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COMMENTARY

Genetic accommodation and the role of ancestral plasticity in the evolution of insect eusociality

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ABSTRACT

For over a century, biologists have proposed a role for phenotypic plasticity in evolution, providing an avenue for adaptation in addition to 'mutation-first' models of evolutionary change. According to the various versions of this idea, the ability of organisms to respond adaptively to their environment through phenotypic plasticity may lead to novel phenotypes that can be screened by natural selection. If these initially environmentally induced phenotypes increase fitness, then genetic accommodation can lead to allele frequency change, influencing the expression of those phenotypes. Despite the long history of 'plasticity-first' models, the importance of genetic accommodation in shaping evolutionary change has remained controversial – it is neither fully embraced nor completely discarded by most evolutionary biologists. We suggest that the lack of acceptance of genetic accommodation in some cases is related to a lack of information on its molecular mechanisms. However, recent reports of epigenetic transgenerational inheritance now provide a plausible mechanism through which genetic accommodation may act, and we review this research here. We also discuss current evidence supporting a role for genetic accommodation in the evolution of eusociality in social insects, which have long been models for studying the influence of the environment on phenotypic variation, and may be particularly good models for testing hypotheses related to genetic accommodation. Finally, we introduce 'eusocial engineering', a method by which novel social phenotypes are first induced by environmental modification and then studied mechanistically to understand how environmentally induced plasticity may lead to heritable changes in social behavior. We believe the time is right to incorporate genetic accommodation into models of the evolution of complex traits, armed with new molecular tools and a better understanding of non-genetic heritable elements.

KEY WORDS: Epigenetics, Eusocial evolution, Genetic accommodation, Plasticity, Social insects

Introduction

A compelling question in evolutionary biology involves the origins and evolution of novel traits. For centuries, biologists have been interested in the diversity of phenotypes across life and how this diversity arose. The role of genetic factors in the origination of novel traits has been especially well studied, including the roles of mutation, genetic drift and recombination in producing

novel genetic combinations and phenotypes (Carroll, 2008). 'Mutation-first evolution' (see Glossary), where a new mutation provides novel phenotypes that can be 'screened' by natural selection, is easily studied when the mutation can be directly linked to the phenotype. Even without knowledge of the phenotypic consequences of alleles, mutation-first evolution studies can be initiated in both natural populations and laboratories simply by documenting changes in allele frequencies over time.

However, novel traits are also suggested to originate independent of new mutations, via the environmental and developmental induction of phenotypes. One of the first biologists to emphasize this was Baldwin, who at the turn of the 20th century suggested a process of 'organic selection' by which fitness differences arising from phenotypic plasticity (see Glossary) during development would, over many generations, lead to genetic change moderating this plasticity (Baldwin, 1896; Baldwin, 1902; Morgan, 1896; Osborn, 1897). Whether plasticity facilitates or slows down evolutionary diversification remains controversial (Pigliucci, 2006), but growing evidence suggests plasticity can influence the evolution of novel traits (Moczek et al., 2011; Pfennig et al., 2010). The potential role of phenotypic plasticity in shaping evolution was more comprehensively discussed a century later by West-Eberhard (2003), who emphasized that selection acts upon phenotypes, not genotypes. Phenotypes are not formed exclusively from genetic factors, but emerge from the integration of genetic, epigenetic (see Glossary) and environmental factors that act during development. While evolution is most commonly defined by changes in allele frequencies, a focus on genetic factors ignores the potential importance of environmental influences on phenotypic variation and evolution.

Other evolutionary biologists have also emphasized the potential importance of phenotypic plasticity in shaping evolution (Moczek et al., 2011; Pfennig et al., 2010; Pigliucci, 2006). Phenotypic plasticity can have large effects on fitness, allowing organisms to adapt to a changing environment and respond appropriately to inputs received during development. Plasticity itself may therefore be a target of selection (Nussey et al., 2005; Pigliucci, 2005; Van Buskirk and Relyea, 1998), and phenotypic plasticity may also lead to the origin of novel phenotypes, preceding or even facilitating evolutionary change (Pfennig et al., 2010; Price et al., 2003). Plasticity-first evolution (see Glossary) (Levis and Pfennig, 2016) emphasizes the phenotype as the subject of selection, which can provide clarity for evolutionary models because phenotypic variation has a clear connection to natural selection.

The process of an environmentally induced phenotype leading to allele frequency change is known as genetic accommodation (see Glossary). Genetic accommodation can lead to either increased plasticity (such as the emergence of polyphenisms; see Glossary) or the fixation of an initially plastic trait, a special case known as genetic assimilation (see Glossary). While genetic accommodation has gained much theoretical support (Moczek et al., 2011;

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Glossary

Adaptive refinement
Increased fitness relative to an ancestral state, possibly facilitated by constitutive expression in the derived lineage related to the ancestral condition.

Ancestral-proxy lineages
Lineages closely related to the derived lineage of interest that display the ancestral character state for the trait of interest; these lineages lack the derived trait of interest while in their natural (ancestral) environment, but may exhibit plasticity for the trait when exposed to a novel environment (indicating pre-existing plasticity).

Cryptic genetic variation
Genetic variation in a population that does not currently contribute to phenotypic outcomes, but that may modify phenotypes following environmental change or new epistatic interactions with novel alleles.

Epigenetic
Referring to any feature of chromatin, DNA or other cellular features other than the DNA sequence itself that may influence gene expression and function, and may lead to heritable changes in transcriptional activity across cell divisions and/or generations.

Eusocial engineering
A proposed coupling of forced association studies with transcriptomics, epigenomics and other molecular analyses to test the mechanisms of genetic accommodation that may have acted on ancestral plasticity in social evolution.

Eusociality
Defined by (1) a reproductive division of labor (a queen that reproduces, workers that do not), (2) overlapping adult generations (often a mother and her daughters or sisters and their offspring), and (3) cooperative care of brood.

Forced association study
A study involving experimentally induced group formation, including of naturally solitary individuals, members of different natural groups or artificially created age classes of individuals; used to study emergent properties of groups or to remove confounds of developmental experience in studying social behavior.

Genetic accommodation
A process by which initially environmentally induced and plastic phenotypes are selected upon, resulting in heritable variation influencing the expression of those phenotypes; genetic accommodation can lead to increased plasticity for the trait (including the emergence of polyphenisms) or decreased plasticity (see genetic assimilation).

Genetic assimilation
A special case of genetic accommodation, where initially plastic traits become fixed through selection on one or more alternative genotypes; this results in reduced phenotypic plasticity over evolutionary time.

Intergenerational inheritance
Transference of environmentally mediated epigenetic changes from parent to offspring.

Mutation-first evolution
A mechanism of evolution in which a novel mutation or novel allele in the population alters a phenotype under selection, leading to changes in allele frequencies; contrast with plasticity-first evolution.

Phenotypic plasticity
The ability of a single genotype to produce multiple phenotypes in response to epigenetic or environmental conditions.

Plasticity-first evolution
A mechanism of evolution in which novel, environmentally sensitive phenotypic variation (i.e. phenotypic plasticity) provides the initial substrate for selection, followed by changes in allele frequencies in the population through selection on cryptic genetic variation (see above) underlying the phenotypic plasticity and/or accommodation on the newly selected trait; contrast with mutation-first evolution.

Polyphenism
Discrete phenotypic forms arising from phenotypic plasticity; often phenotypic forms are very distinct, such as queen and worker castes of complex eusocial insects or color morphs of some butterflies.

Reaction norm
The pattern of expressed phenotypes for a given genotype across one or more environmental variables, typically represented graphically; an individual shows phenotypic plasticity for a trait if the slope of the reaction norm is non-zero, indicating an interaction effect between genotype and environment for the phenotype of interest.

Subsocial
Adult females that protect and/or feed their developing offspring, but disperse or die prior to offspring emergence such that there is no adult generational overlap and no division of labor among adults.

Transgenerational inheritance
Transference of environmentally mediated epigenetic changes across more than two generations.

Pfennig et al., 2010; Pigliucci, 2006; West-Eberhard, 2003), it is difficult to test directly, which has likely influenced debate over the importance of plasticity-first evolution.

Arguments against plasticity-first evolution cite a lack of evidence for the molecular mechanisms enabling environmentally induced traits to become heritable in comparison to those that explain mutation-first evolution (Wray et al., 2014). Indeed, few examples of genetic accommodation have been elucidated to this level in the laboratory or in natural populations (e.g. Casasa and Moczek, 2018; Dworkin, 2005; Jones et al., 2017; Suzuki and Nijhout, 2006; reviewed in Renn and Schumer, 2013; Schlichting

and Wund, 2014). However, it is unclear whether the current low number of genetic accommodation examples reflects actual rarity in nature, or a combination of low research effort together with a lack of known mechanisms. As our ability to study mechanisms of evolution in natural populations continues to improve, we expect more cases of genetic accommodation to be reported.

For many years, the molecular mechanisms that might enable environmentally induced traits to become heritable were unknown, and our lack of knowledge on the relationship between plasticity and genetic changes limited support for plasticity-first models. Recently, evidence for an interplay between plasticity-first

mechanisms and elaboration of phenotypes via new mutations has been reported (Levis et al., 2018), highlighting the importance of considering both plasticity-first and mutation-first models in studies of evolutionary novelty. In addition, recent reports of epigenetic transgenerational inheritance (see Glossary) now provide plausible mechanisms through which genetic accommodation may act, priming the field to further investigate the role of plasticity-first mechanisms, including genetic accommodation, in evolution.

In this Commentary, we briefly review some of these reports, describe the features of social insects that make them good models for studying genetic accommodation, and review current evidence consistent with a role for genetic accommodation in the evolution of eusociality (see Glossary). We end with a description of an empirical method to leverage the inherent plasticity of social insects to further study the mechanisms underlying eusocial evolution; we hope that this approach will lead to novel insights into the role that genetic accommodation has played in the evolution of social behavior.

Epigenetic transgenerational inheritance

There is new evidence for connections between the environment and adaptive phenotypic change across generations, as advances in the field of epigenetics provide plausible mechanisms for transgenerational inheritance. Many studies have demonstrated intergenerational (parent to offspring; see Glossary) or longer-lasting transgenerational effects, across plants, insects and mammals (Agrawal et al., 1999; Benito et al., 2018; Champagne, 2008; Dell and Rose, 1987; Gluckman et al., 2007; Ruden and Lu, 2008; Valtonen et al., 2012). While there is currently more evidence for intergenerational effects, other studies report evidence for transgenerational inheritance (Klosin et al., 2017; Siklenka et al., 2015). Most studies do not address the mechanisms of this inheritance (see Box 1), but a few have produced provocative associations with epigenetic changes such as DNA methylation (Dias and Ressler, 2014; Wei et al., 2014). For example, Dias and Ressler (2014) demonstrated that after adult male mice are subjected to odor fear conditioning, their offspring also exhibit fear of the same odor, despite no direct experience with the learning paradigm or odor. Additionally, they reported that both generations showed differences in DNA methylation at the locus encoding the olfactory receptor responsive to this odor, providing a putative mechanism of inheritance (Dias and Ressler, 2014).

Additional mechanisms of transgenerational inheritance have been identified, including small non-coding RNAs and chromatin remodeling (e.g. Gapp et al., 2014; Greer et al., 2011; reviewed in Houri-Zeevi and Rechavi, 2017; Jablonka and Raz, 2009). For example, male mice that engage in higher levels of voluntary wheel running show altered levels of microRNAs (miRNAs) and tRNA-derived RNAs in their sperm (Short et al., 2017). These males produce male offspring with reduced anxiety and suppressed juvenile fear memory, potentially mediated through post-transcriptional gene regulation by the altered small RNAs in sperm (Short et al., 2017). Rodgers et al. (2015) demonstrated a direct effect of paternal miRNAs on offspring phenotypes through zygotic injection of nine paternal stress-related miRNAs, which led to reduced mRNA stores in zygotes and ultimately stress dysregulation phenotypes in offspring. Benito et al. (2018) also demonstrated a role for miRNAs in mediating synaptic plasticity in the offspring of male mice exposed to an environmental enrichment paradigm. Together, these studies demonstrate the possibly pervasive role of epigenetic mechanisms as mediators of transgenerational inheritance of environmentally induced phenotypes.

In addition to mediating environmental effects on phenotypes, epigenetic changes can also have direct effects on allele frequencies. For example, methylated cytosines make up nearly one-third of all germline and somatic point mutations as a result of increased rates of hydrolytic deamination at methylated cytosines when compared with unmethylated cytosines (Duncan and Miller, 1980; Shen et al., 1994). This leads to a depletion of CpG dinucleotides in genomes that undergo DNA methylation (Flores and Amdam, 2011). Environmental induction of methylation may thus lead to mutation of phenotypically relevant sites (Flores et al., 2013), altering the DNA sequence directly. Additionally, epigenetic marks for open chromatin lead to increased rates of transposable element insertion and meiotic recombination in maize (Liu et al., 2009). Thus, histone modifications that lead to accessible chromatin also increase the probability of genetic change. These mechanisms provide plausible links between phenotypic plasticity and mutation, demonstrating that these processes co-exist. While we are still in the early stages of understanding mechanisms by which parental experience shapes offspring phenotypes, mounting evidence suggests that mechanisms of transgenerational inheritance may be powerful modulators of phenotypic plasticity and, thus, evolution itself.

Phenotypic plasticity in social insects

Social insects have long been models for studying the role of environment on phenotype. Across species, a range of social forms is observed, from solitary to communal to complex eusocial species (Michener, 1974), with multiple independent origins of social phenotypes (Bourke, 2011). The breadth of behavioral plasticity across species provides unique opportunities to compare mechanisms of behavioral plasticity in a phylogenetic context. Additionally, many eusocial species exhibit extreme levels of plasticity between social castes, such as between individuals of different ages or between queens and workers.

Through the study of highly eusocial species such as the western honey bee (*Apis mellifera*), we know that environmental differences during development (e.g. larval nutrition) lead to caste differences, mediated by epigenetic mechanisms (Foret et al., 2012; Kucharski et al., 2008). Social insects have also played a critical role in uncovering the molecular basis of behavioral plasticity, with early transcriptomic studies of honey bees demonstrating for the first time that brain gene expression is predictive of behavioral state (Whitfield et al., 2003). Since then, many gene expression studies of social insects have identified transcriptomic differences associated with numerous phenotypic differences, including differences between queens and workers (Barchuk et al., 2007; Feldmeyer et al., 2014; Pereboom et al., 2005; Toth et al., 2007), differences between worker subcastes (Scharf et al., 2003; Whitfield et al., 2006) and different responses to socially relevant stimuli (Grozinger et al., 2003; Shpigler et al., 2017).

Brain transcriptional plasticity has been further modeled in the honey bee by using a large set of behavioral transcriptomic studies and reconstructing a brain transcriptional regulatory network. This network demonstrated context-dependent plasticity in the relationships between transcription factors and their target genes (Ament et al., 2012; Chandrasekaran et al., 2011), which is likely mediated through epigenetic mechanisms. Changes in DNA methylation and histone modifications have also been implicated in caste-related social behaviors in bees and ants (Herb et al., 2012, 2018; Lyko et al., 2010; Simola et al., 2015). Additionally, in a comparative study across 10 bee species, capturing multiple origins and elaborations of social behavior, sociality was correlated with increases in the occurrence of transcription factor binding sites and numbers of methylated genes, suggesting that eusocial lineages have an increased capacity for regulatory complexity (Kapheim et al., 2015b).

Although we have a good understanding of the mechanisms underlying behavioral plasticity in eusocial insects, less is known about whether phenotypic plasticity is a precursor to eusocial evolution. West-Eberhard (2003) suggested that the worker caste, a defining feature of eusocial colonies (Wilson, 1971), evolved through environmental induction of phenotypically plastic traits. While much indirect evidence suggests an environmentally induced worker origin, including existing plasticity and experimental inducibility in related species (West-Eberhard, 2003), no direct evidence for a plasticity-first origin of worker castes has been demonstrated.

Levis and Pfennig (2016) outlined an empirical approach for assessing plasticity-first evolution in natural populations, with relevance for social insects. They describe characteristics of study systems well suited for studying genetic accommodation and plasticity-first evolution, including knowledge of phylogenetic relationships in the broader taxonomic group and five other criteria. Table 1 outlines these criteria and gives examples of how social insects match many of these characteristics. We review these characteristics in the next section, and describe how features of social insects make them amenable to studies of genetic accommodation.

Social insects as models for studying genetic accommodation

The multiple evolutionary origins of eusociality allow for comparisons of derived lineages with ancestral-proxy lineages (see Glossary) to resolve signals of eusocial evolution. A comparative approach has already been useful in finding patterns of evolution associated with social behavior, leveraging the natural variation in social forms present across bees (Kapheim et al., 2015b; Woodard et al., 2011). Across the hymenopteran social insects, eusociality has evolved at least 10 times (Bourke, 2011), with divergence times from solitary ancestors ranging from 20 to 100 million years ago (mya). While the selective forces acting on different lineages are not always known, some ecological circumstances such as nest-site limitation and parasitism have been implicated as determinants of group living (Gunnels et al., 2008; Langer et al., 2004), suggesting that these factors may be selective agents favoring eusocial evolution.

Social insects also exhibit quantifiable phenotypes that are amenable to molecular analysis, allowing us to explore how plasticity is achieved mechanistically. For example, a key component of eusociality is the presence of a reproductive division of labor between queens and workers. The degree of division of labor can be quantified using the skew in reproduction across adults (Sherman et al., 1995), with more complex eusocial species exhibiting greater skew. Reproductive skew can be measured in a laboratory setting, and has been used to assess whether a division of labor occurs when typically solitary or subsocial (see Glossary) individuals are forced to cohabit (e.g. during forced association studies; see Glossary). Behavioral traits that are not related to reproduction can also be measured in a laboratory setting; such behaviors include excavation (Fewell and Page, 1999), foraging (Tenczar et al., 2014), guarding and nursing (Rittschof et al., 2014; Shpigler and Robinson, 2015). These quantifiable phenotypes could be useful in assessing the mechanisms underlying behavioral plasticity in eusocial colonies, and how these mechanisms may have changed throughout eusocial evolution.

Two social insect groups best match the characteristics outlined in Table 1 – bees and wasps. The multiple evolutionary origins of social behavior within each of these groups allows for phylogenetic comparisons of the mechanisms underlying eusocial evolution. In addition to multiple origins, closely related species display a range of social phenotypes, and growing genomic resources in these groups enable molecular studies of phenotypic plasticity (Ferreira et al., 2013; Jones et al., 2015; Kapheim et al., 2015b; Kocher et al., 2013; Standage et al., 2016). Already, these groups have shown promise in helping us to understand the potential role of genetic accommodation in eusocial evolution, as discussed in the next section.

Evidence for genetic accommodation in eusocial evolution

In addition to suggesting characteristics of ideal study systems for assessing plasticity-first evolution, Levis and Pfennig (2016) summarized four criteria necessary to establish that plasticity-first evolution has occurred, as listed below and in Table 2. Many studies of social insects demonstrate aspects of these criteria, although they were rarely formalized as studies of genetic accommodation (see references in Table 2). Below, we describe the criteria and provide examples from social insect studies that are consistent with each one. For social insects, the 'focal trait' (i.e. the phenotype under examination for testing predictions of genetic accommodation) we

Table 1. Characteristics of study systems well suited to studies of plasticity-first evolution (adapted from Levis and Pfennig, 2016), as well as select examples from social insects

Characteristic	Examples from social insects	Taxa/references
Knowledge of phylogenetic relationships in the broader taxonomic group	Well-resolved phylogenies for many groups of social insects	(Branstetter et al., 2017; Johnson et al., 2013; Romiguier et al., 2016)
Multiple parallel derived lineages, with variable divergence times	Bees: 4–6 independent origins, ranging from 20 to 65 mya Wasps: 4 independent origins, unknown origin dates for all but one lineage (100 mya)	(Bourke, 2011; Brady et al., 2006; Cameron and Mardulyn, 2001; Chenoweth et al., 2007; Schwarz et al., 2007; Thompson and Oldroyd, 2004)
Knowledge of ecological circumstances and selective agents acting on lineages	Nest-site limitation: habitat saturation selects for non-dispersal and group living Parasitism/predation: groups are better protected from parasites or predators	*Exoneura nigrescens* (Langer et al., 2004), *Mischocyttarus mexicanus* (Gunnels et al., 2008), *Xylocopa sulcatipes* (Stark, 1992), *Megalopta genalis* (Smith et al., 2003)
Quantifiable trait that can be induced under laboratory conditions	Reproductive skew, behavioral castes, division of labor; induced in forced association studies or environmental manipulations, or observed naturally in observation nests of social species	*Veromessor pergandei* (Rissing and Pollock, 1986), *Ceratina flavipes* (Sakagami and Maeta, 1987), *Pogonomyrmex barbatus* (Fewell and Page, 1999), *Apis mellifera* (Robinson et al., 1989), *Megalopta genalis* (Jones et al., 2017)
Adequate genomic resources to investigate molecular underpinnings	Genomes per group (from NCBI, accessed 25 February 2018): Ants: 18 Bees: 15 Vespid wasps: 2 Termites: 3	*Acromyrmex echinatior, Atta cephalotes, Atta colombica, Camponotus floridanus, Cyphomyrmex costatus, Dinoponera quadriceps, Harpegnathos saltator, Lasius niger, Monomorium pharaonis, Ooceraea biroi, Pogonomyrmex barbatus, Pseudomyrmex gracilis, Solenopsis invicta, Trachymyrmex cornetzi, Trachymyrmex septentrionalis, Trachymyrmex zeteki, Vollenhovia emeryi, Wasmannia auropunctata*; *Apis cerana, Apis dorsata, Apis florea, Apis mellifera, Bombus impatiens, Bombus terrestris, Ceratina calcarata, Dufourea novaeangliae, Eufriesea mexicana, Euglossa dilemma, Habropoda laboriosa, Lasioglossum albipes, Lepidotrigona ventralis, Megachile rotundata, Melipona quadrifasciata*; *Polistes canadensis, Polistes dominula*; *Cryptotermes brevis, Nasutitermes exitiosus, Zootermopsis nevadensis*
Features amenable to lab rearing, including fast generation time, numerous offspring, etc.	High variability across groups, but many species are easy to maintain in lab colonies, and social species produce many offspring	*Solenopsis invicta* (Banks et al., 1981), *Bombus impatiens* (Cnaani et al., 2002), *Polistes metricus* (Daugherty et al., 2011), *Polistes fuscatus* (Gibo, 1974)

highlight is the presence of a reproductive division of labor between reproductive queen(s) and non-reproductive worker(s), as this is a defining feature of eusociality that is absent in non-eusocial ancestors.

Criterion 1: the focal trait can be environmentally induced in ancestral-proxy lineages

Reproductive division of labor has been induced experimentally in multiple solitary and subsocial species through forced association studies, where typically non-associating females are forced to cohabit. Many examples come from the small carpenter bees (Sakagami and Maeta, 1984, 1989, 1987) and sweat bees (Jeanson et al., 2005, 2008), groups that show high levels of social plasticity and may be especially useful for assessing genetic accommodation (Jones et al., 2017; Kocher and Paxton, 2014; Shell and Rehan, 2017). In some cases, a single species displays both solitary and social forms (Davison and Field, 2016; Smith et al., 2003; Soucy and Danforth, 2002), and in situations where these forms are

Table 2. Criteria for establishing plasticity-first evolution in natural populations (adapted from Levis and Pfennig, 2016), with examples from social insects

Criterion	Example from social insect literature	Taxa/references
Focal trait can be environmentally induced in ancestral-proxy lineages	Induction of castes in artificial multi-female nests of solitary or subsocial species	*Ceratina japonica* (Sakagami and Maeta, 1984, 1987), *Ceratina okinawana* (Sakagami and Maeta, 1989), *Lasioglossum* spp. (Jeanson et al., 2005, 2008)
Cryptic genetic variation is uncovered when ancestral-proxy lineages experience the derived environment	Indirect evidence: inducible social phenotypes, and some genetic differentiation between social and solitary forms	*Ceratina japonica* (Sakagami and Maeta, 1984); *Ceratina flavipes* (Sakagami and Maeta, 1987); *Lasioglossum albipes* (Kocher et al., 2013)
Focal trait exhibits evidence of evolutionary change in regulation/form in derived lineages	Evidence of positive selection on caste-related genes in multiple lineages Social lineages exhibit increases in transcription factor binding site strength/presence and increased numbers of methylated genes	*Solenopsis* spp. (Hunt et al., 2012), *Temnothorax longispinosus* (Feldmeyer et al., 2014), *Apis mellifera* (Harpur et al., 2014; Hunt et al., 2010), *Megalopta genalis* (Jones et al., 2017) 10 bee species comparison (Kapheim et al., 2015b)
Focal trait exhibits evidence of adaptive refinement in derived lineages	Highly specialized queen and worker castes in complex eusocial lineages	Most ant species, *Apis mellifera*

environmentally determined, exploration of this variation may be particularly useful in studies of genetic accommodation.

Criterion 2: cryptic genetic variation is uncovered when ancestral-proxy lineages experience the derived environment

The capacity for variation in eusocial behavior can be uncovered through experimental manipulations of the social environment, as mentioned above for criterion 1. In the primarily solitary bee *Ceratina japonica*, artificial induction of multi-female nests results in a division of labor among females, including skew in reproduction between adults (Sakagami and Maeta, 1984). Similar multi-female nest induction in the related bee *Ceratina flavipes* results in fewer nests that successfully rear brood, but those that do also show evidence of a rudimentary caste system (Sakagami and Maeta, 1987). Whether successful multi-female nests contain females with cryptic genetic variation (see Glossary) enabling division of labor has not been explored. However, populations of the facultatively eusocial *Lasioglossum albipes* with different social forms display genetic differentiation, suggesting that variation in sociality may be facilitated by genetic variation (Kocher et al., 2013). As genomic tools become available for many social insect species, investigations of how cryptic genetic variation influences environmentally induced trait variation will be a critical step in studies of genetic accommodation in social insects.

Criterion 3: the focal trait exhibits evidence of evolutionary change in regulation and/or form in derived lineages

Levis and Pfennig (2016) suggest that both genetically accommodated and assimilated traits will exhibit changes in the slope of the reaction norm (see Glossary) when comparing derived with ancestral-proxy lineages. Genetically assimilated traits would additionally have fixed reaction norms across different environments, compared with more flexible phenotypes in the ancestral-proxy species (Levis and Pfennig, 2016). Finally, the mechanisms underlying changes in reaction norms should be evident, such as changes in hormonal signaling, *cis*-regulatory elements and alternative splicing (Levis and Pfennig, 2016). This criterion is well supported from multiple indirect lines of evidence in social insects. Among obligately eusocial insects, division of labor is fixed compared with ancestral-proxy lineages in which colonies can exist in multiple states, including those without division of labor (e.g. the colony-founding phase of single *Bombus* queens) or species with both solitary and social forms (e.g. *Megalopta genalis* and *Lasioglossum albipes*: Kocher and Paxton, 2014). Many caste-related genes show evidence of positive selection in ants (*Solenopsis* spp.: Hunt et al., 2012; *Temnothorax longispinosus*: Feldmeyer et al., 2014) and bees (*Apis mellifera*: Harpur et al., 2014; Hunt et al., 2010; *Megalopta genalis*: Jones et al., 2017; cross-species comparison: Woodard et al., 2011) relative to genes not related to caste expression. These results suggest a change in usage of these genes relative to their expression in solitary ancestors that affects their evolutionary rates. In addition, computational analysis shows that changes in gene regulatory capacity correlate with the level of eusociality, in two different contexts. First, there are predicted increases in the strength and prevalence of transcription factor binding sites in gene promoters of species with increased levels of eusociality (Kapheim et al., 2015b). Second, there are greater predicted numbers of methylated genes associated with increased levels of eusociality, and the role of gene methylation in controlling expression and splicing in social insects further suggests greater regulatory tuning in social species

(Kapheim et al., 2015b). These *cis*-regulatory and transcription factor differences suggest that genes related to eusociality have experienced evolutionary change in both sequence and regulation.

Criterion 4: the focal trait exhibits evidence of having undergone adaptive refinement in derived lineages

If selection has acted to increase the frequency with which a trait is expressed through genetic accommodation, that trait should experience adaptive refinement (see Glossary) as a result of more consistent exposure to selection (West-Eberhard, 2003). Therefore, genetically accommodated traits in derived lineages should be superior versions of the trait compared with those in ancestral-proxy lineages (Levis and Pfennig, 2016). In the majority of social insect species, a comparison of fitness in solitary and eusocial nests is not possible; however, alternative social strategies of the facultatively eusocial *Megalopta genalis* were found to have similar levels of fitness and to co-exist in evolutionary models based on field-based parameters, potentially explaining the maintenance of facultative eusociality in this species (Kapheim et al., 2015a). Further studies that rear solitary and eusocial species in competition may help elucidate whether eusocial traits have undergone adaptive refinement more directly. In addition, several indirect lines of evidence support the idea that eusociality has undergone adaptive refinement in derived lineages, including extensive elaborations of form and function in queen and workers castes. In some derived lineages, workers have lost the ability to mate and are sterile, while queens have reproductive capacities that are orders of magnitude greater than those of their ancestral-proxy counterparts. This specialization of castes would likely not be possible without the fixed sociality present in these complex eusocial species, where queen and worker traits have been exposed to selection for millions of years. Species with flexible castes or facultative eusociality are less consistently exposed to selection, and do not display the same features as complex eusocial species, suggesting a greater capacity for adaptive refinement in lineages with complex and obligate eusociality. Elaborate chemical communication systems have also evolved in many of these complex eusocial groups, leading to less overt competition over reproduction and highly specialized nestmate recognition systems, providing further evidence for adaptive refinement in derived lineages.

Eusocial engineering

Most studies providing empirical support for genetic accommodation employ artificial selection on experimentally induced phenotypes, some of which may not occur in nature (Suzuki and Nijhout, 2006; Waddington, 1942, 1953). Other empirical studies demonstrate phylogenetic relationships between environmentally sensitive phenotypes in ancestral lineages and more fixed phenotypes in derived lineages (Heil et al., 2004; Santana and Dumont, 2009; West-Eberhard, 2003), but do not examine the underlying mechanisms or show evidence of allele frequency change associated with fixation of the phenotype. Here, we outline a new method that builds upon and extends these approaches.

As discussed above, evidence across the social insects suggests that phenotypic plasticity may have facilitated the origin of eusociality through genetic accommodation. However, many of these lines of evidence come from different lineages and distinct origins of eusocial behavior, making it difficult to assess whether plasticity-first evolution has taken place. We suggest that particular social insect groups can be used to test the predictions of plasticity-first evolution, coupling manipulative experiments with deep molecular probing of the mechanisms involved in shifts between

social forms. Specifically, we propose using experimental environmental induction of novel social traits (as in Table 2, criterion 1) to test the hypothesis that genetic accommodation can act on existing plasticity in social evolution. We call this approach 'eusocial engineering' (see Glossary), and hope that it will enable better understanding of how environmentally induced phenotypes may be involved in gains and losses of eusocial behavior.

Fig. 1 outlines two approaches to eusocial engineering. The forward eusocial engineering technique (Fig. 1A) is as follows. For a species closer to the solitary end of the social spectrum with potential for non-lethal association of females (i.e. communal or facultatively eusocial species), nests are established with pairs or trios of age-matched, mated females of the same generation (mimicking a semisocial origin of eusociality; Michener, 1974), or mother and daughter(s) groups (mimicking a subsocial origin; Michener, 1974). Nests are monitored until successful generation of brood, which may occur in a subset of nests. After emergence of offspring, founding females are analyzed to examine changes in gene expression and epigenetic regulation, and offspring are used to establish new nests to artificially select for high fitness in the induced social environment. While the molecular approaches we suggest may be expensive and require practical considerations,
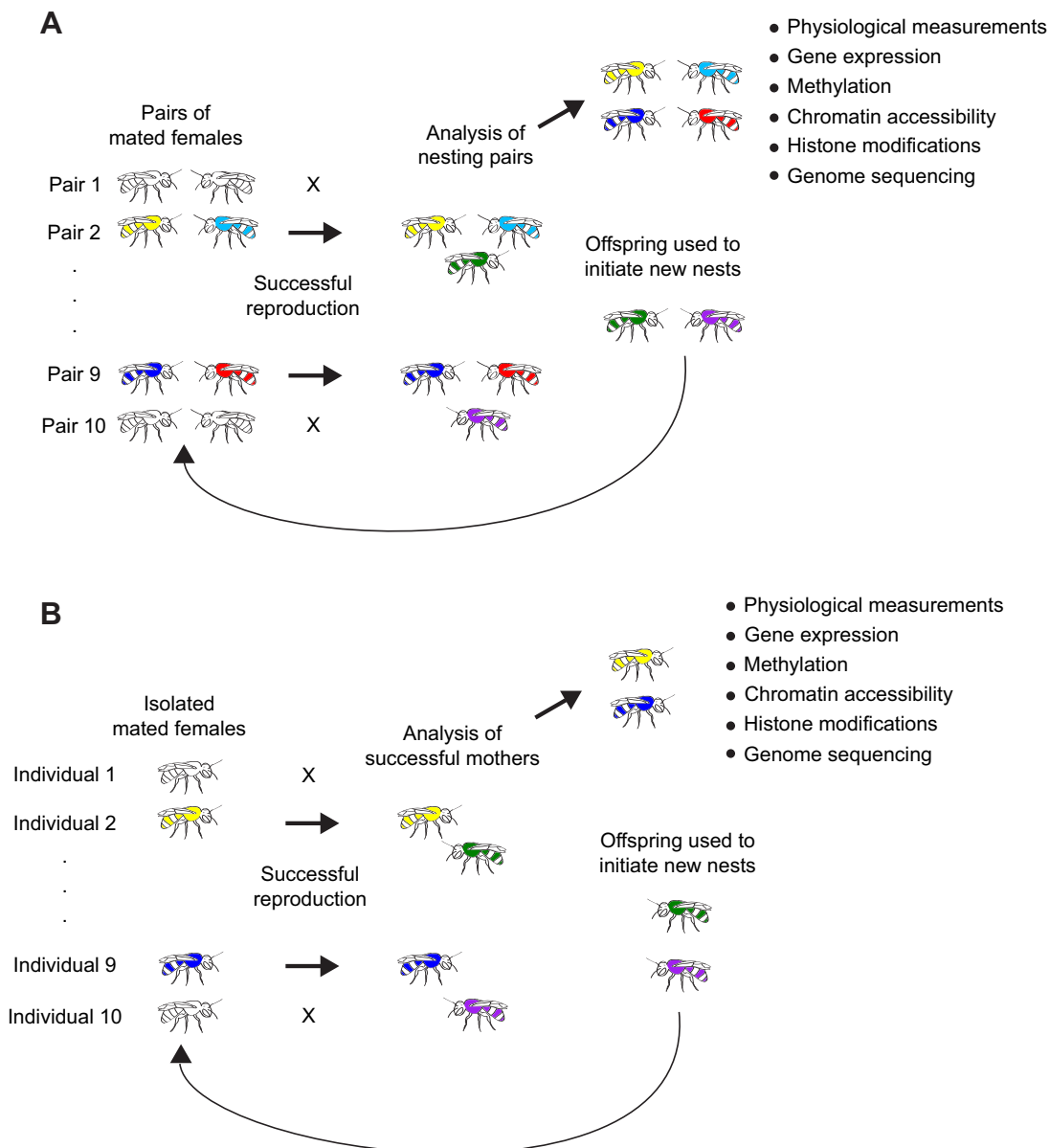

Fig. 1. Schematic diagram outlining forward and reverse eusocial engineering. (A) Forward eusocial engineering. The first generation includes multiple pairs of individuals, some of which may successfully produce offspring (colored pairs, arrows; some pairs fail to produce offspring, denoted by 'X'). Offspring of these successful pairs (shown in green and purple) will be paired again in the next generation, to either successfully produce another generation or fail to produce offspring. This pairing and selection scheme continues for many generations, with sampling of successful pairs throughout to identify transcriptomic, epigenomic and allele frequency changes associated with selection for cooperation. (B) Reverse eusocial engineering. The first generation includes isolated females, some of which may successfully produce offspring (shown in yellow and blue, arrows; some individuals fail to produce offspring in isolation, denoted by 'X'). Offspring of successful females (shown in green and purple) will be isolated again for the next generation. Much like in forward eusocial engineering, successful individuals can be sampled throughout to identify transcriptomic, epigenomic and allele frequency changes associated with selection for solitary reproduction. Many variations of this scheme are possible depending upon the species of interest.

sequencing costs and more tractable techniques for non-model organisms are being developed with increased frequency, bringing these methods within reach of many biologists.

Throughout a study, observations would identify behavioral division of labor, and successful females would be preserved for physiological and molecular measurements. Genotyping of offspring (for females, this would be conducted after their own nests have completed a brood cycle) would identify which founding female successfully reproduced, with ovarian dissection of all females to assess reproductive potential. Transcriptomics on collected foundresses would look for differences in gene expression associated with variation in phenotype induced by the social environment. Genes with plastic expression associated with the social induction would be candidates for selection through genetic accommodation. In addition to differences in gene expression, novel regulation of genes can be assessed by examining differences in chromatin accessibility and methylation using a number of techniques (e.g. ATAC-seq: Buenrostro et al., 2013; ChIP-seq: Barski et al., 2007; bisulfite sequencing: Clark et al., 1994) to address which molecular mechanisms have enabled the plasticity in behavior induced by the novel social environment. Sampling of females throughout many generations of artificial selection would allow a direct test of genetic accommodation by assessing whether candidate genes exhibit allele frequency change or novel variants following selection. In early generations, transcriptomic differences may be observed without reinforcement from epigenetic mechanisms. Epigenetic reinforcement may be expected later, with eventual accommodation of changes resulting in allele frequency change relative to the starting population of individuals. Careful genetic surveying of populations before and after the selection regime would be necessary to understand the role that cryptic genetic variation might play in the emergence of social traits. Longitudinal studies of laboratory bacterial evolution provide excellent experimental guides for this work (e.g. Bohannan and Lenski, 2000).

Reverse eusocial engineering (Fig. 1B) would use species with higher social complexity, and involve the initiation of nests with single females followed by selection on those individuals that successfully reproduce under solitary conditions. As with the forward eusocial engineering scheme above, molecular techniques would be used to assess mechanisms associated with reversions to solitary living, as well as test for genetic accommodation for the environmentally induced solitary phenotype. An additional approach could use selected lines from forward eusocial engineering as a starting point, with molecular monitoring to address how eusociality may be lost when females are forced to rear offspring in isolation.

The foundational behavioral aspects of the forward eusocial engineering approach have already been established, as forced association studies have been successful in multiple species of both ants and bees (Fewell and Page, 1999; Rissing and Pollock, 1986; Sakagami and Maeta, 1987). Reverse eusocial engineering has less behavioral precedence, but eusocial behavior has been lost multiple times in some groups (e.g. Danforth et al., 2003), suggesting that reversion to solitary behavior may be a common phenomenon and therefore important to study. Other work has successfully manipulated the social environment (Robinson et al., 1989, 1992; Ross and Keller, 2002), including changing colony demographics in 'pseudomutant' colonies and comparing the performance of this artificial construct with naturally formed colonies (Wilson, 1985), as well as many instances of queen removals to induce worker reproduction across ants, bees and wasps (e.g. Dietemann and Peeters, 2000; Jones et al., 2017; Reeve and Gamboa, 1987). What is unique about the eusocial engineering approach is the coupling of these manipulative studies with artificial selection and multiple genetic, transcriptomic and epigenetic monitoring approaches, enabling real-time tracking of the plastic and heritable components of environmentally induced traits. With recent advances in transgenic approaches in social insects (Schulte et al., 2014; Trible et al., 2017; Yan et al., 2017), eusocial engineering could be followed by genome editing to directly test the effects of any discovered genetic and epigenetic variants that are found to be associated with transitions in social behavior. We expect that eusocial engineering will be valuable in testing the role of genetic accommodation and phenotypic plasticity in eusocial evolution.

Outside of social insects, phenotype engineering has been previously introduced and implemented in birds; hormonal manipulations were utilized to change behavior and physiology in the dark-eyed junco (Ketterson and Nolan, 1992). This work suggests an approach similar to eusocial engineering may also be fruitful in testing genetic accommodation in non-insects.

Conclusions

An emphasis on plasticity-first evolution does not diminish the importance of mutation-first mechanisms of evolutionary change, but rather adds a potential avenue for scientists to explore for comprehensive analyses of the evolution of complex traits. Selection pressure is agnostic to the mechanism leading to the phenotype, such that individuals that inherited fitness benefits via reversible epigenetic means would survive equally well as individuals with a genetic mutation permanently altering the phenotype, all else being equal. In changing environmental conditions, however, a plastic response may be more advantageous and lead to maintenance of a transgenerational mechanism of inheritance. By contrast, if a population experiences a stable environment, individuals with a fixed and genetically determined phenotype may ultimately edge out those with plastic responses, either because of the costs of plasticity or because of differences in the reliability of phenotypic expression (DeWitt et al., 1998). In this case, the population may experience allele frequency change, completing the plasticity-first model of evolution.

Plasticity-first models of evolution arose before our current knowledge of the possible mechanisms of genetic accommodation, but growing understanding of epigenetics and transgenerational plasticity allows us to now test the predictions of these models. Social insects are well suited for this goal, and an empirical approach that combines behavioral manipulations with 'omics work will open the door to understanding how transcriptional plasticity in the ancestors of eusocial species may have facilitated the evolution of eusocial traits. With this approach applied more broadly in other taxa, we believe that the time has come to consider the role of genetic accommodation more rigorously, in order to determine its significance as a driver of evolutionary change. In doing so, we will not only broaden our understanding of the role of phenotypic plasticity in the origin and elaboration of novel traits but also provide a framework by which multiple modes of evolution may work in concert to influence adaptation.

Competing interests
The authors declare no competing or financial interests.

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