarian filaments (called ovarioles) in each organ – usually 2-14. This correlation between reproductive anatomy and behavior has been taken to support the hypothesis that workers express maternal traits, which in the context of colonies culminate in sib care [36].

Worker life-history progression, including the phenotypic ontogeny from nest to field tasks, has been mapped to four highly epistatic quantitative trait loci (QTL), *pln1-4* [59–62]. This QTL architecture influences the timing (age) of a bee's shift from nurse tasks to foraging, and has pleiotropic effects on reproductive anatomy (ovary size) but also on behavioral features such as sucrose responsiveness and food-related behavior toward pollen. Positional candidate genes for the genetic network underlying the four *pln* QTL have been proposed based on sequence information from the Honey Bee Genome Sequencing Consortium [50]. These regions are highly enriched with genes involved in the nutrient sensing IIS pathway. Together with the TOR (target of rapamycin), this pathway is active in the central (neural) and peripheral (non-neural) tissues of eukaryotes and regulate metabolic responses to food-intake, including growth, reproduction, and lifespan [63,64]. In general, inhibition of insulin/TOR signaling reduces fecundity, boosts survival capacity, and changes body-fat storage in model animals [55,65]. Follow-up studies that associate the transcript level of the honey bee positional candidate genes with size of ovaries and variation in behavior toward pollen, identified responses of the phosphoinositide 3-kinase encoding gene *PDK1* (in *pln3*), and the insulin receptor substrate (IRS) (in *pln4*) [66].

PDK1 is a central nutrient-sensing gene downstream of both TOR and IIS, where *IRS* is a central element (see below). In

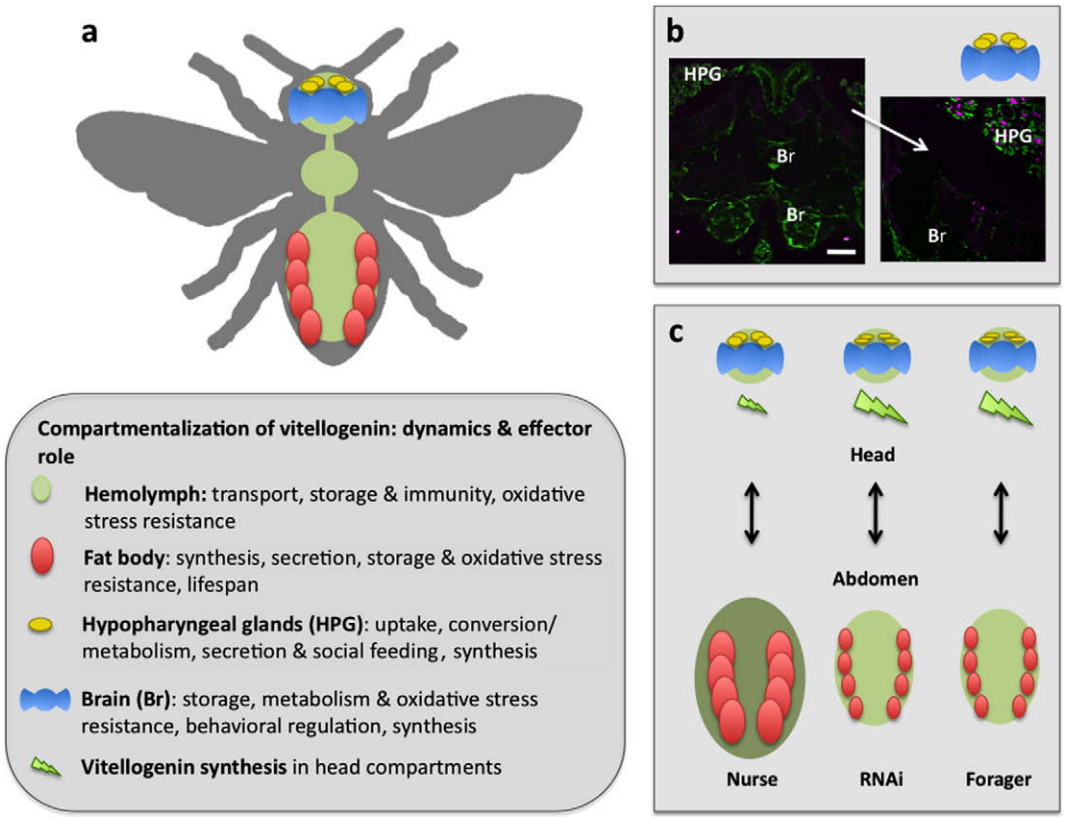

Fig. 2. Vitellogenin – compartmentalization, dynamics and survival promoting effects. Vitellogenin is generally localized in organs that are involved in reproduction, but in honey bee workers the protein is primarily associated with care behavior between siblings (social transfer, see Fig. 1). Compartments important for care behavior are the fat body, hypopharyngeal glands (HPG) and hemolymph (a). The text box in (a) lists vitellogenin related functions for the different compartments. As of yet it is unclear how the brain benefits from vitellogenin (depicted by "?" in (a)). However, recent data suggest the presence of vitellogenin in unexpected locations, such as in various parts of the brain. The confocal microscopic images in (b) show positive anti-vitellogenin immunoreactivity (green, nuclear labeling by DAPI, purple) in the brain and head glands (HPG) of nurse bees (Münch D, Kreibich CD, Amdam, GV, unpublished). Scale bar = 200 μm. Knock down of *vitellogenin* expression by RNA interference (RNAi) supports a regulatory interaction between the brain and the remaining body (c, schematic depiction of Ihle K, Amdam GV; unpublished). In nurse bees (left) with larger abdominal fat body (large red icons), the abdominal *vitellogenin* expression is high compared to foragers. (The difference in fat body *vitellogenin* expression is indicated by a darker green color of the large circular icon for nurse bees, left, as compared to a lighter green color and smaller size for the similar icon for foragers, right.) However, in forager brain tissue (upper portion of the figure), *vitellogenin* RNA levels are higher than levels measured in nurse bee brains. (The different *vitellogenin* RNA levels for brain tissue are again indicated by a different size of the green lightning bolts). Similarly, *vitellogenin* knockdown, induces down-regulation of *vitellogenin* RNA levels in nurse abdominal compartments (small red fat body and light green circular icons) but a likely compensatory up-regulation in brain tissue (compare lightning bolts between "RNAi", middle, and "Nurse", left).

concurrence, RNAi mediated gene repression of *IRS*, *TOR* or both in honey bee larvae causes queen-destined individuals to develop worker traits: queens can only emerge after abundant larval feeding, while restricted diet in early life results in workers [67]. The role of nutrient sensing in this extreme reproductive differentiation of queens (up to 300 oocyte-producing ovarioles) and workers (2–14 largely quiescent ovarioles) has lead to the hypothesis that varying larval nourishment (quantity, quality and frequency of feeding) can affect the worker phenotype as well [68]. Thereby, differences in sib care behavior by adult bees can affect the development of larvae, not only toward worker or queen fate but also as a source of worker phenotypic variability. Worker larvae that receive a bit more food than average would emerge with a larger ovary, an elevated responsiveness to sucrose, and a bias toward pollen foraging behavior as mature adults. In this way, sib care can change phenotypic predisposition.

Relationships between IIS, worker behavioral variation and vitellogenin are also supported [69]. When *IRS* is suppressed by RNAi in adult bees, workers show a change in behavior that is conditional on their level of vitellogenin: If *IRS* is suppressed in a low vitellogenin background, then foragers reduce their carbohydrate (nectar) loading but continue to collect pollen quantities as before. If *IRS* is suppressed in a high *vitellogenin* background, on the other hand, then foragers also reduce their nectar loading. Yet, in parallel they significantly increase their loading of pollen. It has been shown before that *vitellogenin* expression biases workers to collect protein-rich food [44], and it has been suggested that vitellogenin affects life-history and aging by interacting with IIS-transduction [49,50]. The data from the *IRS* RNAi experiments are consistent with this hypothesis.

The IIS signaling cascade is initiated when insulin/insulin-like peptides (ILP) bind with the insulin receptors at the cell surface, leading to phosphorylation of IRS inside the plasma membrane, and to subsequent activation of second messengers in the cell. While vitellogenin is the most heavily phosphorylated protein in honey bee hemolymph and fat body (Havukainen, H., Halskau, Ø., Wolschin, F., Amdam, G.V., unpublished) it is difficult to envision how vitellogenin could affect IRS phosphorylation and downstream events. As to the alternative point of interference, the upstream signaling initiation: two *ILP* encoding genes as well as *IRS* are expressed in honey bee fat body but none of these transcripts are negatively correlated with *vitellogenin*. Instead, one of the *ILP* encoding genes *ILP1* may increase in response to juvenile hormone or foraging [23,49] while the other, *ILP2*, increase in response to amino acids – which also upregulate vitellogenin [70]. These and other associations lead us to propose that *ILP2* may communicate nutrient surplus and connect positive vitellogenin status to reduced IIS-transduction as an antagonist of honey bee insulin

receptors [70]. In summary, the understanding of the influence of vitellogenin on IIS in honey bees is incomplete, but the prospect of understanding how negative life span influences of nutrient signaling can be reversed by the sib care factor vitellogenin is highly interesting [49,31,71].

8. Conclusion

Aging research is application-oriented. Yet, history of science exemplifies how studies of curious cases shaped our understanding of fundamental processes. Studies of senescence often refer to a "healthy range" for age influencing factors: reasonable exercise is beneficial, excessive exercise can result in wear and tear. This balance is a common and generic theme in aging, and is present in bee as well. However, in highly social insects these aspects of survival are contrasted by an extreme diversification of aging phenotypes that is linked to sibling interactions. Such multimodal aging patterns not only challenge assumptions on aging as a "fixed fate", but also indicate how manipulation of social interactions can be instrumental in influencing life outcomes.

Unfavorable genetic and epigenetic predispositions in IIS signaling and lipid metabolism correlate with negative influences also on human lifespan. Vitellogenin represents a sib care related molecule with the potential to drive these well-established aging modulators in honey bees. Studying potentially generic cause-effect relationships by redirecting pleiotropic networks that modulate care behavior, nutritional profile and aging, thus, can be relevant to modern gerontology.

Acknowledgments

Gro V. Amdam was supported by the Research Council of Norway (#175413, 180504, 185306 and 191699), the National Institute on Aging (NIA P01 AG22500), the PEW Charitable Trust, and the Wissenschaftskolleg zu Berlin.

References

[1] Keller, L. and Jemielity, S. (2006) Social insects as a model to study the molecular basis of ageing. Exp. Gerontol. 41, 553–556.
[2] Garland Jr., T. and Kelly, S.A. (2006) Phenotypic plasticity and experimental evolution. J. Exp. Biol. 209, 2344–2361.
[3] Eaves, L. (1976) A model for sibling effects in man. Heredity 36, 205–214.
[4] Laviola, G. and Alleva, E. (1995) Sibling effects on the behavior of infant mouse litters (*Mus domesticus*). J. Comp. Psychol. 109, 68–75.
[5] Schwabl, H., Mock, D. and Gieg, J. (1997) A hormonal mechanism of parental favouritism. Nature 386, 231.
[6] Amdam, G.V. and Omholt, S.W. (2002) The regulatory anatomy of honeybee lifespan. J. Theor. Biol. 216, 209–228.
[7] Winston, M.L. (1987) The Biology of the Honey Bee, Harvard University Press, Cambridge, Massachusetts.
[8] Free, J.B. (1965) The allocation of duties among worker honeybees. Zool. Soc. Lond. 14, 39–59.
[9] Crailsheim, K. (1990) The protein balance of the honey bee worker. Apidologie 21, 417–429.
[10] Remolina, S.C., Hafez, D.M., Robinson, G.E. and Hughes, K.A. (2007) Senescence in the worker honey bee *Apis mellifera*. J. Insect Physiol. 53, 1027–1033.
[11] Robinson, G.E., Page, R.E., Strambi, C. and Strambi, A. (1992) Colony integration in honey bees: mechanisms of behavioral reversion. Ethology 90, 336–348.
[12] Dukas, R. (2008) Mortality rates of honey bees in the wild. Insect. Soc. 55, 252–255.
[13] DeVeale, B., Brummel, T. and Seroude, L. (2004) Immunity and aging: the enemy within? Aging Cell 3, 195–208.
[14] Wille, H. and Rutz, W. (1975) Beziehungen zwischen Juvenilhormontiter und Hämozyten erwachsener Sommerbienen (*Apis mellifera* L.). Schweiz. Landwirtschaft. Forsch 14, 339–353.
[15] Amdam, G.V., Aase, A.L., Seehuus, S.C., Kim Fondrk, M., Norberg, K. and Hartfelder, K. (2005) Social reversal of immunosenescence in honey bee workers. Exp. Gerontol. 40, 939–947.
[16] Vance, J.T., Williams, J.B., Elekonich, M.M. and Roberts, S.P. (2009) The effects of age and behavioral development on honey bee (*Apis mellifera*) flight performance. J. Exp. Biol. 212, 2604–2611.
[17] Giurfa, M. (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J. Comp. Physiol. A 193, 801–824.
[18] Behrends, A., Scheiner, R., Baker, N. and Amdam, G.V. (2007) Cognitive aging is linked to social role in honey bees (*Apis mellifera*). Exp. Gerontol. 42, 1146–1153.
[19] Scheiner, R. and Amdam, G.V. (2009) Impaired tactile learning is related to social role in honeybees. J. Exp. Biol. 212, 994–1002.
[20] Seehuus, S.C., Krekling, T. and Amdam, G.V. (2006) Cellular senescence in honey bee brain is largely independent of chronological age. Exp. Gerontol. 41, 1117–1125.
[21] Corona, M., Hughes, K.A., Weaver, D.B. and Robinson, G.E. (2005) Gene expression patterns associated with queen honey bee longevity. Mech. Ageing Dev. 126, 1230–1238.
[22] Harman, D. (1981) The aging process. Proc. Natl. Acad. Sci. USA 78, 7124–7128.
[23] Ament, S.A., Corona, M., Pollock, H.S. and Robinson, G.E. (2008) Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. Proc. Natl. Acad. Sci. USA 105, 4226–4231.
[24] Ikeya, T., Galic, M., Belawat, P., Nairz, K. and Hafen, E. (2002) Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Curr. Biol. 12, 1293–1300.
[25] Medawar, P.B. (1952) An Unsolved Problem of Biology, H.K. Lewis, London.
[26] Williams, G.C. (1957) Pleiotropy, natural selection and the evolution of senescence. Evolution 11, 398–411.
[27] Kirkwood, T.B.L. (1977) Evolution of ageing. Nature 270, 301–304.
[28] Engels, W. and Fahrenhorst, H. (1974) Alters- und kastenspezifische Veränderungen der Haemolymph-Protein-Spektren bei *Apis mellificia*. Roux's Arch. 174, 285–296.
[29] Lee, R.D. (2003) Rethinking the evolutionary theory of aging: Transfers, not births, shape senescence in social species. Proc. Natl. Acad. Sci. USA 100, 9637–9642.
[30] Amdam, G.V. and Page Jr., R.E. (2005) Intergenerational transfers may have decoupled physiological and chronological age in a eusocial insect. Ageing Res. Rev. 4, 398–408.
[31] Seehuus, S.C., Norberg, K., Gimsa, U., Krekling, T. and Amdam, G.V. (2006) Reproductive protein protects functionally sterile honey bee workers from oxidative stress. Proc. Natl. Acad. Sci. USA 103, 962–967.
[32] Fluri, P., Lüscher, M., Wille, H. and Gerig, L. (1982) Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. J. Insect Physiol. 28, 61–68.
[33] Amdam, G.V., Norberg, K., Omholt, S., Kryger, P., Lourenco, A.P., Bitondi, M.M.G. and Simoes, Z.L.P. (2005) Higher vitellogenin concentrations in honey bee workers may be an adaptation to life in temperate climates. Insectes Sociaux 52, 316–319.
[34] Ruttner, F. (1988) Biogeography and Taxonomy of Honeybees, Springer-Verlag, Berlin.
[35] Byrne, B.M., Gruber, M. and Ab, G. (1989) The evolution of egg yolk proteins. Prog. Biophysiol. Mol. Biol. 53, 33–69.
[36] Amdam, G.V., Csondes, A., Fondrk, M.K. and Page, R.E. (2006) Complex social behavior derived from maternal reproductive traits. Nature 439, 76–78.
[37] Fluri, P., Wille, H., Gerig, L. and Lüscher, M. (1977) Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees (*Apis mellifera*). Experientia 33, 1240–1241.
[38] Amdam, G.V., Norberg, K., Hagen, A. and Omholt, S.W. (2003) Social exploitation of vitellogenin. Proc. Natl. Acad. Sci. USA 100, 1799–1802.
[39] Münch, D., Amdam, G.V. and Wolschin, F. (2008) Ageing in a eusocial insect: molecular and physiological characteristics of life span plasticity in the honey bee. Funct. Ecol. 22, 407–421.
[40] Crailsheim, K. (1986) Dependence of protein metabolism on age and season in the honeybee (*Apis mellifica carnica* pollm). J. Insect Physiol. 32, 629–634.
[41] Nakamura, A., Yasuda, K., Adachi, H., Sakurai, Y., Ishii, N. and Goto, S. (1999) Vitellogenin-6 is a major carbonylated protein in aged nematode, *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. 264, 580–583.
[42] Ando, S. and Yanagida, K. (1999) Susceptibility to oxidation of copper-induced plasma lipoproteins from Japanese eel: protective effect of vitellogenin on the oxidation of very low density lipoprotein. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 123, 1–7.
[43] Li, Z., Zhang, S., Zhang, J., Liu, M. and Liu, Z. (2009) Vitellogenin is a cidal factor capable of killing bacteria via interaction with lipopolysaccharide and lipoteichoic acid. Mol. Immunol. 46, 3232–3239.
[44] Nelson, C.M., Ihle, K., Amdam, G.V., Fondrk, M.K. and Page, R.E. (2007) The gene *vitellogenin* has multiple coordinating effects on social organization. PLoS Biol. 5, 673–677.
[45] Guidugli, K.R., Nascimento, A.M., Amdam, G.V., Barchuk, A.R., Omholt, S., Simoes, Z.L. and Hartfelder, K. (2005) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. FEBS Lett. 579, 4961–4965.
[46] Flatt, T., Tu, M.P. and Tatar, M. (2005) Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. Bioessays 27, 999–1010.
[47] Jaycox, E.R., Skowronek, W. and Guynn, G. (1974) Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of a juvenile hormone mimic. Ann. Entomol. Soc. Am. 67, 529–534.
[48] Amdam, G.V. and Omholt, S.W. (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. J. Theor. Biol. 223, 451–464.

[49] Corona, M., Velarde, R.A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K.A. and Robinson, G.E. (2007) Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc. Natl. Acad. Sci. USA 104, 7128–7133.
[50] Hunt, G.J. et al. (2007) Behavioral genomics of honeybee foraging and nest defense. Naturwissenschaften 94, 247–267.
[51] Amdam, G.V., Simoes, Z.L., Hagen, A., Norberg, K., Schroder, K., Mikkelsen, O., Kirkwood, T.B. and Omholt, S.W. (2004) Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. Exp. Gerontol. 39, 767–773.
[52] Ding, F. and Dokholyan, N.V. (2008) Dynamical roles of metal ions and the disulfide bond in Cu, Zn superoxide dismutase folding and aggregation. Proc. Natl. Acad. Sci. USA 105, 19696–19701.
[53] Tufail, M. and Takeda, M. (2008) Molecular characteristics of insect vitellogenins. J. Insect Physiol. 54, 1447–1458.
[54] Brandt, B.W., Zwaan, B.J., Beekman, M., Westendorp, R.G.J. and Slagboom, P.E. (2005) Shuttling between species for pathways of lifespan regulation: a central role for the vitellogenin gene family? Bioessays 27, 339–346.
[55] Tatar, M., Bartke, A. and Antebi, A. (2003) The endocrine regulation of aging by insulin-like signals. Science 299, 1346–1350.
[56] Simonet, G., Poels, J., Claeys, I., Van Loy, T., Franssens, V., De Loof, A. and Broeck, J.V. (2004) Neuroendocrinological and molecular aspects of insect reproduction. J. Neuroendocrinol. 16, 649–659.
[57] Hancock, R.G. and Foster, W.A. (2000) Exogenous juvenile hormone and methoprene, but not male accessory gland substances or ovariectomy, affect the blood/nectar choice of female *Culex nigripalpus* mosquitoes. Med. Vet. Entomol. 14, 373–382.
[58] Vancassel, M., Foraste, M., Strambi, A. and Strambi, C. (1984) Normal and experimentally induced changes in hormonal hemolymph titers during parental behavior of the earwig *Labidura riparia*. Gen. Comp. Endocrinol. 56, 444–456.
[59] Hunt, G.J., Page, R.E., Fondrk, M.K. and Dullum, C.J. (1995) Major quantitative trait loci affecting honey-bee foraging behavior. Genetics 141, 1537–1545.
[60] Page, R.E., Fondrk, M.K., Hunt, G.J., Guzman-Novoa, E., Humphries, M.A., Nguyen, K. and Greene, A.S. (2000) Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. J. Heredity 91, 474–479.
[61] Rueppell, O., Pankiw, T., Nielson, D., Fondrk, M.K., Beye, M. and Page, R.E. (2004) The genetic architecture of the behavioral ontogeny of honey bee workers. Genetics 167, 1767–1779.
[62] Rueppell, O., Pankiw, T. and Page, R.E. (2004) Pleiotropy, epistasis and new QTL: the genetic architecture of honey bee foraging behavior. J. Heredity 95, 481–491.
[63] Oldham, S. and Hafen, E. (2003) Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. Trends Cell Biol. 13, 79–85.
[64] Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J. and Leopold, P. (2003) A nutrient sensor mechanism controls *Drosophila* growth. Cell 114, 739–749.
[65] DiAngelo, J.R. and Birnbaum, M.J. (2009) Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol. Cell Biol. 29, 6341–6352.
[66] Wang, Y., Amdam, G.V., Rueppell, O., Wallrichs, M.A., Fondrk, M.K., Kaftanoglu, O. and Page Jr., R.E. (2009) PDK1 and HR46 gene homologs tie social behavior to ovary signals. PLoS ONE 4, e4899.
[67] Patel, A., Fondrk, M.K., Kaftanoglu, O., Emore, C., Hunt, G. and Amdam, G.V. (2007) The making of a queen: TOR pathway governs diphenic caste development. PLoS ONE 6, e509.
[68] Linksvayer, T.A., Fondrk, M.K. and Page Jr., R.E. (2009) Honeybee social regulatory networks are shaped by colony-level selection. Am. Nat. 173, E99–E107.
[69] Wang, Y., Mutti, N.S., Ihle, K.E., Siegel, A., Dolezal, A.G., Kraftanoglu, O. and Amdam, G.V. (2010) Down-regulation of honeybee IRS gene biases behavior toward food rich in protein. PLoS Genet 6, e1000896.
[70] Nilsen, K.A., Ihle, K.E., Frederick, K., Smedal, B., Fondrk, M.K., Hartfelder, K. and Amdam, G.V. (2008). Dual role of insulin-like peptides in the integration of nutritional state, endocrine signaling and social behavior in honeybee workers. M.S. thesis, Norw Univ Life Sci.
[71] Remolina, S.C. and Hughes, K.A. (2008) Evolution and mechanisms of long life and high fertility in queen honey bees. Age (Dordr) 30, 177–185.