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Review

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Parent-of-origin effects, allele-specific expression, genomic imprinting and paternal manipulation in social insects

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Haplo-diploidy and the relatedness asymmetries it generates mean that social insects are prime candidates for the evolution of genomic imprinting. In single-mating social insect species, some genes may be selected to evolve genomic mechanisms that enhance reproduction by workers when they are inherited from a female. This situation reverses in multiple mating species, where genes inherited from fathers can be under selection to enhance the reproductive success of daughters. Reciprocal crosses between subspecies of honeybees have shown strong parent-of-origin effects on worker reproductive phenotypes, and this could be evidence of such genomic imprinting affecting genes related to worker reproduction. It is also possible that social insect fathers directly affect gene expression in their daughters, for example, by placing small interfering RNA molecules in semen. Gene expression studies have repeatedly found evidence of parent-specific gene expression in social insects, but it is unclear at this time whether this arises from genomic imprinting, paternal manipulation, an artefact of cyto-nuclear interactions, or all of these.

This article is part of the theme issue 'How does epigenetics influence the course of evolution?'

1. Introduction

In this review, we explore the role of epigenetic inheritance and genomic imprinting in the evolution of social insects. Our goals are fourfold: (i) To describe the kinship theory of genomic imprinting (KTGI) [1–5] for diploid organisms and to contrast this with haplo-diploid social insects [6,7]. (ii) To extend the KTGI theory by suggesting that in social insect species where queens mate multiply, males may be selected to directly manipulate gene expression in their daughters to the detriment of the daughters of other males. (iii) To describe the epigenetic mechanisms by which parents might influence the reproductive success of their daughters. (iv) To summarize the known evidence for parent-specific gene expression, and for parent-of-origin effects on phenotypes, particularly reproductive phenotypes. By these means, we hope to show that epigenetic inheritance plays an important role in shaping the structure of insect societies.

A companion paper in which we describe the role of epigenetic effects on the evolution of caste (i.e. queen and worker dimorphism) in social insects also appears in this issue [8]. Some background information on epigenetic mechanisms appears in the companion paper, which we do not repeat here. However, we reference that paper where it may be helpful in understanding this one.

2. The kinship theory of genomic imprinting—diploid organisms

With the exception of the sex chromosomes, diploid organisms have two alleles at every locus, one inherited from the father (the individual's 'patrigene') and one inherited from the mother (the individual's 'matrigene') [7]. For most genes, it is

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advantageous for the patrigene and the matrigene to be similarly expressed. First, for any given gene there is likely to be an optimal expression level, and both the patrigene and matrigene are selected so that their combined expression level equals that expression level [1]. Second, equal expression provides some protection against potential defects in one or other allele [9]. Third, there may be heterozygote advantage—when the simultaneous expression of both alleles confers greater fitness than expression of a single allele, even if the level of the expression is held constant [10].

Despite the general conclusion that equal expression of patrigenes and matrigenes is expected, Haig [1–3] has pointed out that there are some circumstances in which the selective forces acting on the expression level of a particular gene differ depending on whether the gene is in its patrigenic or matrigenic form. Such genes are likely to be those that help the offspring gain additional resources from their mother. Mothers provide benefits to their offspring that help the offspring survive and thrive [11]. At a minimum, an animal mother bequeaths her yolk-filled eggs to her offspring. But maternal commitments can be much more expensive: the massive endosperm provided by some plants, or the nutrients transferred to developing embryos during gestation by mammals are obvious examples [12]. Beyond this, a mother may need to provide months or years of lactation or other maternal care.

In cases where a gene is involved in an offspring acquiring or soliciting resources from its mother, one needs to consider the fitness of matrigenes and patrigenes separately. The reason for this is that a mother's genes are in all her offspring with equal frequency $r_m = 1/2$. By contrast, when females mate with multiple males (polyandry), a father's genes are only present in his own offspring $r_p = 1/2$. They are not present in his daughter's half-sisters $r_p = 0$. This means that there can be selection on a gene to be differentially expressed depending on whether it is in its patrigenic or matrigenic form.

Consider a parental investment that provides a benefit B to an offspring at a cost C to other offspring. Increased investment by the mother increases the fitness of patrigenes whenever $B > r_pC$ [5,6,13]. Since r_p is less than $1/2$ under polyandry, this equation can be re-written as $B > (1/2n)C$ where n is the typical number of lifetime mates of a female [5]. r_m is always $1/2$ so matrigenes and patrigenes are under divergent selection whenever $1/2\ C > B > (1/2n)C$. Note that if the selection is successfully acting on the patrigene to increase its expression, then there may also be selection on the matrigene to decrease its expression level. In what has been called 'loudest voice prevails' [1], this can lead to complete silencing of the matrigene and optimal (from the patrigene's perspective) expression of the patrigene. A gene that is differentially expressed in its patrigenic and matrigenic form is said to be 'imprinted' [1–3,14,15]. The word arises because it is necessary that such a gene be epigenetically marked in some way so that it is expressed appropriately in its matrigenic and patrigenic form in the offspring. About 100 imprinted genes are known from mammals [16].

3. The kinship theory of genomic imprinting in haplo-diploid social insects

Social insects differ from most other animals in that the nurturing of offspring (the brood) is performed not by the mother, but by the collective workers. This means that differential selection on matrigenes and patrigenes is not directed towards gaining additional resources from the mother (the queen), but from the colony as a whole. There are large fitness benefits at stake for those females that can become reproductive (either as queens or as laying workers) [17–19]. As we shall see, the genetic architecture of insect colonies means that the selective pressures on matrigenes and patrigenes are often different—and this provides conditions in which genomic imprinting could potentially evolve [1,7,20,21].

(a) Monandrous species

Bees, wasps and ants are haplo-diploid; the females are diploid and the males are haploid [22]. Haplo-diploidy generates conditions suitable for the evolution of genomic imprinting, even in the absence of polyandry, a necessary condition in diploid organisms [1,3]. In most social insects, queens are monandrous—they mate with a single male [23], and there is only one queen per colony [24]. In these species, all workers in a colony share 100% of their patrigenes and 50% of their matrigenes. This means that patrigenes are equally present in the sons of a given worker ($r_p = 1/2$) as they are in the sons of that worker's sisters ($r_p = 1/2$) (figure 1*a*, [20]). This also means that there is no selection on patrigenes to increase the reproductive success of their bearer beyond what is optimal for the colony. Reproduction by an unmated sister is equally valuable to a patrigene as personal reproduction by its bearer [7,26]. Not so for matrigenes. A matrigene in a focal worker has a $1/4$ chance of being in the son of a sister ($r_m = 1/4$) and a $1/2$ chance of being in the worker's own son. So genes that are related to worker reproduction are predicted to be selected to increase reproductive behaviour in their matrigenic form and not in their patrigenic form in monandrous, haplo-diploid eusocial insects [26].

This kind of selection, if it exists, is particularly likely to occur in bumblebees. In bumblebees, workers initially cooperate to produce female workers and queens, all of which are daughters of the founding queen. However, at a certain point in the colony's development, it switches its focus from the production of workers to the production of new queens and males. Some or many of these males are worker laid, particularly if the queen dies [27–29]. Furthermore, worker mothers are often reproductive parasites from other colonies [30,31]. Thus, particularly after the colony commences male production, we might predict that bumblebee matrigenes are under selection to increase the reproductive success of their bearers, which could result in the evolution of genomic imprinting. And indeed, there is some evidence for imprinting in bumblebees. First, there is evidence of biased parent-of-origin gene expression in *Bombus terrestris* [26,32]. Second, genes with maternally biased expression are enriched for reproductive functions [26]. Third, there is some association between DNA methylation, a molecular mark that could potentially be used to distinguish patrigenes and matrigenes, and gene expression [32]. We conclude that these findings are supportive of a role for genomic imprinting via DNA methylation in bumblebees, while not being definitive. For that, we would need to show a strong association between methylation marks and maternal origin of over-expressed matrigenes.

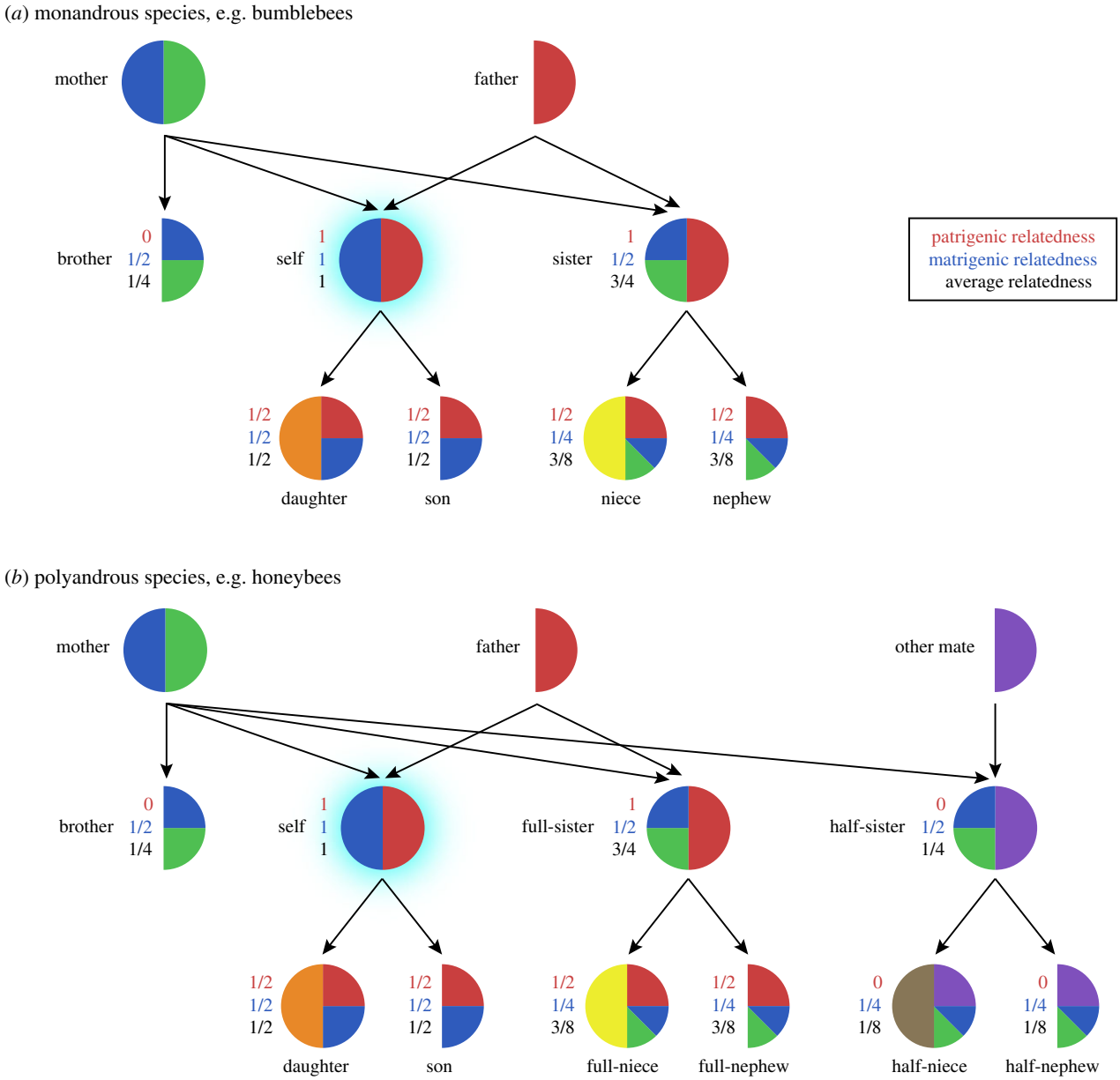

Figure 1. Pedigree showing coefficients of relatedness (patrigenic, matrigenic and average) in haplo-diploid social insect colonies. The relatedness coefficients for the individual labelled 'self' and highlighted with a light blue background to her brothers, sisters, nephews and sons are indicated. Patrigenic genes (red) are distinguished from matrigenic genes (blue) in the highlighted individual. (*a*) Monandrous species. Matrigenic genes have a probability of being present in the focal worker's son of 1/2, but only 1/4 in the sons of sisters. By contrast, patrigenic genes are present in nephews and sons with equal frequency 1/2. Therefore, there is no selection for patrigenes to evolve differential expression to favour worker reproduction, but there is selection on matrigenes to do so. (*b*) Polyandrous species. The focal worker is in a colony with a single queen that has mated with two males. As with monandry, matrigenic relatedness to sons is 1/2 and 1/4 in nephews. However, patrigenic relatedness to full sisters is 1 and 0 to half-sisters. This selects for imprinting of paternal alleles that enhance worker reproduction. Reciprocally, because the colony as a whole is disadvantaged by worker reproduction, matrigenes may be imprinted to reduce it. Under a manipulation model, fathers are predicted to epigenetically manipulate their daughters reproductive success as they are unrelated to the non-daughters in the colony. In the F_2 daughters, the orange, yellow and brown genomes are derived from unrelated fathers. Modified from Queller & Strassmann [25].

(b) Polyandrous species

In some of the most highly eusocial species like neotropical army ants [33], African driver ants [34], leaf-cutter ants [35] and honeybees [36], queens are extremely polyandrous (10–100 matings per queen). Polyandry changes the coefficients of patrigenic relatedness within colonies while matrigenic relatedness is unchanged. The decline in patrigenic relatedness changes the likelihood of imprinting evolving away from matrigenes towards patrigenes [6,7,20,21]. Matrigenes are present in all of a colony's workers with equal frequency ($r_m = 1/2$). By contrast, under polyandry, patrigenes are only present in a particular male's daughters $r_p = 1$. They are not present in half-sisters $r_p = 0$ (figure 1). Therefore there is the potential for selection on patrigenes to be expressed in ways that maximize the benefits that their worker bearers can extract from living in a colony of half-sisters, while minimizing their payments to the common pool [21].

More formally, the fitness of a patrigene will be increased if the benefit of patrigenic expression in a given worker exceeds the total costs of this expression to other workers (i.e. the total loss to the colony):

$$r_p B > \overline{r_p}\, C,$$

where $\overline{r_p}$ is the average patrigenic relatedness of workers in

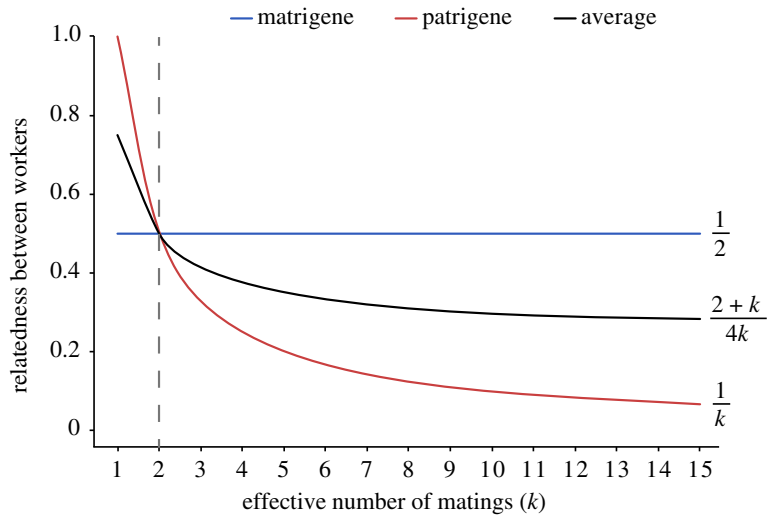

Figure 2. Patrigenic, matrigenic and average relatedness of workers in a colony as a function of the effective number of matings (k) of the queen.

the colony. In the context of this inequality, consider a worker carrying an imprinted patrigene providing a fitness benefit B to that worker. $r_p = 1$ to that worker and her full sisters, but the average relatedness to patrigenes in the totality of workers in the colony, $\overline{r_p}$, is $1/k$ where k is the effective number of matings (figure 2). This means that the costs to the patrigene of any selfish behaviour it causes are rapidly discounted as k increases, but there is no change in the benefit to the patrigene.

For a maternally imprinted gene, we have:

$$r_m B > \overline{r_m} C.$$

Here the relatedness of the matrigene to other matrigenes in the colony is 1/2. Therefore, matrigenic imprinting for selfish behaviour only evolves if the benefits exceed the costs to the average worker, which is unlikely.

Figure 2 shows how the relatedness of matrigenes between sisters, $\overline{r_m}$, remains constant as 1/2 for all levels of polyandry. But the average relatedness of a patrigene rapidly declines with increasing mating frequency to near 0 (figure 2). Analogous arguments can be made for those ant and termite species where there are multiple single-mated queens in a colony [24]. Here, both matrigenes and patrigenes can be subject to selection for imprinting [7,21,37].

There is, of course, an unresolved question here. If we accept the argument that in monandrous species like bumblebees and stingless bees, there can be selection for matrigenic imprinting and over-expression of genes related to worker reproduction because matrigenes are more related to genes in sons than in nephews [26], then this argument should also hold for polyandrous species. However, the strength of selection for imprinting would be much stronger for patrigenes than for matrigenes in polyandrous species because patrigenes are completely unrelated to genes in half-sisters whereas matrigenes are always related by 1/2 (figures 1 and 2).

4. Direct manipulation of gene expression by male parents

The KTGI is a gene-centred hypothesis that focuses on the different kinds of selective pressures on genes in their matrigenic and patrigenic forms in offspring. Another potential way in which genomic conflicts may play out in social insects is the possibility that in polyandrous species fathers are selected to directly influence gene expression in their daughters in ways that enhance the likelihood that their daughters become reproductive. Note that we are not suggesting conflicts between queens and fathers, but between individual fathers. The difference between imprinting and paternal manipulation is subtle. Under an imprinting model, the gene itself is under selection to alter its expression between its patrigenic and matrigenic forms. All that the parents must do is provided an epigenetic mark to the imprinted gene that designates its provenance (figure 3). Under a paternal manipulation model, the differentially expressed gene itself is not under selection, but fathers manipulate its expression in the early embryo via epigenetic processes transferred in semen. Importantly, in haplo-diploids, all genes present in a father are present in his female offspring with equal frequency. Thus, there is unlikely to be a conflict between manipulating genes in the father and any of the patrigenes of their offspring. By contrast, matrigenes may be under selection to avoid manipulation by fathers [1,38].

There are at least three plausible epigenetic mechanisms by which paternal manipulation could occur, which we discuss below. Explanations of these mechanisms are provided in the companion paper [8] and in [39–41].

(a) DNA methylation

In diploid mammals where imprinting is present (e.g. mice and humans), DNA methylation is an important means by which patrigenes and matrigenes are distinguishable in the embryo, leading to the differential expression [16,42]. DNA methylation is also a candidate as an imprinting and/or paternal manipulation mechanism in social insects, and the mechanism on which the most work has been done. Methylation patterns are heritable in social insects [43–45] and often reflective of the underlying DNA sequence [46]. Interestingly, in the thelytokous Cape honeybee, diploid female embryos produced asexually (without a father) have lower methylation levels than sexually produced diploid female embryos with a father [47]. Cape honeybee males might methylate the genome of their sperm cells to influence their expression in offspring. Since methylation is a mutagen [48], methylation of sperm DNA is likely to have a fitness cost, suggesting mitigating

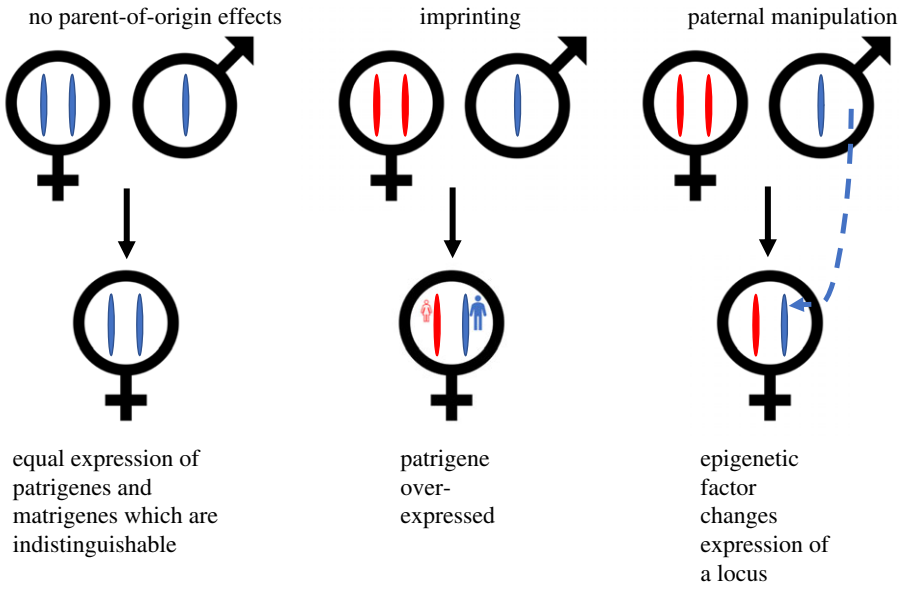

Figure 3. Contrasting models for parent-of-origin effects in haplo-diploid species. Imprinting occurs if a locus is epigenetically marked to identify its parent of origin and is under selection to be expressed differently in its patrigenic and matrigenic form. Under parental manipulation one parental sex may be selected to influence gene expression in the embryo, perhaps by including small RNA molecules in the eggs, sperm or seminal fluid.

fitness benefits for fathers engaging in methylation of their sperm [43]. Paternal manipulation and/or imprinting could explain why paternal methylation occurs despite its costs.

The problem with this interpretation, as we have discussed in the companion paper [8], is that there is little evidence that methylation affects gene expression in social insects [43,44,49,50]. Further, when Smith *et al*. [51] examined genes that showed strong Parent-of-Origin (PoO) expression differences in honeybees, they did not see an association with methylation—the most differentially expressed genes were hypo-methylated. We conclude that while it would be imprudent to rule out a role for methylation in paternal imprinting or paternal manipulation, there is currently no evidence that it does so.

(b) Small RNA molecules as a medium of paternal manipulation?

In *Caernorhabditis elegans*, small RNA molecules (18–32 bp long, hereafter s-RNAs) move between tissues and escape germline reprogramming [52], and by these means can be responsible for transgenerational epigenetic inheritance of immune functions that can last for 20 generations [53]. Tissue-specific s-RNAs are readily identified in insects generally [54] and social insects in particular [55,56]. s-RNAs are strong candidates for epigenetic inheritance in social insects and therefore provide a plausible mechanism by which one sex could directly influence the expression of a particular gene in their offspring.

There are no studies that have directly addressed the question as to whether s-RNAs are involved in paternal manipulation or genomic imprinting in social insects, but there are some intriguing hints. First, in honeybees, mandibular gland secretions fed to larvae by workers contain double-stranded RNAs that auto-propagate within the larva and cause gene knockdown that lasts until adulthood [57]. Second, the jewel wasp *Nasonia vitripennis* uses a maternally derived RNA of the sex-determining gene *transformer* to establish female development in embryos [58]. These facts demonstrate that s-RNAs can be transferred vertically and horizontally in Hymenoptera, including to embryos, where they can profoundly influence gene expression and development. It remains to be seen whether s-RNAs are responsible for the strong PoO effects observed in social insects.

(c) Histone marks

As we have noted in the companion paper [8], histone states affect transcription, and are propagated mitotically [59]. Transgenerational inheritance of histone states is known from yeast, plants and nematodes [60] but is thus far unknown from insects. We therefore have no evidence that it contributes to imprinting or paternal manipulation in social insects.

5. Evidence for parent-of-origin phenotypic effects in social insects

A PoO phenotypic effect may be defined as a tendency for offspring to resemble one parent more than the other. PoO effects are efficiently discovered by performing reciprocal crosses between two strains that have divergent phenotypes. The offspring of reciprocal crosses are genetically the same on average, so the direction of the cross should make no difference to their phenotype. However, if the direction (who is the father and who is the mother) makes a difference, then there is a PoO effect [61]. In the older literature of quantitative genetics, these were called 'reciprocal effects' [62] or 'maternal effects' [63].

To our knowledge, five studies have shown significant phenotypic PoO effects in social insects (table 1). When considering reciprocal crosses, it is important to note that it is not really possible to distinguish a positive paternal effect from a negative maternal effect and *vice versa*. For instance, Gibson *et al*. [70] performed reciprocal crosses between a gentle European strain of honeybees and a more aggressive Africanized strain. They waved leather patches in front of the hybrid colonies and found that the time to the first sting was shorter when the father was of the Africanized strain (i.e. these

Table 1. Studies showing parent-of-origin effects in social insects.

	species	evidence	reference
termite	*Reticulitermes separatus*	strong maternal effect for caste determination as shown by reciprocal crosses; this was originally interpreted as genetic caste determination based on a two-allele sex-linked locus	[64,65]
honeybee	*Apis mellifera*	strong paternal effect for stinging behaviour in reciprocal crosses	[66]
		strong paternal effect for queen-like characters in workers in inter-subspecies backcrosses	[67]
		strong paternal effect for ovary size in workers in inter-subspecies backcross to Africanized strain	[68]
		strong paternal effect for ovary size and presence of a rudimentary spermatheca in workers in inter-subspecies reciprocal crosses	[69]
		strong paternal effect for stinging behaviour in hybrids with Africanized fathers	[70]
		strong paternal effect for ovary size and likelihood of ovary activation in workers with an Africanized father in reciprocal crosses	[71]
		strong paternal effect for ovary size and likelihood of ovary activation in workers with an African father in reciprocal crosses	[72]
Argentine ant	*Linepithema humile*	significant maternal and paternal effects on worker behaviour as determined by reciprocal crosses	[73]

colonies were more defensive). They interpreted this as a *maternal* effect causing shorter times to stinging when the mother was European. But you can easily make the reciprocal conclusion that having an Africanized father decreases the time to first sting.

It actually makes more sense when the hybrid's phenotype is closer to one or other parent's to say the effect is from that parent. Indeed, Guzmán-Novoa *et al*. [66] interpreted similar findings to Gibson *et al*. [70] by concluding that hybrid colonies with an Africanized *father* were more aggressive, rather than that colonies with a European *mother* were more aggressive. Regardless, whichever way you wish to interpret these data it is clear that that the direction of the cross makes a difference to aggression, and the difference was in the same direction in both experiments. It is also clear that there is a PoO effect for ovary size in honeybees (table 1). This is exactly what one expects if there is paternal manipulation of daughter reproduction or genomic imprinting, whereas we see no reason why PoO effects for aggression would be expected.

6. What kinds of genes might be subject to imprinting and parental manipulation?

For most genes, the interests of male and female parents and patrigenes and matrigenes are precisely aligned—to build a better daughter—so imprinting or parental manipulation of offspring gene expression is unlikely to evolve in the majority of genes [12]. However, as we have seen, there can be differential selection on matrigenes and patrigenes, and fitness advantages for males to directly manipulate the expression of a minority of genes in their daughters. It is therefore useful to consider what kind of genes are more likely to be imprinted or paternally manipulated.

Female phenotypes that are likely to enhance the fitness of patrigenes and fathers [7] are those that:

(1) *Bias the reproductive output of a colony away from sons towards daughters* [74]. In haplo-diploid organisms, fathers are unrelated to sons of their mate; only to grandsons. So processes that lead to the imprinting of patrigenes can only spread via daughters. Imprinting or direct paternal manipulation of patrigene expression that affected sex ratios would be difficult or impossible to identify via expression studies, and measuring the effect of such genes phenotypically would be challenging.
(2) *Increase the likelihood that a daughter will activate her ovaries and produce larger numbers of eggs* [20]. This phenotype is readily measured as associations between paternity and proxies of daughter fecundity: number of ovarioles, frequency of ovary activation in queenless and queenright colonies, number of worker offspring, etc. All of these characters show strong paternal effects [19,69,75–80]. They are also selectable and heritable [81].
(3) *Increase the likelihood that the larval daughter will be reared as a queen* [82]. There is strong evidence that there is a weak relationship between paternity and the likelihood of being reared as a queen [83–87]. So genes that make some larvae more attractive for rearing as a queen, for example, by producing more brood pheromone that elicits larval feeding [67], are candidates for paternal manipulation and imprinting.

7. Allele-specific gene expression

Biased allele expression towards a patrigene or matrigene underlies PoO phenotypic effects. PoO expression effects can potentially arise from cyto-nuclear interactions and incompatibilities [88], from imprinting [61,70], or from parental manipulation, and these three causes can potentially be confounded. Cyto-nuclear incompatibilities arise because mitochondrial proteins coded in the nucleus must be co-adapted to the systems present in the mitochondria [89]. In inter-subspecies hybrids, the maternally derived part of

Table 2. Evidence for allele-specific gene expression in social insect workers.

	species	experiment	expression bias	reference
honeybees	*Apis mellifera*	reciprocal crosses between European and Africanized subspecies	46 transcripts showed allele-specific expression biases, the majority to the maternal allele	[91]
		reciprocal crosses between European and Africanized subspecies	parentally biased expression, particularly towards paternal alleles, particularly in workers with activated ovaries	[71]
		reciprocal crosses between *A. m. scutellata* and *A. m. capensis*	parentally biased expression, particularly towards paternal alleles in female embryos	[51]
bumblebees	*Bombus terrestris*	assays for allele-biased expression in three candidate genes	paternally biased expression of two genes associated with worker reproduction; maternally biased expression of a gene associated with suppression of worker reproduction	[92]
		assays for mono-allelic expression	19 genes were mono-allelically expressed and methylated; 555 loci showed putative allele-specific expression	[32]
		reciprocal crosses between strains	maternally and paternally biased gene expression with greater maternal bias	[26]
		RNAseq and bisulfite sequencing of reproductive and sterile workers	allele-specific expression, but no relationship to methylation patterns	[50]

the nuclear genome is co-adapted with the maternally derived mitochondrial genome, but the paternally derived part of the nuclear genome may be inappropriately matched to the maternally derived mitochondria and, as a result, certain alleles may be over or under-expressed in ways that reduce fitness in F_1 hybrids. Over-expression of maternal alleles is more likely than over-expression of paternal alleles when cyto-nuclear incompatibilities are in play [90] because the maternal genome is expected to be co-adapted to the cytoplasmic genome with which it has been co-inherited over evolutionary time [89]. By contrast, selection for imprinting of patrigenes or direct paternal manipulation to increase the reproductive success of daughters is more likely to cause over-expression of the patrigene relative to expression of the matrigene [7,51,71].

Though the number of studies and taxonomic breadth remains small at this time, there is strong evidence for allele-specific expression in social insect workers (table 2). Mostly, the expression bias is in the direction predicted under the KTGI (§3 and [1,7,93]), i.e. a bias towards the expression of the paternal allele, especially for genes related to worker reproduction in polyandrous species [51,70,71,92], but towards maternal alleles in monandrous species [26]. One study of a polyandrous species did report a bias towards the expression of maternal alleles [91], which might be indicative of cyto-nuclear incompatibilities. The jury is still out and more data are needed.

One final remark on allele-specific gene expression. In social insects, the 'loudest voice prevails' principle does not seem to hold as it often does in mammals [1]. RNAseq studies show biases in gene expression towards (mostly) the paternal or (less often) maternal allele in social insects (table 2), but rarely complete silencing. The significance of this is unknown, but it may suggest that allele-specific expression differences are a consequence of paternal manipulation rather than genomic imprinting. In mammals, imprinted genes often show complete silencing, and this is infrequent in social insects.

8. Conclusion

The focus of this review has been parent-of-origin effects, genomic imprinting and the possibility of parental manipulation. The evidence for PoO effects and allele-specific expression are strong, but the means by which they are mediated (genomic imprinting or direct parental manipulation) are unknown. We are also unclear about mechanisms. Although DNA methylation patterns are highly variable among male honeybees, and highly heritable [45], the evidence that methylation patterns influence gene expression or alternative splicing is being increasingly questioned (see §4 and [8]). Rather than DNA methylation, we suspect that if direct parental manipulation occurs, this will be mediated by s-RNA molecules secreted into the seminal fluid that go on to affect gene expression in the early embryo. However, there is a major caveat to this hypothesis. Hymenopteran queens store semen acquired from a single mating event throughout life. The spermathecal fluid contains substances secreted by the mother—which should give ample opportunity for queens to neutralize any molecules contributed by fathers.

Data accessibility. This article has no additional data.

Authors' contributions. B.P.O. and B.Y. wrote the paper jointly.

Competing interests. We declare that we have no competing interests.

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