

**Patterns of reproductive differentiation and reproductive plasticity in the major evolutionary transition to superorganismality**

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## Highlights

- The major transition to superorganismality has occurred several times in insects
- Understanding superorganismality requires a multi-level selection approach
- Plasticity is transferred between levels of complexity across this transition
- Mechanistic patterns of reproductive control across insect sociality are elusive

## Abstract

Major evolutionary transitions in individuality are characterised by the formation of new levels of biological complexity from the cooperation of previously independent lower-level units. The evolution of superorganismality in insects is one such major transition, and is characterised by an extreme division of reproductive labour between ancestrally autonomous units, in the form of queen and worker castes. Here, we discuss the nature of plasticity in the emergence of castes across the major transition to superorganismality in insects. We identify key changes in plasticity which act at different levels of selection: a loss of reproductivity plasticity at the individual level is matched by a gain in plasticity at the colony level. Taking multi-level selection into consideration has important implications for formulating testable hypotheses regarding the nature of plasticity in a major transition from a lower to a higher level of biological complexity.

## Introduction

The evolution of life on earth has been shaped by a number of major transitions in individuality [1,2]. Each of these transitions has involved the formation of new, more complex individuals from the cooperation of previously independently-replicating units [1,2]. Canonical major evolutionary transitions include the transition from independently-living unicells to multicellular organisms [3,4], the formation of the eukaryotic cell from the conjunction of prokaryotic cells [5,6], and the evolution of highly cohesive superorganismal insect societies from solitary ancestors [7\*\*,8\*\*].

Despite having played a large role in the evolutionary history of life, major evolutionary transitions in individuality have proven difficult to study. Transitions such as the formation of the genome and the formation of the eukaryote cell occurred in the distant past, and probably only once each. By contrast, the transition to complex insect sociality has occurred more recently, and multiple times independently, in the corbiculate bees, termites, ants and vespine wasps [7\*\*,9]. Superorganismal colonies are easily decomposed and manipulated, and therefore provide excellent models with which to study major evolutionary transitions and unravel the proximate and ultimate foundations that underlie major shifts in individuality [8\*\*].

Here, we review the role that reproductive plasticity has played in the transition from a solitary to a superorganismal lifestyle. A fundamental aspect of each major transition is the functional differentiation and division of labour among the lower-level units that together constitute a new higher-level unit of individuality [1,2]. The evolution of multicellularity, for example, has involved a transition from phenotypically flexible unicells to higher-level organisms with an obligate division of reproductive labour between germ and somatic cells [3]. This is mirrored in the transition to insect superorganismality, which is defined by a reduction in phenotypic plasticity and a division of reproductive labour between reproductive queens and non-reproductive workers [7\*\*,8\*\*].

We argue that the emergence of a fixed reproductive division of labour does not so much represent a loss of reproductive plasticity among lower-level units but a transfer of plasticity between levels of selection – from the individual to the colony. We review the proximate mechanisms that may underlie this shift and the ultimate pressures that may have driven it.

## **Social organisation and reproductive plasticity**

Insect sociality covers a broad spectrum of social systems of varying levels of social complexity. While a range of classification systems have been proposed [7\*\*,10,11], most insect species broadly fall into one of three categories (Box 1). Solitary breeding is characterised by individuals that are able to reproduce independently and represents the likely ancestral state for all social lineages. Cooperative breeding involves the formation of social groups with division of reproductive labour but in which most individuals retain a significant degree of reproductive flexibility. Finally, superorganismal species exhibit an irreversible division of reproductive labour, cementing the colony as the unit of selection. Here we outline the broad trends in reproductive differentiation and reproductive plasticity across the major transition from cooperative breeding to superorganismality (Figure 1).

### **Shifts in reproductive plasticity across solitary and cooperative breeders**

Solitary breeding is the likely basal state (or 'ground-plan') for all social insect lineages. Solitary insects perform reproductive tasks sequentially, switching frequently between them in response to environmental cues such as seasonal fluctuations in the availability of resources [12,13] or expected mortality risk [14,15], as well as in response to their physiological state. The sequential execution of reproductive and non-reproductive tasks by the same individual, and the capacity to modify their expression in response to external and internal conditions, implies high reproductive plasticity at the level of the individual. This, however, comes at the cost of trade-offs when the optimisation of the phenotype to the opposing selective demands of each task is limited by the time and energetic costs of continuously remodelling traits [16,17]. These costs will be particularly large in unpredictable environments and for traits that take a long time to modify [18]. The need to facultatively switch between reproductive modes should be reflected in the mechanisms of reproductive differentiation in solitary species (Box 2).

Intriguingly, among the solitary insects with the most pronounced sequential division of reproductive labour are progressively provisioning Eumenid (e.g. *Synagris cornuta* & *Zethus miniatus*) or Sphecid wasps (e.g. *Ammophila pubescens*), which are likely to represent the ancestral state of the social wasps. After laying eggs, females in these species go through extended periods of brood care and provisioning while having diminished ovaries, in effect cycling between distinct periods of reproductive and non-reproductive labour [19–22\*]. It has been proposed that the uncoupling of reproductive and non-reproductive phenotypes in solitary insects such as progressively provisioning

wasps has acted as a ground plan for the division of labour that occurs in cooperative insect societies [21–24].

A prerequisite for this major transition is the transition from solitary to cooperative breeding, which involves a shift from sequential to parallel task execution in the form of a division of reproductive labour: cooperative groups are able to divide reproductive and non-reproductive tasks between multiple individuals. Thus, cooperatively breeding insect groups typically contain one (or a few) dominant individual(s) that performs the vast majority of reproductive activity on the nest, and a (usually larger) number of non-reproductive individuals that perform most foraging, nest building and offspring care. This parallelisation of roles likely represents an efficient solution to the trade-offs between reproductive and non-reproductive investment [25,26], although it also entails a source of potentially costly conflict in fitness interests between reproductive and non-reproductive specialists.

Reproductive and non-reproductive phenotypes within typical cooperatively breeding insect societies are significantly more stable than those expressed within the lifetime of a solitary individual; yet, substantial task plasticity persists in these societies. Non-reproductives are capable of upregulating reproductive traits in response to the loss of a dominant reproductive across cooperatively breeding bees [27,28], wasps [29,30] and termites [31]. Nonetheless, the rate at which individuals are able to transition between roles appears to be limited. While behavioural changes may be apparent within days [27,28], time to physiological upregulation of reproductive tissues appears to be in the order of weeks or months [30,31]. Additionally, there is evidence that non-reproductive individuals may experience a loss of reproductive plasticity as they age, for example in *Polistes canadensis* [32] and *Metapolybia cingulata* [33] paper wasps. The transition from solitary to cooperative breeding thus appears to involve some loss in reproductive plasticity, but of a minor degree: the majority of individuals in a cooperatively breeding society will remain reproductively totipotent for the majority of their adult lives. This should be reflected in the proximate mechanisms underlying plasticity at this level of sociality (Box 2).

### **Transfer of plasticity from the individual level to the colony level in the superorganismal society**

The major transition from cooperative breeding to superorganismality is itself defined by the evolution of obligate and irreversible differentiation of reproductive phenotypes and non-reproductive phenotypes, cementing the reproductive division of labour [7\*\*,8\*\*]. Superorganismal castes are

determined during development, after which non-reproductive individuals are unable to transition to a fully reproductive role. This reduction in reproductive plasticity commits non-reproductives to indirect fitness strategies and shifts the target of selection from the individual to the colony. Selection will then favour traits that maximise indirect fitness gains, opening the door for strong task specialisation. Accordingly, superorganismal castes are typically morphologically differentiated, exhibit a reversal of the usual negative trade-off between reproductive activity and longevity [34], and may even include specialised phenotypic subdivisions of the non-reproductive caste. Relative to reproductively plastic organisms, the mechanisms that underpin reproductive differentiation in superorganismal species are expected to include a significantly larger role for high-stability molecular mechanisms (Box 2).

Adult individuals in superorganismal colonies are much less reproductively plastic than adult individuals in cooperative species, which themselves are somewhat less reproductively plastic than solitary individuals. It is therefore tempting to infer a relatively straightforward pattern of decreasing reproductive plasticity across the spectrum of insect sociality from solitary to cooperatively breeding to superorganismal insect species. Such a pattern only holds, however, when one focuses exclusively on a single level of individuality, namely that of the individual adult organism. The nature of a major evolutionary transition is that it results in the generation of a higher level of individuality: reproductive plasticity must therefore be considered at both the level of the individual organisms that constitute the superorganismal society and the higher level of the superorganism itself.

There are significant analogies between the organisation of complex multicellular organisms and the organisation of superorganisms: the queen is the reproductive tissue of a superorganism, while workers are its somatic tissue [35,36]. Just as a solitary multicellular organism can be said to possess reproductive plasticity if it is able to facultatively vary its investment into reproductive and non-reproductive traits, the superorganism is reproductively plastic if it has the capacity to facultatively vary investment into the production of workers and sexual offspring. The ability of a superorganism to engage in this kind of plastic response is a function of its sociogenome, a composite of the genetic traits of all individuals that together constitute the superorganism and the interactions of those traits to produce colony-level feedback loops [37,38].

Sociogenome-mediated interactions between constituent individuals allow superorganisms to plastically mediate reproductive investment in much the same way that multicellular organisms do. Though the individuals within a superorganism are not perfectly analogous to the cells of a multicellular individual – the latter being fully clonal and therefore almost entirely insulated from internal reproductive conflict, while the former retain the potential for conflict since workers are usually not clonal and are often capable of producing males despite having limited fertility – the high degree of task parallelisation and specialisation has facilitated similar adaptations in each system. Like multicellular organisms, superorganismal colonies may upregulate investment into reproduction (i.e. the production of sexuals) in response to seasonality and may store energy within non-reproductives to later transfer to sexual brood [39,40]. Both multicellular organisms and superorganisms typically transition from somatic to reproductive investment having reached some growth threshold [40\*]. Ant colonies appear to possess a form of terminal investment, switching to the production of sexuals in response to queen mortality [25,42]. Honeybee colonies respond to the loss of a queen by rearing replacement queens [43]. Thus, while superorganismal colonies lack the individual-level reproductive plasticity necessary for non-reproductives to directly replace reproductives, at the superorganism level these colonies are able to plastically respond to the loss of a queen.

Viewed at a single level of selection, the transition to superorganismality might be generalised as a simple reduction in reproductive plasticity, producing individuals that cannot adapt their reproductive roles in response to changing environments. However, such an approach would overlook the opposing patterns of plasticity at the different levels of selection. Specifically, we highlight here that the transition should be viewed as a transferral of plasticity from the lower-level organism to the higher-level superorganism. Just as it would be meaningless to argue that an adult mammal lacks phenotypic plasticity because it is largely comprised of unipotent cells, so too must we recognise that the transition to superorganismality can only be understood by considering the superorganism as a unified whole that is the greater than the sum of its parts.

## **Conclusions**

The major evolutionary transition to insect superorganismality is one of our best models for understanding the way in which lower-level units are able to come together to form new (higher) levels of biological complexity. Here we highlight that the current view of the process of the major transition to superorganismality overlooks the effects of multi-level selection. We have briefly

summarised how the shifts in reproductive division of labour have made this radical shift in complexity possible. The patterns of differentiation involved in this transition are likely to be of general relevance to other shifts in individuality, such as the transition to multicellularity. In order to fully appreciate these commonalities, however, social insect researchers must embrace the status of the superorganismal colony as an individual in its own right.

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## Box 1: Levels of insect sociality

[Accompanying images uploaded separately]

**Solitary insect species**, in which individuals reproduce independently and rely heavily upon direct reproduction to pass on their genes, comprise the majority of all insects. Solitary breeding is the basal state for all insect lineages.

**Cooperatively breeding insect species** (commonly referred to as ‘primitively eusocial’ species) are those in which individuals form cohesive groups with division of labour between reproductive and non-reproductive individuals. Within such species, some proportion of individuals are reliant upon indirect fitness gains from altruistic behaviour directed towards relatives. However, commitment to reproductive roles within such societies is not complete: individuals retain the plasticity to switch between non-reproductive and reproductive roles. Model examples of cooperatively breeding insects include halictid bees, paper wasps and many ‘lower’ termites.

**Superorganismal insect species** (commonly referred to as ‘complex eusocial’ species) are those in which reproductive roles are irreversibly determined during development, resulting in extremely cohesive groups in which the majority of individuals are morphologically distinct workers. Superorganismal species include honeybees, vespine wasps, most ‘higher’ termites, and the large majority of ant species.

Not all species will fit squarely within these categories: some insects may facultatively switch between solitary and cooperatively breeding social systems dependent upon prevailing ecological conditions, for example in the case of carpenter bees and sweat bees [44\*]. Nonetheless, the majority of species possess a social structure that is approximately described by one of these three broad categories and there are clear adaptations that differentiate the three groups from one another, most notably the presence of reproductive division of labour and the subsequent obligate fixation of that division of labour [38,45]. As such it is possible to make meaningful predictions about the proximate and ultimate factors that have contributed to the evolutionary transition between each category.

It is well-established that the transition to superorganismality represents a major evolutionary transition in individuality [1,2,6], but disagreement exists regarding the exact nature of this transition. It is possible to conceptualise the major transition as spanning the entire range of sociality from solitary to superorganismal species [45], with cooperatively breeding species representing stable but

approximately intermediate stages within this transition. A recent set of publications have argued against this view, proposing instead that there is a unique and qualitative evolutionary discontinuity between cooperatively breeding and superorganismal species [7,46,47]. Within this model, the transition to cooperative living is only a difference of degree, whereas the transition to superorganismality is a difference of kind: as such, only the latter is a true major evolutionary transition in individuality.

In this paper we have employed terminology that reflects this latter model, referring to ‘cooperatively breeding’ and ‘superorganismal’ species to emphasise a qualitative distinction between the two systems. Nonetheless, it should be noted that the continuous model of insect social evolution remains significantly more prominent in the social insect literature than the discontinuous model that we have advocated here. Descriptors that imply a continuous scale of social complexity, such as simple (or primitive) and complex (or advanced) eusociality, are therefore widely employed in the literature at present. Regardless of which model best describes the true nature of this major evolutionary transition, species must necessarily pass through a cooperatively breeding stage with plastic castes before they can reach the point of obligate, irreversible caste differentiation, so we consider all three stages here.

**Photo credits, clockwise from top left:** *Ammophila nigricans* © Judy Gallagher ; *Andrena* sp. © Judy Gallagher ; *Polistes dominula* © Dimitri Dresde ; *Vespula vulgaris* © S. Rae ; *Nasutitermus luzonicus* © Diaz Geo ; *Atta* sp. © Gordon Milligan ; *Apis mellifera* © Phin Hall ; *Lasioglossum* sp. © Katja Schulz

## **Box 2: Proximate mechanisms of plasticity across the spectrum of sociality**

Although every social lineage has a unique evolutionary history, there are likely to be broad commonalities in the mechanisms that underpin reproductive differentiation at each level of insect social evolution. As reproductive roles become less flexible across the transition from solitary breeding to superorganismality, we predict that more stable ('committed') mechanisms of differentiation (such as epigenetic modifications) will become increasingly important relative to more changeable mechanisms (such as hormonal regulation). The development of more sophisticated bioinformatic and molecular assays is now making it possible to test this prediction [8\*\*]. Several possible mechanisms of reproductive differentiation and molecular commitment, and the evidence for their roles across the transition to superorganismality, are reviewed below.

### **Hormonal regulation**

As hormones can be produced quickly and are relatively short-lived, hormonal regulation of reproductive differentiation might be expected to be most prominent in solitary species that must switch frequently between reproductive modes. In practice, however, the weight of evidence suggests that endocrine regulation remains highly important at all levels of sociality. Juvenile hormone (JH) is the primary regulator of reproduction in basal solitary insects [48,49\*], including in progressively provisioning wasps that may represent the basal state for wasp sociality [50,51]. This role appears to be maintained in both cooperatively breeding and superorganismal species. JH is associated with reproductive differentiation in cooperatively breeding bees [52], termites [53] and wasps [54,55]; and likewise in ants [56,57] and honeybees [58,59]. It thus appears that JH is a mediator of reproductive differentiation across insect lineages of all degrees of sociality. However, we predict that in less reproductively plastic lineages additional mechanisms will be necessary to stabilise the expression of reproductive and non-reproductive phenotypes.

### **DNA methylation**

Methylation offers a stable means of altering gene expression levels. Drawing comparisons with mammalian development (in which levels of epigenetic modification increase with increasing cell differentiation) Patalano et al 2012 [60] have proposed that reduced reproductive plasticity in the transition to insect sociality ought to be associated with increasing evidence of methylation, while

Maleszka et al 2014 [61] have emphasised the potential role of methylomic degeneracy in producing functionally divergent castes from a single genome. In the last decade the role of methylation in social insect differentiation has been the topic of extensive research [62–64], but it has proven difficult to establish a consistent pattern of association between methylation and social complexity [65,66\*]. In cooperatively breeding species, methylation appears to be associated with caste in some species of wasps [65] and bees [67]. Two separate studies, however, have found no significant reproductive role for methylation in the cooperatively breeding paper wasps *Polistes canadensis* [68] and *Polistes dominula* [69], and indeed both these species appear to have lost a key methyltransferase gene. Whether this loss of epigenetic machinery is a lineage-specific trait or rather a more general signature of social complexity remains unknown. In superorganismal ants, several papers have linked caste to levels of methylation [70–72], but the methodology of these and other methylation studies has been called into question [73].

### **Histone acetylation**

Histone acetylation alters the accessibility of chromatin, and thus joins DNA methylation as a possible stable source of reproductive differentiation. To our knowledge there is no evidence of a role for histone acetylation in reproductive differentiation in solitary or cooperatively breeding insect species. The discovery of histone deacetylase inhibitor (HDACi) as a component in the royal jelly of honey bees suggests a role for this mechanism in superorganismal reproductive caste determination [74], as does the recent finding that caste-specific chromatin modification patterns are present early in honey bee development [75\*]. Royal jelly also contains noncoding microRNAs (miRNAs), another possible reproductive regulator [76]. Histone acetylation additionally appears to play a role in the determination of ant worker subcastes [77,78], strengthening the evidence that this mechanism is suited to the generation of stable phenotypes.

### **Genetic differentiation**

In general, allelic variants that bias individuals towards either a reproductive or a non-reproductive role should become fixed or lost, since reproductives have a clear advantage over non-reproductives in passing on their genes directly. Several exceptions to this rule have, however, become known in both ants [79] and superorganismal termites [80]. Such examples probably represent a form of evolutionary ‘cheating’ by queen-biasing variants and are unlikely to be widespread mechanisms of caste differentiation.

**Figure 1:** The evolutionary transition from solitary to cooperative breeding involves a shift from sequential execution of reproductive and non-reproductive tasks by individuals to parallel execution of tasks by groups of cooperating individuals, but with little or no loss of reproductive plasticity: at this stage in the major transition, most or all group members are able to switch between tasks if the opportunity arises. The transition from cooperative breeding to superorganismality is defined by a dramatic and putatively irreversible loss of reproductive plasticity at the level of the lower-level organism, but the simultaneous generation of a higher-level superorganism with its own colony-level plasticity. Arrows indicate the likely ancestral trajectory of modern superorganismal species; note we do not wish to imply an inevitable evolutionary trajectory for extant solitary or cooperatively breeding species.

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**Figure 1**

