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NEWS AND VIEWS

PERSPECTIVE

Social pleiotropy and the molecular evolution of honey bee vitellogenin

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In this issue of *Molecular Ecology*, Kent *et al.* (2011) describe the adaptive evolution of honey bee vitellogenin that belongs to a phylogenetically conserved group of egg yolk precursors. This glyco-lipoprotein leads a double life: it is central to egg production in the reproductive queen caste, and a regulator of social behaviour in the sterile worker caste. Does such social pleiotropy constrain molecular evolution? To the contrary; Kent *et al.* show that the *vitellogenin* gene is under strong positive selection in honey bees. Rapid change has taken place in specific protein regions, shedding light on the evolution of novel vitellogenin functions.

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Vitellogenin was identified in honey bees about 40 years ago and its reproductive role in queens was instantly recognized (Engels 1974). Queen bees are responsible for egg-laying, but curiously, vitellogenin was also found in considerable amounts in worker bees, which are helper females that normally are sterile. The workers' expression of vitellogenin was initially seen as evolutionary baggage; an unavoidable consequence of selection for extreme rates of vitellogenin synthesis in queens. But later studies revealed pleiotropic functions of the protein (Fig. 1). Vitellogenin can coordinate social behaviour in workers, which expend the protein in larval food, and it enhances stress resistance, immunity and survival in both workers and queens (see (Nelson *et al.* 2007; Seehuus *et al.* 2006) and references therein). While some species have several *vitellogenin* gene copies that may accommodate caste-specific functions, the honey bee has only one *vitellogenin*. Thus, this gene is socially pleiotropic through its influence on several fitness traits that are partly separated in queen and worker castes (Kent *et al.* 2011).

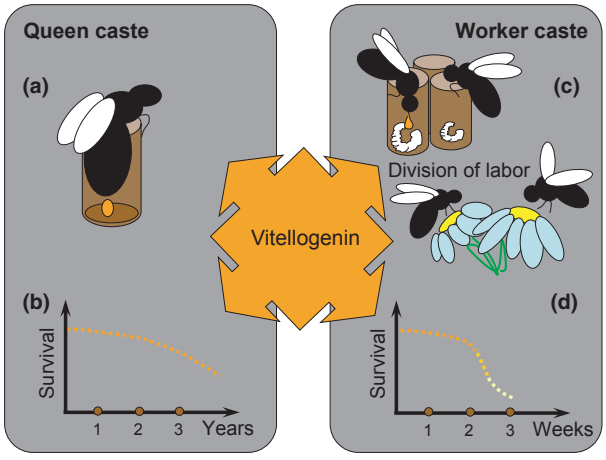

Fig. 1 Socially pleiotropic functions of vitellogenin in the honey bee *Apis mellifera*. In queens (a–b), vitellogenin (orange) is an egg-yolk protein precursor that may also enhance longevity through effects on metabolism and oxidative stress resistance. Queens can survive several years and they maintain constant high blood levels of vitellogenin, even during periods when they do not lay eggs (see (Seehuus *et al.* 2006) and references therein). In workers (c–d), vitellogenin influences social behaviour: Nest workers that care for larvae have high vitellogenin levels and use vitellogenin-derived products in larval food. When the bees' blood levels of vitellogenin decline 2–3 weeks later, the workers switch from caregiving to risky foraging activities. The result is a division of labour between nest and field activities (Nelson *et al.* 2007). The high vitellogenin levels of nest workers have been functionally connected to increased oxidative stress resistance and immunity (Seehuus *et al.* 2006).

It may be difficult to accumulate selectable mutations in a pleiotropic gene while retaining or increasing its fitness contribution to two or more traits. Kent *et al.* (2011) therefore set out to test whether honey bee *vitellogenin* experiences this constraint. They sequenced *vitellogenin* and seven other genes in 41 honey bee workers from Africa, East- and West Europe, where honey bee population sizes, structures and histories are known. With this knowledge base, it is unlikely that changes or differences in population sizes are misinterpreted as signatures of selection in the data. Moreover, the seven additional genes served as reference points that would be equally affected by issues of population dynamics and genetic drift. These included *Erk7* and *for* which are genes that, similar to *vitellogenin*, are expressed in both workers and queens and implicated in worker behaviour (see (Kent *et al.* 2011) for details and

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references). Sequence information from one worker from each of three other bee species added to the dataset.

The high average pair-wise nucleotide differences, linkage disequilibrium and skew in the allele frequency spectrum that Kent *et al.* (2011) estimated from the bees documented allelic variation for *vitellogenin* and suggested recurrent positive selection acting on this gene. Among several statistical approaches, the authors employed McDonald–Kreitman tests to compare the ratio of nucleotide substitutions that have no effect on the protein sequence (synonymous) to those that change the amino acid sequence (nonsynonymous), and flagged relative rates of nonsynonymous versus synonymous divergence as positive selection. The *vitellogenin* gene showed this high relative rate in comparisons between the four bee species of the dataset: the honey bee *Apis mellifera*, Asian honey bee *A. cerana*, giant honey bee *A. dorsata* and dwarf honey bee *A. florea*. The specific analyses of honey bee *vitellogenin*, however, revealed significant linkage disequilibrium only for European populations, and not African. We believe this new finding supports the hypothesis that changes in *vitellogenin* occurred to accommodate colony survival in colder climates during and after the prehistoric migrations of *A. mellifera* from Africa to Europe (Amdam *et al.* 2005; Seehuus *et al.* 2006). Overall, Kent *et al.* (2011) could assign a higher recent and ongoing rate of adaptive protein evolution to *vitellogenin* than the seven reference genes and concluded that social pleiotropy does not constrain honey bee vitellogenin adaptation by selection.

This positive selection on honey bee vitellogenin begs the question of how genetic changes translate to the level of protein structure and function. Vitellogenin is large (180 kDa) and has a complex domain structure. Are there degrees of freedom to sculpt parts of this molecule for novel functions while retaining its conserved role in yolk production? There are examples of much smaller proteins evolving several new features under considerable constraints, for example alpha-lactabumin (a milk protein) evolved from lysozyme into a substrate modifier and secretory nutritional protein while retaining its overall structure (Qasba & Kumar 1997). Kent *et al.* (2011) discuss 64 single nucleotide polymorphisms (SNPs) that are unequally distributed in the *vitellogenin* sequence. Roughly dividing the protein in two parts, the N-terminal domain (N-sheet) is resistant to change, while the major lipid-binding cavity is sprinkled with SNP hotspots (Fig. 2a, b).

The N-sheet contains the phylogenetically conserved, putative receptor-binding domain of vitellogenin that presumably is important for uptake to the ovary (Li *et al.* 2003). This domain can be cleaved off the mature protein (Fig. 2a) in a process that appears to be regulated tissue-specifically in honey bee workers (Havukainen *et al.* 2011). The biological significance of this dynamic is unknown, but current evidence supports the conclusion of Kent *et al.* (2011) that the N-sheet is central to vitellogenin's conserved function in reproduction, while the remaining protein has been freer to evolve.

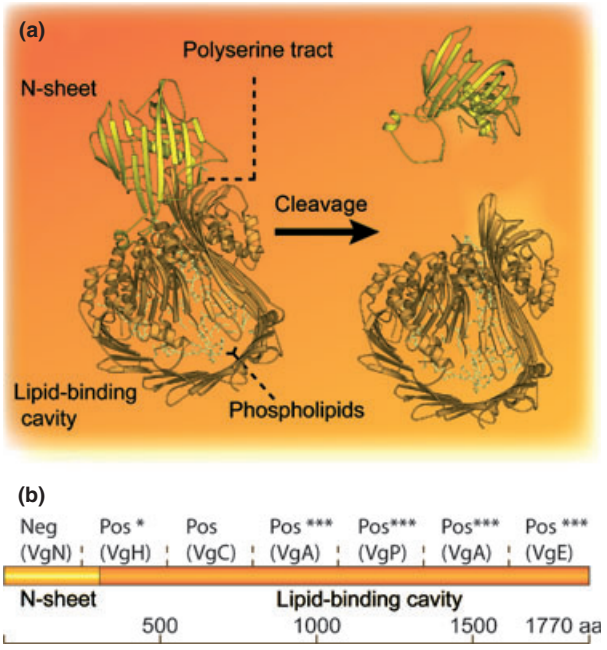

Fig. 2 An illustration of the honey bee vitellogenin protein based on the lamprey lipovitellin X-ray structure (Anderson *et al.* 1998). (a) Vitellogenin's conserved N-sheet can get cleaved from the major lipid binding cavity, forming two independent fragments of unknown function in worker tissues (Havukainen *et al.* 2011). (b) Linear presentation of the 1770 amino acid residues of vitellogenin. The approximate locations of regions under positive or negative selection are adapted from Kent *et al.* (2011), Fig. 1, Table S4).

This remaining part of vitellogenin is dominated by a lipid-binding cavity (Fig. 2a, b) where any structural analysis must proceed with caution. This is because the only available vitellogenin X-ray structure is from a vertebrate (Anderson *et al.* 1998), and insect vitellogenins have features that are missing from this structural template: The most significant is a linker coil (~80 amino acids long) with a tract of serine residues between the N-sheet and the subsequent α-helical domain of the lipid-binding cavity (Fig. 2a) (Tufail & Takeda 2008). If such insect-specific sequence elements are forced into the cavity region of Vg during structural modelling, the positions of amino acids are displaced relative to the conserved domain fold in the cavity. These displacements occur in the homology model by Kent and coworkers. SNP locations are accordingly shifted from their true positions in the tertiary Vg structure, and the model's predictions about specific, physical connections (at 4–5 Å resolution) between individual SNPs and lipids become very difficult to interpret.

This discussion, however, does not detract from the overall conclusion that polymorphisms in the lipid-binding cavity of vitellogenin can alter ligand-binding properties (Kent *et al.* 2011). These properties may explain how

vitellogenin regulates worker behaviour, and SNPs that alter vitellogenin binding- and transport-efficacies could translate into adaptive phenotypic differences. For example, a proposed ligand of vitellogenin is juvenile hormone, which can promote flight behaviour and foraging in worker bees. The binding-affinity of vitellogenin to circulating juvenile hormone appears to be weak (Nilsen *et al.* 2011), but may be biologically relevant because vitellogenin can be present in large amounts (up to 100 μg/μL blood). Other suggested ligands are fatty acids from brood pheromone, which might partly explain why workers with different vitellogenin levels, and thus different ligand binding capacities, have separate behavioural responses to the pheromone: Workers with high vitellogenin levels respond with larval care-behaviour, while bees with low vitellogenin levels respond with increased protein (pollen) collection (see Smedal *et al.* (2009) and references therein). Alternatively, the general lipid load of vitellogenin might vary in size or composition based on structural features of the cavity and influence several organism features.

In closing, Kent *et al.* (2011) suggest that genes involved in social pleiotropy, like *vitellogenin,* may be less constrained in molecular evolution than genes involved in other forms of pleiotropy. This can occur if socially pleiotropic alleles usually have neutral or positive effects across castes. But, can socially pleiotropic loci be actively protected from antagonistic alleles? For honey bee *vitellogenin*, we believe the answer might be yes: This gene is not only expressed by workers and queens, but also expressed by larvae before their caste fate is set (Guidugli *et al.* 2005). Such young larval bees can be cannibalized at low but measurable rates by workers (see (Aase *et al.* 2005) and references therein). This *vitellogenin*, thereby, is expressed in a bipotent larval form that can be selectively discarded by the colony. The functions of *vitellogenin* in bee larvae are not well understood, but we have shown that *vitellogenin* knockdown larvae are viable but more frequently cannibalized than controls (Aase *et al.* 2005). We speculate that *vitellogenin* affects larval quality traits that can be assessed by the adult workers. Thereby, if bipotent larvae are negatively affected by antagonistic social pleiotropy, then the frequency of antagonistic *vitellogenin* alleles in honey bee populations could be suppressed and allow vitellogenin to be more effectively sculpted to the needs of the colony.

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