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Essay

The developmental genetics and physiology of honeybee societies

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Eusocial animal societies, as diverse as those found in the ants, bees, wasps, shrimp and naked mole-rats, are structured around one or few reproductive females. The remaining females are helpers called 'workers' that are mostly sterile. A paradigm in studies of eusociality is that worker sterility is a key to societal functions because advanced sociality cannot be achieved when there is conflict over reproduction. Yet, traits such as sensory responsiveness, foraging and hoarding behaviour that change between female reproductive life stages also vary between workers. This variation is central to worker division of labour, a complex social trait believed to be instrumental for the ecological success of animal societies. Thus, we took a step back from established views on worker sterility and societal functions, and hypothesized that division of labour can be better understood if adaptive variation in worker behaviour is seen as emerging from pre-existing mechanisms associated with female reproduction. In exploring this reproductive ground plan hypothesis (RGPH) in honeybee workers, we established that variation in foraging division of labour correlates with ovary size and is affected by expression changes in vitellogenin, an egg yolk protein precursor. Here, we explain and reconcile the RGPH with data on honeybee sensory sensitivity, genomic mapping, transcript and endocrine profiling, and link our discussion with Ihle et al. (2010, this issue, pp. 1001–1006). The findings bring together mechanistic and evolutionary explanations of honeybee worker behaviour. This essay suggests that a broader view on worker reproductive traits can increase the understanding of animal social behaviour.

BACKGROUND

Eusocial animal societies are characterized by reproductive division of labour between primary reproductives and mostly sterile helpers called workers. Workers display different biases in the kinds of behavioural tasks they perform, which are often associated with changes in physiology that are correlated with age, as well as differences in adult morphology. This striking level of social organization is believed to be the cause of the enormous ecological and evolutionary success of social species, including the advanced societies of ants, bees, wasps and termites (Oster & Wilson 1978).

Recently, Hölldobler & Wilson (2008) resurrected the early 20th century metaphor of the insect society as a superorganism (Wheeler 1911), conjuring images of a distributed organism with systems equivalent to physiology, reproduction, communication and information processing (nervous system). The superorganism metaphor works well at the phenomenological level of the colony but does not explain the genetics or developmental biology of social evolution. There is no single 'superorganismal' genome that natural selection can act on. Instead, each individual in a colony is a product of development derived from that individual's genome. Natural selection must change this genome to influence development and behaviour. The challenge is to understand how natural selection on colonies changes genomes, development and individual behaviour (i.e. to reveal the developmental evolution of the social structure; Page & Amdam 2007).

There are many definitions of the superorganism (Page & Mitchell 1991; Mitchell 2003), but central to all is the reproductive division of labour between the fertile reproductives and the workers. The definitions imply that developmental evolution of worker sterility is key to social harmony because advanced sociality cannot be achieved when there is conflict over reproduction (Wilson & Sober 1989). In ants, bees and wasps, which make up the majority of the social insect taxa, division of labour is exclusively female, and the female reproductive process is a potential source of conflict. To reduce conflict, natural selection has acted on reproductive gene networks, reducing or eliminating their functionality in workers relative to their fertile sisters, the queens (Wilson 1971; Khila & Abouheif 2008). Functional systems of worker reproductive biology, consequently, are seen as something natural selection will

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abolish as societies evolve more complexity and become more 'superorganismal'.

In this essay, we discuss a different view. We place worker reproductive biology at the forefront of developmental evolution of complex social behaviour, an approach taken previously towards reproductive division of labour between queens and workers, and towards worker age polyethism (temporal change in task performance) by West-Eberhard (1987, 1996). The regulatory networks of reproductive maturation and plasticity that coordinate expression of female physiology and behaviour are exploited by natural selection to adapt social structures. This is possible because correlation of female physiology and behaviour is central not only to successful reproduction in solitary species (Klowden 1990; Clements 1992; Atchley et al. 2005), but also to worker division of labour (Seeley 1982; Hölldobler & Wilson 1990).

We focus on the honeybee, *Apis mellifera*, the best-studied social insect (Honey Bee Genome Sequencing Consortium 2006). We describe how artificial selection on stored colony food resources altered the social foraging behaviour of honeybees through effects on worker reproductive biology. We believe this response to colony-level selection exemplifies how developmental evolution of complex social structures can occur: the influence of reproductive biology on female food-related behaviour, an apparently ubiquitous trait in animals (Clements 1992; Clarke & Ossenkopp 1998; Atchley et al. 2005), can be evolutionary co-opted to produce division of labour between workers with different behavioural biases in food collection and food hoarding (Amdam et al. 2004b, 2006). This reproductive ground plan hypothesis (RGPH, see below for details) refocuses the discussion regarding the role of worker reproduction from one where worker ovaries lead to competition and discord to one where the reproductive system of workers has been co-opted and is now a facilitator of cooperation and social organization.

HONEYBEE DIVISION OF LABOUR AND STORED COLONY RESOURCES OF NECTAR AND POLLEN

Honeybee workers demonstrate a striking division of labour that is physiologically based, in which bees of different ages perform different tasks (Seeley 1982; Robinson 1992). Younger bees perform tasks within the nest, such as feeding larvae, constructing and maintaining the nest and processing honey, while older bees forage. This division of labour is further divided into specialists that perform some tasks more frequently than other individuals. For example, foragers can specialize on collecting pollen, a protein source, or nectar, a source of carbohydrate. This specialization is best observed as a foraging bias or 'preference', measured as the ratio of the two substances collected by the individual bee: some bees collect relatively more pollen, others more nectar (Page et al. 2000). The collective activities of foraging workers provide food for adults and developing larvae within the nest and results in the adaptive storage of surplus honey (from nectar) and pollen by colonies.

ARTIFICIAL SELECTION ON STORED POLLEN: EFFECTS ON SOCIAL BEHAVIOUR

We (Page & Fondrk 1995) conducted a bidirectional selection programme for high and low levels of surplus pollen storage by honeybee colonies (pollen hoarding) and demonstrated a strong response to selection. We looked at individual behavioural traits that changed as a consequence of selection on the colony-level phenotype. We found that workers from the strain selected for increased pollen storage (high pollen-hoarding strain) initiate foraging about 10 days earlier in life than low pollen-hoarding strain bees (Pankiw & Page 2001). High strain bees are more likely to bias their foraging (specialize) towards pollen, while low strain bees are more likely to specialize on nectar (Page & Fondrk 1995; Page et al. 1995; Fewell & Page 2000; Pankiw & Page 2001). Furthermore, when high strain bees forage for nectar, they accept nectar with lower concentrations of sugar, and also respond to lower concentrations of sucrose solution when analysed with a proboscis extension response (PER) test (see also Fig. 1).

Pollen-hoarding strain phenotypes reflect genotypic differences, validated by cross-fostering experiments where high strain bees were reared by low strain colonies and vice versa (Calderone & Page 1992) and by co-fostering where strains were reared together by 'wild-type' (unselected commercial) honeybees (Pankiw & Page 2001; Scheiner et al. 2001). The genotypes, however, are not fixed. The breeding scheme includes planned outcrosses to the original source population (California, U.S.A., Page & Fondrk 1995) and maintains within-strain variability. Recent genome sequencing of two low strain sisters covering 92% of AT-rich and 96% of GC-rich

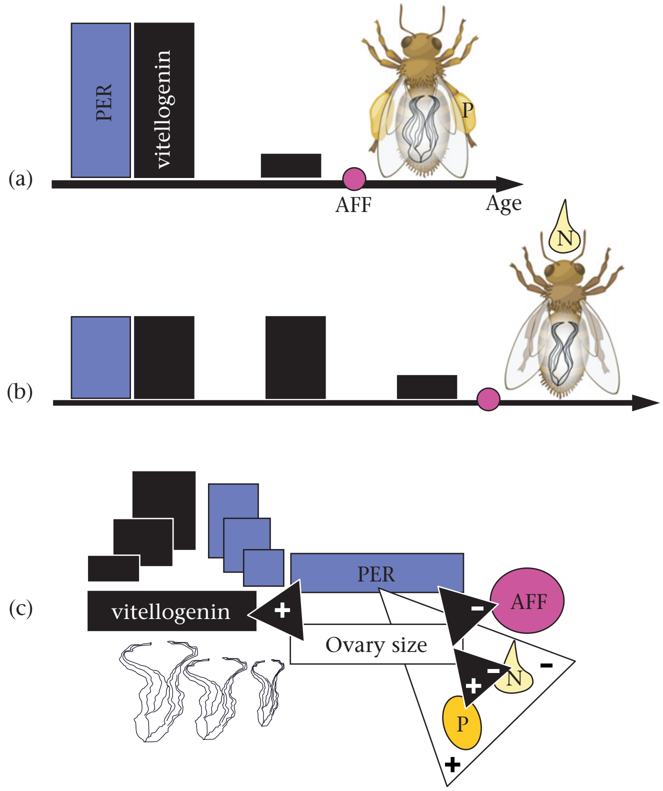

Figure 1. Variation in foraging behaviour correlates with differences in sensory and reproductive traits in worker honeybees. Trait associations in selected high (a) and low (b) pollen-hoarding strain bees. Horizontal arrows show the timeline of worker ontogeny (Age). High strain bees emerge as adults with a larger ovary (white line-drawings inside bees), elevated sucrose responsiveness measured by the proboscis extension response (PER, blue bars), and develop a higher peak titre of vitellogenin yolk protein (black bars) as young adults compared with low strain bees. The vitellogenin level of high strain bees then drops rapidly and workers initiate foraging earlier in life than bees with low strain genotype. This difference in 'age at first foraging' (AFF) is indicated by violet circles, panel (a) versus (b). As foragers, high strain workers bias their collecting towards pollen ('P', bee in (a)), while low strain workers are biased towards nectar ('N', bee in (b)). (c) The corresponding trait correlations in wild-type (unselected) worker bees, which show considerable phenotypic variation (illustrated by ovary, PER, and vitellogenin symbols of various sizes). Black, connecting triangles indicate positive (+) correlations between vitellogenin expression, PER and ovary size, and between ovary size and pollen foraging, and negative correlations (−) between PER and age at foraging onset, and between ovary size and foraging onset. The white triangle specifies that PER is also correlated with foraging choice directly. These associations in wild-type bees reflect the same relationships as those seen in selected pollen-hoarding strain workers.