The Architecture of the Pollen Hoarding Syndrome in Honey Bees: Implications for Understanding Social Evolution, Behavioral Syndromes, and Selective Breeding

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Abstract:

Social evolution has influenced every aspect of contemporary honey bee biology, but the details are difficult to reconstruct. The reproductive ground plan hypothesis of social evolution proposes that central regulators of the gonotropic cycle of solitary insects have been co-opted to coordinate social complexity in honey bees, such as the division of labor among workers. The predicted trait associations between reproductive physiology and social behavior have been identified in the context of the pollen hoarding syndrome, a larger suite of interrelated traits. The genetic architecture of this syndrome is characterized by a partially overlapping genetic architecture with several consistent, pleiotropic quantitative trait loci (QTL). Despite these central QTL and an integrated hormonal regulation, separate aspects of the pollen hoarding syndrome may evolve independently due to peripheral QTL and additionally segregating genetic variance. The characterization of the pollen hoarding syndrome has also demonstrated that this syndrome involves many non-behavioral traits, which may be the case for numerous "behavioral" syndromes. Furthermore, the genetic architecture of the pollen hoarding syndrome has implications for breeding programs for improving honey health and other desirable traits: if these traits are comparable to the pollen hoarding syndrome, consistent pleiotropic QTL will enable marker-assisted selection, while sufficient additional genetic variation may permit the dissociation of trade-offs for efficient multiple trait selection.

Keywords: Apis mellifera | pleiotropy | behavioral syndrome | honey bee health | social behavior | correlated evolution | artificial selection | ovary

Article:

1 Behavioral syndromes and pleiotropy in social insects

Behavioral traits have generally been perceived as highly plastic and independent of other traits because they are the outcome of a complex central nervous system that integrates numerous external and internal influences. However, our increasing understanding of the proximate mechanisms of behavior has led to the realization that different behavioral traits are commonly correlated with each other. Correlation of different behavioral traits or the same trait across different contexts has been defined as a behavioral syndrome to parallel the concept of life history syndromes (Sih et al. *2004*). If the behavioral syndrome in question has a genetic basis, it parallels pleiotropy, the genetic description for one or multiple genes concomitantly influencing several phenotypes.

Studies of behavioral syndromes and studies of pleiotropy have focused on the resulting trade-offs between multiple correlated traits and the possibility of evolutionary constraint due to genetic covariance (Agrawal and Stinchcombe, *2009*). The simple logic behind the trade-off is that an advantageous change in trait A may result in a non-advantageous change in the correlated trait B, potentially constraining the optimization of both traits (Price and Langen, *1992*). While the possibility of such trade-offs through pleiotropy has long been realized in the fields of quantitative genetics (Lande, *1982*) and life history theory (Williams, *1957*; Stearns,*1989*), the ideas has only recently found traction in the study of behavior (Sih et al. *2004*; Jandt et al. *2013*). The correlation between traits depends on the proximate mechanisms and genetic architecture that are responsible for their inter-dependence. Strong genetic covariance, high penetrance, and low intra-individual plasticity increase the possibility of trade-offs. Thus, the underlying genetic architecture of any syndrome of correlated traits is central to understanding the evolution of these traits (Roff, *2011*).

Social insects are particularly interesting because of their strong phenotypic differentiation among closely related individuals and the additional colony level that results from the integration of many individuals. The integration of individuals into a colony leads to a new layer of behavioral syndrome and potential constraint (Jandt et al. *2013*), particularly when individuals are closely related and therefore genetically similar. Similar to a genetic correlation between the two sexes (Delph et al. *2010*), genetic correlations between different castes and colony members may constrain the evolutionary optimization of specific phenotypes. The phenotypic plasticity that leads to differences among closely related individuals has presumably evolved to reduce constraints imposed by pleiotropy and facilitate social evolution (Gadagkar, *1997*).

Honey bees are a highly derived clade and numerous changes have occurred during their social evolution from a solitary ancestor (Arias and Sheppard, *2005*). It is difficult to determine how social evolution and adaptations to the new social organization have progressed in honey bees because fossil evidence does not resolve many behaviors (Engel, *1998*). Furthermore, causes and consequences of social evolution are hard to establish because honey bees present only one singular transition to sociality (Arias and Sheppard, *2005*). Therefore, the phenotypic and genotypic architectures of the present species need to inform us about plausible processes that have led to honey bee sociality. One hypothesis that is testable by studying current species is the

reproductive ground plan hypothesis of social evolution in honey bees (Amdam et al. *2004*). The reproductive ground plan hypothesis proposes that the gonotropic cycling of a hypothetical solitary ancestor has been co-opted by social evolution to facilitate intra-group specialization: The temporal phenotypic plasticity between reproductive and non-reproductive states has been transformed into stable differences among individuals (Page and Amdam, *2007*; Amdam and Page, *2010*). The reproductive ground plan hypothesis predicts that phenotypic and genetic associations that characterize the insect gonotropic cycle will still be apparent as sets of correlated traits or syndromes in modern honey bees (Wang et al. *2009*; Graham et al. *2011*). Below, I describe how the phenotypic and genetic architecture of the pollen hoarding syndrome (PHS) supports the reproductive ground plan hypothesis and what the results suggest more generally about behavioral syndromes and the prospects for selective breeding in honey bees.

2 Phenotypic associations of the pollen hoarding syndrome

Social insects, including the Western Honey Bee, *Apis mellifera* (L), protect and regulate their colony environment to rear brood and store food. The two principal food sources, pollen and nectar, are brought into the colony by foragers that may specialize on one of these resources or collect both (Fewell and Page,*1993*). In contrast to stored nectar or honey (Fewell and Winston, *1996*), the amount of pollen in the honey bee hive is tightly regulated (Fewell and Winston, *1992*; Dreller et al. *1999*). Artificial selection has repeatedly demonstrated a substantial genetic component for the amount of pollen stored, or pollen hoarding (Hellmich et al. *1985*; Page and Fondrk, *1995*). Pollen hoarding is a complex social trait because the amount of pollen stored in the hive is a function of available space, pollen collection, and consumption, and the active regulation occurs by adjusting the level of pollen collection by the foragers (Dreller and Tarpy, *2000*). Therefore, it is not surprising to find artificial selection for pollen hoarding associated with corresponding changes in the probability of individual forager to collect pollen and their pollen load sizes (Page et al. *2000*; Pankiw and Page *2001*). Consistently, workers from the strain selected for high-pollen hoarding performed more recruitment dances for pollen sources and also follow such dances more (Waddington et al. *1998*; Page et al. *2012b*).

However, detailed studies of the high- and low-pollen hoarding strains have revealed a number of correlated changes in other traits with less obvious connections to pollen hoarding (Page et al. *2012b*). These include behavioral traits such as an earlier transition from in-hive to foraging activities (Pankiw and Page, *2001*), higher locomotor activity after emergence (Humphries et al. *2005*), increased sensitivity to several stimuli (Tsuruda and Page, *2009*), and increased learning performance (Scheiner et al. *2001*) in the strain selected for high-pollen hoarding. Workers from this strain are also smaller (Linksvayer et al. *2009a*), die earlier (Amdam et al. *2007*; Rueppell et al. *2007*), complete pupation at a later age (Amdam et al. *2010*), and have larger ovaries (Amdam et al. *2006a*). At the physiological level, the high-pollen hoarding workers differ in brain biochemistry (Humphries et al. *2003*) and the endocrine dynamics of vitellogenin, juvenile hormone, and ecdysteroids (Page and Amdam, *2007*; Amdam et al. *2007*, *2010*).

A substantial part of the phenotypic trait associations of the PHS has also been found in unselected honey bees in the commercial North American bee population (Amdam et al. *2006a*; Page et al. *2012b*), Africanized honey bees (Pankiw, *2003*; Page et al. *2012b*), and in the closely related *Apis cerana* (Rueppell et al. *2008*). Thus, the connections between the studied traits are robust, suggesting co-regulation at a fundamental level (Amdam et al. *2004*). Presumably, the basic relation between nutrition and reproduction preceded the evolution of social honey bees and has been sophisticated to regulate certain aspects of their social organization (Toth and Robinson, *2007*). This concept of cooption of reproductive regulatory modules by social evolution has been proposed as the reproductive ground plan hypothesis of social evolution in honey bees (Amdam et al. *2004*; Amdam and Page, *2010*).

3 Genetic architecture of the pollen hoarding syndrome

The phenotypic studies of the PHS and associated phenotypes have been accompanied from their beginning by genetic analyses, yielding an understanding of the genetic architecture of the behavioral syndrome from quantitative trait loci mapping, transcriptome analyses, and candidate gene studies.

4 Quantitative trait loci

The first detailed genetic study of the pollen hoarding trait revealed two quantitative trait loci (QTL: *pln1* and*pln2*) that together explained 59 % of the pollen hoarding variation in a backcross between the selected strains (Hunt et al. *1995*). In a second mapping study of a cross between these selected strains after several more generations confirmed one of these QTL and identified another (*pln3*) explaining 10 % of the observed variation (Page et al. *2000*). Finally, a third study confirmed *pln1*, *pln2*, and *pln3* by demonstrating an effect on individual foraging behavior, reported a fourth QTL (*pln4*), and demonstrated complex interactions between these loci (Rüppell et al. *2004*).

As predicted, based on the phenotypic associations, the *pln* QTL also were shown to have pleiotropic effects on other aspects of the PHS. Either alone or interactively, *pln1–pln3* affected the age of first foraging (Rueppell et al. *2004*) and all four *pln* QTL had an effect on the sucrose responsiveness of honey bees (Rueppell et al. *2006a*). Most importantly for the reproductive ground plan hypothesis, the ovary size of worker honey bees was affected by *pln2*, *pln3* (Wang et al. *2009*), and *pln4* (Ihle et al., unpublished), and hormonal dynamics were affected by *pln3* (Ihle et al., unpublished). In all instances, mapping studies of these other traits have revealed additional QTL that usually exceeded the pleiotropic effects of the *pln* QTL in the specific crosses of the high and low pollen hoarding strains: Three additional QTL for the age of first foraging were identified (Rueppell et al. *2004*, *2009*), one for sucrose responsiveness (Rueppell et al. *2006a*), and three for worker ovary size (Rueppell et al. *2011*). One QTL for the age of first foraging (*aff2*) also showed an effect on worker ovary size and two worker ovary size QTL showed effects on hormonal dynamics (Ihle et al., unpublished). Single effects of

the *pln3* and *aff3* QTL on the life expectancy of honey bee workers have also been demonstrated (Dixon et al. *2012*). However, most conceivable tests for pleiotropy at the QTL level remain to be explored.

Outside the selected pollen hoarding strains, *pln1* was confirmed to affect pollen hoarding in a cross between European and Africanized honey bees, (Page et al. *2000*). Analyses of two parallel backcrosses between these two populations in a later experiment have further demonstrated overlap between the previously identified behavioral QTL and QTL for worker ovary size: Markers for two of the three age of first foraging QTL (*aff2* and *aff4*) and two of the four pollen hoarding QTL (*pln1* and *pln2*) showed significant effects on ovary size (Graham et al. *2011*). The high genetic overlap between reproductive and social traits supports the reproductive ground plan hypothesis of social evolution in honey bees (Wang et al. *2009*; Graham et al. *2011*) and suggests a partially overlapping genetic architecture of the different aspects of the PHS with a set of common core regulators (Page et al. *2012b*).

The identified QTL do not represent specific genes but designate genome intervals that contain positional candidate genes (Hunt et al. *2007*). The exceptionally high recombination rate of the honey bee genome typically results in small QTL regions (Hunt et al. *2007*), facilitating the identification of the genetic element that is responsible for each QTL effect. The PHS is one of very few examples where the connection from QTL to candidate genes has been made (see below). However, this is not true for all QTL and complementary data, such as gene expression patterns, are a powerful complement to QTL maps.

5 Transcriptome studies

Complementary to QTL mapping, the study of transcriptome patterns generates functional candidate genes for complex traits that could be the cause or consequence of the phenotype studied. Among the traits of the PHS, the gene expression changes that are related to the transition from in-hive tasks to foraging have been analyzed in most detail. Multiple gene expression signatures for the behavioral transition from in-hive tasks to foraging exist (Whitfield et al. *2006*) and some of the central functional candidate genes (e.g. the MAP-kinase *ERK7*) have also been suggested by the *aff* QTL (Rueppell, *2009*). The PHS links into the central endocrine core of the regulation of this life history transition with juvenile hormone and vitellogenin co-regulation, affecting insulin-like signaling (Amdam et al. *2007*; Nelson et al. *2007*; Nilsen et al. *2011*). However, the transcriptome studies provide little corroborating evidence for an involvement of these endocrine regulators.

The second trait of the PHS that has been studied by transcriptomic approaches is the ovary. Representational difference analysis of the developing ovary in worker and queen-destined larvae revealed a small number of differentially expressed genes (Humann and Hartfelder, *2011*). Two of these transcripts were long non-coding RNAs that are located near the *aff2* QTL that has repeatedly exhibited an influence on worker ovary size (Graham et al. *2011*; Ihle et al.,

unpublished). However, sequence analysis of a part of both genes failed to identify allelic variants in the original mapping population that may be responsible for the QTL effects (B. Vannasane and O. Rueppell, unpublished data). Transcriptome comparisons of the adult worker ovary between the high- and low- pollen hoarding strains revealed more than 2,000 differentially expressed genes with significant overlap to a similar number differentially expressed genes in the honey bee brain in response to hormonal treatment and the transition from in-hive to forager status (Wang et al. *2012*). Although these numbers of differentially expressed transcripts are frustratingly large in the context of searching for singular candidate genes, overlap between multiple studies (Whitfield et al. *2006*) and the combination of positional and functional candidates improve the prospects of finding the underlying molecular causes of the observable social phenotypes.

6 Candidate genes

Informed by the genomic studies (Hunt et al. *2007*, Amdam et al., *2007*) and theoretical considerations (Amdam et al. *2004*), several specific genes have been studied in the context of the PHS. The vitellogenin gene encodes a principal egg yolk protein that has been co-opted by social evolution to fulfill additional functions (Amdam et al. *2003*). Although the vitellogenin gene is not located in any of the QTL intervals, it has been studied in great detail and RNAi-mediated gene knockdown experiments show extensive effects on central traits of the PHS, such as gustatory responsiveness, foraging specialization, and the age of transitioning to foraging activity (Amdam et al. *2006b*; Amdam et al. *2007*; Ihle et al. *2010*). Vitellogenin effects on the PHS are not localizing to the described QTL, suggesting that vitellogenin levels may be controlled by segregating genetic variation in other genome locations.

Multiple genes in the insulin-like signaling pathway have been identified as candidate genes for the *pln* QTL (Hunt et al. *2007*, Amdam et al., *2007*), and three genes have been experimentally associated with the PHS: expression differences of nuclear hormone receptor homolog *HR46* and phosphoinositide-dependent kinase-1 (*PDK1*) were associated with the selected strains and directly with worker ovary size (Wang et al. *2009*). Additionally, down-regulation of the insulin receptor substrate causes honey bee foragers to collect more pollen relative to nectar without affecting sucrose responsiveness (Wang et al. *2010*). Vitellogenin levels affect insulin-like signaling in honey bees (Nilsen et al. *2011*) and the inverse seems also likely because insulin-like signaling affects juvenile hormone (Mutti et al. *2011*), which may decrease vitellogenin levels (Page et al. *2012b*).

7 Conclusions

The PHS relates to many behavioral and life history traits in honey bees although its initial study began with a specific behavioral trait, pollen hoarding. The artificial selection program generating the high and low pollen hoarding lines has proven to be a powerful investigative tool (Conner, *2003*). Particularly, the search for mechanisms explaining this specific phenotype led to

the discovery that numerous other traits are involved, resulting in the best characterization of a behavioral syndrome in social insects so far. The mechanistic emphasis of the described studies has resulted in an integrative understanding of the proximate causation of the PHS (Page and Amdam, *2007*; Page et al. *2012b*). However, the fitness consequences of the coordinated specialization or honey bee workers within colonies remain to be explored. The PHS involves numerous developmental, physiological, behavioral, morphological, and life history traits (Page et al. *2012b*). Specifically, the connections between reproductive physiology and behavioral specialization have been predicted by the reproductive ground plan hypothesis and the direct phenotypic and genetic correlations support the reproductive ground plan hypothesis of social evolution in honey bees. The reproductive ground plan hypothesis may exemplify a more general pattern of social evolution by modification of fundamental, preexisting life history regulators. The consistency and number of relations among traits and genetic effects is remarkable, given that honey bees cannot be inbred and all complex traits of the PHS depend on the interaction of the current genetic variation at multiple loci with a changing environment.

8 Implications for studies of behavioral syndromes

The studies of the PHS at the phenotypic and genetic level have demonstrated a central role for reproductive physiology in social evolution (Amdam et al. *2006a*; Page and Amdam, *2007*). Even though the PHS initially was described as a set of correlated behavioral traits (Page and Fondrk, *1995*; Page and Erber, *2002*), the underlying hormonal differences affect also development, physiology, and life history (Page et al. *2012b*). Studies of the PHS exemplify the connection between different levels of biological organization from molecular differences in single genetic regulators to colony-level resource allocation changes. Thus, the PHS may also be conceptualized as a life history syndrome instead of a behavioral syndrome. This conclusion prompts the general question how much the concept of behavioral syndromes differs from the concept of established syndromes in ecology and evolution (Pianka, *1970*). Life history syndromes inevitably involve correlated behavioral traits. It should be realized that behavior integrates with most other aspects of biology, such as reproductive and nutritional physiology (Page and Amdam, *2007*; Toth and Robinson, *2007*). The study of behavioral syndromes that fail to make these connections will only represent an incremental advance over the analysis of single behavioral traits in behavioral ecology.

The growing understanding of the genetic architectures and underlying mechanisms of animal behavior needs to be integrated with phenotypic studies measuring correlations among different behavioral contexts. The relatively weak but significant behavioral correlations that are characterized in many studies of behavioral syndromes (Zsolt Garamszegi et al. *2012*) suggest partially overlapping genetic architectures that are similar to the PHS (Rueppell et al. *2004*, *2006a*; Graham et al. *2011*) and can be explored to identify central control elements (Hunt, Amdam et al., *2007*; Wang et al. *2009*; Page et al. *2012a*). Due to its complexity and plasticity, natural behavior is more challenging to understand than most other kinds of traits, but the example of the PHS provides a cause for optimism beyond the study of social insects.

9 Implications for selective breeding for honey bee health

Pleiotropy and genetic covariance among traits may prevent optimization of single traits by natural or artificial selection. The large number of traits integrated in the PHS and complex correlations across castes (Rueppell et al. *2006b*; Page et al. *2012a*) suggest that fundamental processes underlie the PHS that prevents independent evolution. Alternatively, the coordinated multi-trait evolution of the different members of the honey bee colonies may have been beneficial, leading to trait linkage and pleiotropy (Lande, *1984*). Most traits of the PHS are of little direct interest to the applied problems of declining honey bee health (vanEngelsdorp and Meixner, *2010*) and selective breeding to increase the quality of honey bees for pollination and general apiculture (Rinderer et al. *2010*). Disease syndromes, such as colony collapse disorder (vanEngelsdorp et al. *2009*) or brood disease syndrome (vanEngelsdorp et al. *2013*), also combine multiple disease symptoms but are conceptually unrelated to life history and behavioral syndromes discussed above. Nevertheless, the study of the PHS and the artificially selected high- and low-pollen hoarding strains for over 20 years (Page et al. *2012b*) provides important lessons for the current efforts to identify genes for disease resistance and apply this knowledge in selective breeding programs. One direct lesson is the potential for individual drone selection via artificial insemination based on male–female trait correlations (Rueppell et al. *2006b*; Page et al. *2012a*).

Honey bees have been bred for resistance against the ectoparasitic *Varroa* mite and commercially available stocks in the USA include the Russian honey bees (Bourgeois and Rinderer, *2009*), the Minnesota hygienic honey bees (Spivak et al. *2009*), and the *Varroa* sensitive hygiene stock (Harris, *2007*). These selection programs have all been successful, relying on different selection and breeding strategies. Although the principal mechanism of resistance of at least two of these stocks appears to be hygienic behavior (Harris,*2007*), no “hygienic syndrome” has emerged and multiple mechanisms of resistance are possible (Rinderer et al. *2010*). The existence of a “hygienic syndrome” cannot be excluded and a detailed characterization of these mite-resistant stocks with respect to a wider array of life history and behavioral traits remains to be performed. A direct test failed to link hygienic behavior to the PHS (Goode et al. *2006*), even though Africanized honey bees combine increased mite resistance (Camazine, *1986*) with multiple aspects of the PHS (Pankiw, *2003*; Graham et al. *2011*).

In order to enable marker-assisted selection (Rinderer et al. *2010*), QTL studies of the hygienic behavior of the Minnesota hygienic (Lapidge et al. *2002*; Oxley et al. *2010*) and the *Varroa* sensitive hygiene stocks (Tsuruda et al. *2012*) have been conducted. Additional QTL studies have targeted mite grooming behavior (Arechavaleta-Velasco et al. *2012*) and mite reproductive success (Behrens et al. *2011*). A QTL search was also performed for resistance to chalkbrood (*Ascosphaera apis*) (Holloway et al. *2012*). However, over 70 different honey bee diseases are known (Schmid-Hempel, *1998*) and studies of the genetic architecture of honey bee resistance to most of them are still lacking, even though a genetic basis for resistance against

most diseases probably exists (Kulincevic and Rothenbuhler, *1975*; Bailey and Ball, *1991*; Palmer and Oldroyd, *2003*; Huang et al. *2012*).

The studies of the PHS have demonstrated that QTL effects can be relatively robust across time and different mapping populations (Page et al. *2000*; Rüppell et al. *2004*; Ihle et al., unpublished), which increases the prospects for marker associated selection of honey bees. However, the QTL studies of *Varroa*resistant stocks have not yielded consistent QTL and show no overlap with transcriptome analyses (Le Conte et al. *2011*; Tsuruda et al. *2012*). A tighter control of breeding and the identification of causative SNPsmay increase the prospects for consistent findings in honey bee health research and enable marker-assisted selection. Marker-assisted selection is a highly desirable breeding strategy in honey bees, given the practical limits to breeding and numerous desirable traits that could be selected for in addition to disease resistance (Laidlaw and Page, *1997*). Genetic studies of the PHS have revealed also pleiotropy and epistasis (Rüppell et al. *2004*), non-additivity and genetic heterogeneity (Linksvayer et al. *2009b*; Graham et al. *2011*), and complex trait relations (Page et al. *2012a*), which may limit the success of phenotypic selection. In contrast, marker-assisted selection may increase long-term breeding success by enabling breeders to maintain and combine specific genetic variants and to cross “adaptive valleys” from one local optimum to another on the selective landscape (O'Hagan et al. *2012*).

The prospects for multi-trait selection also relate to another central question for the field of honey bee health research raised by studies of the PHS: How much overlap in the resistance to different honey bee diseases exists and is there a “healthy” syndrome in honey bees? Hygienic behavior counteracts a series of brood diseases (Spivak and Gilliam, *1998*) but how other desirable traits relate to this behavior and to each other remains to be studied. The phenotypic and genomic studies of trait relations are important to determine the prospects for combined selection on multiple traits. Comparisons between European and Africanized honey bees suggest potential trade-offs between disease resistance and less desirable behavioral traits, such as defensiveness. However, it is unclear whether these traits are genetically linked or could be dissociated by selective breeding. Studies of the PHS have shown that correlated sets of complex traits most likely have a partially overlapping genetic architecture consisting of central and peripheral regulators. Depending on the trait correlations and the genetic architecture, either the central or the peripheral regulators will present the basis for progress during social evolution and for selective breeding.

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