

Ageing and the costs of reproduction: insights from
Euglossa viridissima, an orchid bee on the cusp
of sociality

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1. General introduction

1.1. The fecundity/longevity trade-off

Reproduce infinitely and live forever – such are the elusive traits of an imaginary “Darwinian Demon” capable of maximising all aspects of fitness (Law 1979). Trade-offs between the life history traits fecundity and longevity have however been demonstrated in a large number of studies spanning a wide range of taxa (Fowler & Partridge 1989; Kenyon 2010, Holliday 1994) and are thought to arise due to limited energetic resources and to the pleiotropic effects of central regulatory pathways (van Noordwijk & de Jong 1986; Leroi 2001; Flatt *et al.* 2005). While it is well established that trade-offs such as that between fecundity and longevity play a central role in shaping evolutionary life history trajectories, there is still limited understanding regarding how such trade-offs evolve (Houle 1991; Roff & DeRose 2001).

1.2. Remoulding of the trade-off in eusocial insects

Eusocial insects, characterised by a reproductive division of labour, remarkably seem to escape the widespread fecundity/longevity trade-off. Indeed, in eusocial insect colonies, reproductive individuals (queens, and in the case of termites, also kings) exhibit extraordinarily long lifespans, often hundreds of times longer than that of non-social insects of comparable body size (Heinze & Schrempf 2008), while their non-reproductive counterparts (workers, and in the case of termites, also soldiers) are relatively short-lived (Carey 2001). This goes against the idea that reproduction and lifespan trade off with one another. While the remarkable life histories of eusocial insects have been extensively studied, the mechanisms underlying the apparent remoulding of the fecundity/longevity trade-off with sociality remain poorly understood.

Mathematical models of resource acquisition and allocation suggest that the positive association between fecundity and longevity in social insects may be due to caste differences in resource acquisition (van Noordwijk & de Jong 1986), as queens are supplied with abundant resources by workers and may use these resources to maximise both reproduction and longevity. Experiments in bumble bees showed



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that when workers were left to “choose” whether or not to reproduce, those that did so lived longest, whereas when reproduction was experimentally induced in a random subset of workers, thus uncoupling reproductive investment from other traits, a negative association between reproduction and lifespan was observed (Blacher *et al.* 2017). This suggests that a positive association between reproduction and lifespan can be observed when only “high quality” individuals reproduce, and one may hypothesise that this is the case of social insect queens, which are fed high quality resources during development (von Rhein 1933; Smith *et al.* 2008).

While this may help explain the reshaping of life histories with sociality, it does not exclude the possibility of a remoulding of physiological pathways allowing eusocial insect queens to forego the costs of reproduction. Empirical studies have indeed supported the rewiring of multiple physiological and genetic pathways in eusocial insects, including pathways linked to ageing and/or reproduction, or even known to mediate the relationship between these two traits (Box 1).

Box 1. Proposed mechanisms underlying the unique life histories of eusocial insects:

remoulding of physiological and genetic pathways

Rewiring of endocrine pathways

A notable example of the remoulding of an endocrine pathway with sociality is the network comprised of the insulin-like/IGF1 (IIS), juvenile hormone (JH) and vitellogenin (Vg). An upregulation of this network is associated with increased reproductive investment at the expense of longevity in solitary insects, but remarkably, a rewiring of the network in eusocial insects may allow them to escape this trade-off (reviewed in Rodrigues & Flatt 2016).

Maternal heterochrony hypothesis

As queens and workers of a same species share the same genome, their vast differences in life histories may be underpinned by a shift in the timing of genetic pathways, such as those underlying maternal care. It has been suggested that in eusocial insects, pathways underlying presumably costly maternal care behaviours are expressed pre-reproductively towards siblings, thus effectively only in workers (Linksvayer & Wade 2005; empirical support: Toth *et al.* 2007; Rehan *et al.* 2014).

**Box 1.** (continued)**Caste-specific differences in somatic maintenance**

Empirical studies suggest that social insect reproductives invest more in somatic repair compared to their worker counterparts (Lucas *et al.* 2016), which may help explain the observed caste differences in ageing trajectories. Along these lines, studies in termites suggest that genes involved in transposable element silencing are downregulated with age in workers, but not in reproductives, which may also help explain caste-specific differences in longevity (Elsner *et al.* 2018)

1.3. Insights from facultatively social species

Corbiculate bees comprise over 600 species that span the social spectrum, including solitary, communal, facultatively social and obligate eusocial species (Noll 2002; Ramirez *et al.* 2010). They provide an excellent opportunity for comparative studies between solitary and eusocial nesting individuals to uncover the molecular mechanisms underlying the reversal of the fecundity/longevity trade-off which seemingly accompanies the transition to eusocial behaviour. Particular insight can be gained from facultative eusocial species, which allow direct comparisons between individuals that share a genotype and at times even environmental conditions, as solitary and social forms can sometimes be found within the same population (Cocom Pech *et al.* 2008; Rehan *et al.* 2014).

The first chapter of this thesis reviews possible insights to be gained from the study of facultatively social insects into the mechanisms underlying the transition to eusociality and the reversal of the fecundity/longevity trade-off. This thesis then focuses on the study of a facultatively social orchid bee species, *Euglossa viridissima* (Figure 1), with the aim of uncovering mechanisms underlying differential ageing in solitary *versus* social forms of this species, and shedding light on the costs of reproduction in relation to sociality. *E. viridissima* is part of the orchid bee tribe Euglossini, consisting of solitary, communal, parasocial or facultatively social species (Ramirez *et al.* 2010). Euglossini is the only tribe within the clade of the corbiculate bees (which also contain bumblebees, honeybees and stingless bees) that does not exhibit some form of advanced eusociality (Michener 2007), and although the position of Euglossini within the corbiculate bees has been widely debated, it is generally accepted



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that orchid bees were ancestrally solitary (Kawakita *et al.* 2008; Ramirez *et al.* 2010). This suggests that the facultatively social *E. viridissima* potentially represents an initial step in the transition from solitary to obligately eusocial, thus offering a unique opportunity to shed light on the mechanisms underpinning the remoulding of the fecundity/longevity trade-off in the early stages of social evolution.

Using next-generation sequencing, I explored transcriptomic signatures of ageing in solitary and social females from a same population in the Yucatán Peninsula of Mexico, as well as juvenile hormone titres in relation to age and sociality (Chapter 2). I then used an experimental approach to investigate potential differences in the costs of brood provisioning in social *versus* solitary forms of *E. viridissima* (Chapter 3).

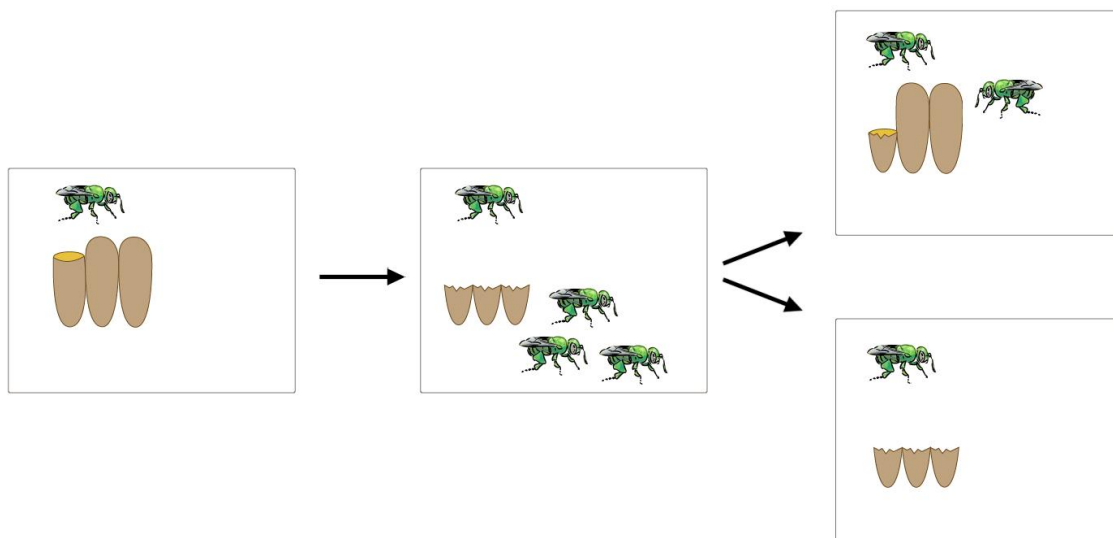


Figure 1. Life cycle of *Euglossa viridissima*. All nests are initiated by a solitary foundress (left). She builds brood cells and each cell is then filled with provisions on top of which a single egg is laid, then the cell is capped. The mother then remains in the nest until the brood emerges, *circa* 2 months later (center). Upon emergence of the first brood, either (top right) one or several daughters remain in the maternal nest and help the mother provision and care for a second brood, or (bottom right) all offspring from the first brood leave the maternal nest, supposedly to found their own nests.



Facultative social insects can provide insights into the reversal of the longevity/fecundity trade-off across the eusocial insects

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Abstract

In eusocial insects, reversal of the fecundity/longevity trade-off and extreme differences in life histories between castes of the same species garner scientific and public interest. Facultative social species at the threshold of sociality, in which individuals are socially plastic, provide an excellent opportunity to understand the causes and mechanisms underlying this reversal in life history trade-off associated with eusociality. We briefly present the ultimate factors favoring sociality and the association between fecundity and longevity in facultative eusocial insects, including kin selection and disposable soma, as well as proximate mechanisms observed in such species, such as differences in hormone titers and functions. Potential genetic underpinnings of lifespan and fecundity differences between castes are discussed and future research directions are proposed.

Key words: social plasticity, Hymenoptera, bee, ultimate, proximate, genetic



Introduction

In many organisms, a negative correlation exists between fecundity and longevity (Stearns 1992; Harshman & Zera 2007). This trade-off stems in part from the limited internal resources that organisms have at their disposal; an individual cannot invest at the same time in both reproduction and body maintenance (Harshman & Zera 2007; Braendle *et al.* 2011). Advanced eusocial insects like the honey bee (*Apis mellifera*), with perennial societies and marked worker-queen dimorphism, are a notable exception to this rule (Monroy Kuhn & Korb 2016; Rueppell *et al.* 2016); queens, the reproductive individuals, have high egg-laying capacities and extended longevity whereas non-reproductive workers have a relatively short lifespan (Keller & Genoud 1997; Keller 1998), a phenomenon termed reproductive division of labor.

Reproductive division of labor is a corollary of the highly fecund and long-lived reproductives of advanced eusocial insects. By outsourcing most of the risky tasks to workers, queens reduce their extrinsic mortality (*e.g.* exposure to predators and pathogens) as well as many of the costs linked to colony maintenance and provisioning of offspring (*e.g.* defense, foraging for brood food, brood care). For example, the intense flight activity of eusocial bee and wasp workers to collect brood provisions likely results in oxidative damage and hence shortens individual lifespan, as in solitary insects like *Drosophila* (Williams *et al.* 2008; Lane *et al.* 2014). In addition, ontogeny, morphology and physiology all differ tremendously between queens and workers in advanced eusocial species, which may also help explain caste differences in lifespan and fecundity (Rueppell *et al.* 2016).

The positive association between longevity and fecundity is also seen in primitively eusocial species (eusocial species with an annual life-cycle; Table 1) such as bumble bees and some sweat bees, with less marked worker-queen dimorphism and in which queens establish nests and provision brood alone at the start of the season (Figure 1; for discussion of the terminology concerning social organization, see Kocher & Paxton 2014). Clearly, mechanisms exist that allow reversal of the fecundity/longevity trade-off across a taxonomically wide range of species exhibiting a diversity of social organizations.

Facultative social species, in which social structure varies from solitary to social within a single species, provide an excellent opportunity for investigating the reversal of the longevity/fecundity trade-off.



For some species, sociality is a derived state whereas others are ancestrally social and secondarily solitary (Wcislo & Danforth 1997). They allow direct comparison of solitary and social phenotypes within a single species (*e.g.* *Halictus rubicundus* (Field *et al.* 2012); *Megalopta genalis* (Kapheim *et al.* 2012)). The absence of clear morphological and physiological differences between behavioral castes (worker-queen) in such societies even suggests plasticity in behavior; individuals may be able to switch between castes depending on the social and physical environment (Smith *et al.* 2009; Field *et al.* 2010). Individuals of such species that are seemingly able to switch between solitary and social behavior are also termed socially plastic.

Here we review variation in life history and aging across facultative social insects, particularly facultative eusocial species. First, we present a brief overview of the lifespan and life history traits in solitary, facultative eusocial and eusocial Hymenoptera, particularly focusing on bees (Hymenoptera, Apiformes) because eusociality has evolved four times independently within the taxon, with some species thought or known to be facultative eusocial (Kocher & Paxton 2014). Then we present the evolutionary (ultimate) forces and proximate mechanisms that may have promoted life history differences between individuals in facultative eusocial species. We discuss molecular mechanisms that might have led to life history variation among reproductive and non-reproductive individuals. Finally we outline directions for future research on facultative eusocial species that may help shed light on why advanced eusocial species like the honey bee show a marked reversal in the fecundity/longevity trade-off.

Life history traits and lifespan across a range of social organizations

Solitary aculeate Hymenoptera, including many solitary bees and wasps, tend to invest heavily in brood cell provisioning (Wilson 1971; Michener 1974). Adult female lifespan is generally short, with low per capita offspring production (Table 1).

Though a diversity of social organizations have been defined in the social insects (*e.g.* see Kocher & Paxton 2014), we focus on a number of key ‘levels’ of social organization, including the advanced



(and often perennial) eusocial species like the honey bee with cooperative brood care, overlapping generations, colony founding by swarming, discrete morphological caste differences (reproductive division of labor), and a well characterized reversal of the longevity/reproduction trade-off. In these, queens have extended lifespans whereas workers do not (Table 1). Primitive eusociality in bees and wasps comprises species with an (usually) annual life cycle in which queens found colonies alone at the start of the season (*i.e.* they act as solitary nest provisioners) but the colony then develops into a eusocial society with more-or-less differentiated castes (queen and worker, see Figure 1). Queen longevity in such species (*e.g.* *Bombus terrestris*, Table 1 and references therein) is far greater than in solitary species and also than that of their workers, but lower than in queens of advanced eusocial species (Table 1). Worker longevity in primitive eusocial bees and wasps is poorly known but does not seem to differ dramatically from that of solitary females (Table 1). In facultative eusocial species that exhibit either solitary or eusocial behavior, the lifespan of individuals following different behavioral paths is also poorly known (Table 1, Figure 1); better documentation of the longevity of additional species is needed to inform on whether eusociality *per se* is linked to extended lifespan of reproductives over non-reproductives or whether reversal of the trade-off evolved later in social evolution.

Ultimate explanations for life history differences between behavioral castes

Understanding the ultimate factors that favor the emergence and maintenance of eusocial behavior is important for gaining insight into the novel selection pressures which arise under altered social conditions, which in turn may lead to differences in life histories between social castes. The leading ultimate factors promoting the evolution of eusociality include kin selection (following Hamilton's kin selection theory, also known as inclusive fitness theory (Hamilton 1964; Mueller 1991; Bourke 2011; Yagi & Hasegawa 2012)), ecological variables which impact on the costs and benefits of exhibiting eusocial behavior (Korb & Heinze 2008) (*e.g.* levels of parasitism, availability of nesting sites (Wcislo & Cane 1996; Boff *et al.* 2015)), and parental manipulation of offspring (acting in concert with kin selection, (Kapheim 2015a); for an overview of ultimate factors, see Figure 2).



Eusocial life, by definition, includes reproductive division of labor, thus new selection pressures arise which favor fecundity and longevity differences between individuals of the same colony (Parker 2010). Intriguingly, one facultative eusocial species at the threshold of eusociality, *Halictus rubicundus*, also exhibits marked differences in lifespan, with foundress queens outliving workers (Table 1). Though these data suggest that reversal of the longevity/fecundity trade-off occurred early in social evolution, we note that *H. rubicundus* is likely ancestrally eusocial (Wcislo & Danforth 1997) and secondarily facultative eusocial; analysis of facultative eusocial species that are ancestrally solitary will help test whether the trade-off is concomitant with eusociality *per se*.

Once a eusocial colony is established, reproductive conflicts among nestmates often continue to be expressed (Korb & Heinze 2016); if these intracolony evolutionary conflicts are suppressed to a sufficient extent, the colony itself may be viewed as the unit of selection. Under this superorganism perspective (Keller & Genoud 1997; Rueppell *et al.* 2015), the queens can be viewed as analogous to the colony's 'stem cells'; they produce the majority of the colony's offspring, while workers provision the brood and help maintain the colony. Workers can therefore be considered as analogous to the soma (Kramer *et al.* 2016; Monroy Kuhn & Korb 2016). Disposable soma theory (Kirkwood 1977) thus provides one explanation for the long lifespan of queens in relation to workers, including in the facultative eusocial species, *H. rubicundus* (Table 1). The higher lifespan of queens is further promoted by the nest as a location for reproduction and brood care, which decreases a queen's extrinsic mortality, therefore allowing selection to favor increased longevity in queens (Keller & Genoud 1997; Heinze & Schrempf 2008; Johnson & Carey 2014; Southon *et al.* 2015).

According to other evolutionary theories of aging (mutation accumulation theory, antagonistic pleiotropy (Medawar 1952; Williams 1957)), extrinsic mortality is one of the most important factors driving the evolution of lifespan differences between castes (Heinze & Schrempf 2008). Workers, unlike queens, perform all the risky foraging tasks and are therefore more exposed to environmental risks such as predation, bad weather and diseases. They exhibit all the qualities of the disposable soma (Heinze & Schrempf 2008), as selection favors the production of a high number of poorly defended and disposable offspring. In addition, extended parental care, as exhibited by primitively eusocial foundresses, at least with respect to their first brood (Figure 1), theoretically promotes an increase in



their longevity (Figure 2); the maintenance of high fertility later in life through intergenerational transfers (*e.g.* resource provisioning at the proximate level) promotes the maintenance of high selection against senescence, which would otherwise decrease in species lacking parental care (Lee 2003).

Quantification of longevity differences between queens and workers of additional facultative eusocial species (*e.g.* *Euglossa viridissima*, Table 1) is needed to demonstrate the role of extrinsic mortality as an ultimate explanation for life history differences between queens and workers. Detailed within-colony observations of facultative eusocial species may also shed light on the importance of extended parental care in promoting long lifespan in individuals following the reproductive (queen) ontogenetic trajectory.

Proximate mechanisms for life history differences between behavioral castes

Hormones

Division of labor at the early stages of sociality is established and maintained mostly through behavioral dominance rather than through hard-wired physiological differences (Hartfelder 2000). In advanced eusocial species, in contrast, the principal proximate mechanisms at the endocrine level for longevity and fecundity differences among castes revolve around juvenile hormone (JH), ecdysteroid and insulin titers (see Bloch *et al.* 2002 for a review). JH in particular has been considered a major regulator of life history variation in advanced eusocial insects such as the honey bee due to its pleiotropic functions in insect development and reproductive physiology (Hartfelder 2000; Rodrigues & Flatt 2016). In highly eusocial species such as *A. mellifera* and ants, JH loses the gonadotropic function it has in solitary species and is mainly involved in generating polymorphic castes and controlling division of labor (Hartfelder 2000; Rueppell *et al.* 2015). Indeed, experimental application of JH to queens of the (advanced eusocial) ant *Lasius niger* actually reduced egg-laying and increased mortality, possibly via reduced immunocompetence (Pamminger *et al.* 2015).

An open question is whether JH and other endocrinological markers play an important proximate role as life history pacemaker at the brink of eusociality (for an overview of proximate factors, see Figure 2). In the facultative social *M. genalis*, JH and vitellogenin (Vg, the major egg yolk protein triggered



by JH, see Rodrigues & Flatt 2016) titers in queens are higher than in their worker or solitary counterparts, and reproductive females exhibit higher JH and Vg titers than non-reproductive females (Kapheim *et al.* 2012; Smith *et al.* 2013), as in *Drosophila*. In advanced eusocial species, long lifespan (and high reproductive output) is associated with low JH titer in queens whereas short lifespan (and low reproductive output) is associated with rising JH titer in workers. These data suggest that JH retains its gonadotropic function in early stages of social evolution and that the decoupling of JH and egg production only occurred later in advanced eusocial species. Analysis of JH titers in queen and worker castes of additional facultative eusocial species is needed to test whether JH acts as a fundamental pacemaker of aging at the very beginnings of eusociality, or whether it has been co-opted in that role only in advanced eusocial species, as the data on the facultative social *M. genalis* suggest (Smith *et al.* 2013); experimental manipulation of JH in facultative eusocial species might be a particularly productive avenue of research (see *e.g.* Pamminer *et al.* 2015), and would help test this hypothesis.

Mating status

Insemination has also been proposed as a proximate factor explaining life history differences between castes in advanced eusocial species, since transfer of sperm and seminal fluids can trigger oogenesis and affect longevity (honey bees (Engels 1974); ants (Schrempf *et al.* 2005; Oettler & Schrempf 2016)). Mating is thought to favor development of the queen phenotype in the facultative eusocial *H. rubicundus* (Yanega 1988; Yanega 1992) but not in the facultative eusocial *M. genalis* (Kapheim *et al.* 2012). Experimental studies in facultative eusocial species could help elucidate whether and how mating and insemination can act as important proximate factors in the early stages of sociality and longevity differences between castes.

Nutrition

Differences in food consumption between individuals have also been proposed as a proximate mechanism for caste determination and life history variation; in advanced eusocial species, female larvae receiving different amounts of protein and sugar exhibit differences in body size, amount of fat stores and hormone (JH and Vg) titers, which in turn predispose them to become either workers or gynes (Kapheim *et al.* 2011; Kapheim *et al.* 2012). This can be linked to the maternal manipulation of



larval food in societies with few workers; for example, in the facultative social sweat bee *M. genalis*, maternal manipulation of larval food leads to size differences in offspring, which predispose them to stay in the nest and work (Kapheim *et al.* 2015a). These predispositions can, indirectly, lead to differences in longevity between individuals *via* the different factors (other than nutrition) promoting life history differences between behavioral castes. Better nourishment may directly lead to extended lifespan within a caste rather than indirectly through caste differentiation, as seen in facultative eusocial orchid bee (*E. viridissima*) foundresses (May-Itzá *et al.* 2014); impacts on longevity of a restricted diet are not known.

Foraging activity and oxidative stress

Oxidative stress has been proposed as a mechanism underlying differences in life histories between castes in advanced eusocial species (honey bees (Corona *et al.* 2007; de Verges & Nehring 2016)), as it is related to activity and risk-taking, which are higher in workers than in queens. Differences in levels of oxidative stress can then be linked to differences in lifespan observed between castes (Figure 2). Moreover, differences in Vg titers between queens and workers can help explain the differences in oxidative stress levels found between these castes because of the antioxidant function of Vg, as has been demonstrated in honey bees (Seehus *et al.* 2006). However, this link has yet to be explored in facultative eusocial species.

Genetic underpinning of life history variation in facultative social insects

Interest in the genetic underpinning to the origin of female castes and the evolution of social behavior is growing (Elsner *et al.* 2016), driven in part by developments in sequencing technologies, and accompanied by multiple, non-mutually exclusive hypotheses (Rehan & Toth 2015) with which to interpret – to date – largely descriptive genomic and transcriptomic studies. Principal among hypotheses is the ‘ovarian’ or ‘reproductive ground plan hypothesis’ (West-Eberhard 1987; West-Eberhard 1996; Figure 3), which posits that gene networks regulating foraging and reproductive phases in solitary insects became decoupled in social insects; workers display the foraging phase whereas queens are



‘stuck’ in the reproductive phase (for support, see *e.g.* Amdam *et al.* 2006). An alternative, though not mutually exclusive, hypothesis is ‘maternal heterochrony’ (Linksvayer & Wade 2005), which posits changes in the timing of expression of genes underpinning offspring care; it does not necessarily require decoupling of gene networks/regulatory pathways underpinning foraging and reproduction (for support, see *e.g.* Rehan *et al.* 2014). Recent genomic analysis of 10 bee species spanning multiple origins of sociality rather suggests independent genetic pathways to social behavior in different lineages (Kapheim *et al.* 2015b).

Although genomic and transcriptomic analyses of facultative social species are scarce (but see Kocher *et al.* 2013; Jones *et al.* 2015), they offer the possibility to examine whether and how genetic differences underpin the early stages of caste differentiation and life history differences among totipotent individuals (Kocher & Paxton 2014; Rehan & Toth 2015). A recent study of eusocial societies, comparing transcriptomes and methylomes of individuals retaining plastic (queen/worker) phenotypes (*Dinoponera* ants and *Polistes* wasps), found surprisingly few differences at the transcriptional level; reproductive and non-reproductive individuals differed mainly in subtle aspects of their transcriptional network organization (Patalano *et al.* 2015), though the termite *Zootermopsis*, which exhibits considerable worker behavioral plasticity, differs markedly from ants and wasps in its caste-specific transcriptional profiles (Terrapon *et al.* 2014). Differences in DNA methylation among castes of ants and wasps – but not termites - also seemed to play a minor role (Patalano *et al.* 2015; but see Terrapon *et al.* 2014), possibly because of a reduced DNA methylation system in primitively eusocial species (Holman *et al.* 2016; Standage *et al.* 2016). It remains an open question as to whether changes in genetic programming during the transition from solitary to eusocial behavior, as well as those accompanying the extended lifespan exhibited by eusocial queens, are mostly subtle (Gadau 2015; Patalano *et al.* 2015; Rehan & Toth 2015). Facultative eusocial species may be a particularly fruitful group with which to address genetic programming.



Future directions

Facultative social species, particularly those in which individual behavior is plastic and can vary between solitary and eusocial life histories, provide excellent models for understanding – at ultimate and proximate levels – the reversal of the longevity/fecundity trade-off seen in advanced eusocial species. On the one side, facultative social species may highlight whether reversal of the longevity/fecundity trade-off in advanced eusocial species is a direct consequence of sociality *per se*, as opposed to changes induced by obligate eusociality, such as large colony size and a perennial life cycle. On the other, our review suggests that facultative eusocial species exhibit some of the life history and physiological traits fundamental to advanced eusocial species with morphological and physiologically hard-wired caste differences.

One important step will be to determine just how plastic are behavior and physiology in facultative social species; are individuals totipotent or do subtle differences in nutrition or morphology underlie behavioral castes and corresponding differences in life histories (Kapheim *et al.* 2012; Kapheim *et al.* 2013)? A second step is to move from descriptive studies, which by definition take a correlative approach, to manipulative experiments (Korb 2016), for which facultative eusocial species are ideally placed because individuals are likely plastic in responses to social, ecological or physiological cues. Factors that we have highlighted and that warrant attention include the roles of insemination, JH titer and flight activity (oxidative damage), all of which may underpin life history differences and that are known to differ dramatically between solitary and advanced eusocial species. Transcriptome analysis of behavioral castes may also help highlight proximate mechanisms regulating life history variation (Rehan *et al.* 2014; Patalano *et al.* 2015; Rueppell *et al.* 2015; von Wychetzki *et al.* 2015; Standage *et al.* 2016), especially when undertaken with experiments manipulating insemination, JH titer, flight activity or reproduction. In contrast, the role of methylation in modulating life history seems to be important for advanced but not primitively eusocial species (Patalano *et al.* 2015; Standage *et al.* 2016); it may play only a minor role in modulating life history differences in facultative eusocial species.

Thirdly, explicit consideration of the phylogeny of social evolution (Kocher & Paxton 2014; Rehan & Toth 2015) will help provide a more meaningful understanding of the reversal of fecundity/longevity



trade-offs in advanced eusocial species. For example, the two origins of eusociality in sweat bees (Halictidae) have led to one known species with a multi-annual life-cycle (*Lasioglossum marginatum*; Plateaux-Quénu 1959) and solitary behavior appears to be a secondarily derived trait in facultative eusocial halictine sweat bees (Wcislo & Danforth 1997; Gibbs *et al.* 2012). The two independent origins of eusociality in the family Apidae (in Apinae and in Xylocopini) have, in contrast, led to two advanced eusocial lineages (honey bees, stingless bees, both Apinae) and sociality may represent a derived trait in many facultative eusocial apid lineages (Cardinal & Danforth 2011); analysis of facultative eusocial apid species may therefore provide excellent insight into why and how advanced eusocial species seem to have escaped the longevity/fecundity trade-off of solitary species.

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**Table 1:** Life history traits of representative Hymenoptera varying in social behavior (for references, see supplementary material Appendix A, p. 92)

Species	Social organization	Climate and diapause	Experimental conditions	Longevity mean (range)	Principal factors affecting longevity	Size of nest (N workers)	N ovarioles per ovary	References
<i>Andrena vaga</i> (Andrenidae)	Solitary	Temperate, diapause not included in longevity	Field	15 days (4-38)	Climate, through climate-dependent activity patterns	n.a.	3?	Straka <i>et al.</i> , 2014
<i>Anthophora plumipes</i> (Apidae)	Solitary	Continental, diapause not included in longevity	Field	14 days (4-41)		n.a.	?	
<i>Eulaema meriana</i> (Apidae)	Solitary	Tropical, diapause not included in longevity	Lab	91 days (62-120)	?	n.a.	?	Ackerman & Montalvo, 1985
<i>Halictus rubicundus</i> (Halictidae)	Facultative eusocial	Temperate, hibernation included queen longevity	Field	Solitary female: 1 year Queens: 1 year Workers: few weeks	?	8	3	Yanega, 1988, 1990, 1993
<i>Euglossa viridissima</i> (Apidae)	Facultative eusocial	Tropical, diapause not included in the solitary females' longevity	Field and semi-natural	Solitary: 6 months Queens and workers: unknown	Temperature?	1-2	?	Dressler, 1982; Skov & Wiley, 2005; May-Itzá <i>et al.</i> , 2014
<i>Lasioglossum umbripenne</i> (Halictidae)	Primitive eusocial	Tropical or temperate, queen longevity includes 8 month dormant period	Field	Queens: 1 year Workers: 1 month	?	60-84	3	Wille & Orozco, 1970
<i>Lasioglossum marginatum</i> (Halictidae)	Primitive eusocial, multi-annual colony	Temperate, diapause included in queen and worker longevity	Field	Queens: 5-6 years Workers: 9 months	?	200-400	3	Plateaux-Quénu, 1959, 1962
<i>Bombus terrestris</i> (Apidae)	Primitive eusocial	Temperate, queen longevity includes 6-9 month diapause	Field and lab	Queens: 1 year Workers: 36 days working in the field; 72 days working in the nest	?	< 400	4	Roman & Szczesna, 2008; Goulson, 2010; Amsalem <i>et al.</i> , 2015
<i>Apis mellifera</i> (Apidae)	Advanced or perennial eusocial	Temperate and tropical, no diapause but longevity includes overwinter (where specified)	Field and lab	Queens: 1-2 years, max 8 Workers: 15-38 days in summer, 30-60 days in spring and fall, 150-200 days in winter	Climate, through climate-dependent activity patterns	10,000-20,000	> 150	Remolina & Hughes, 2008; Galizia <i>et al.</i> , 2012

n.a., not applicable

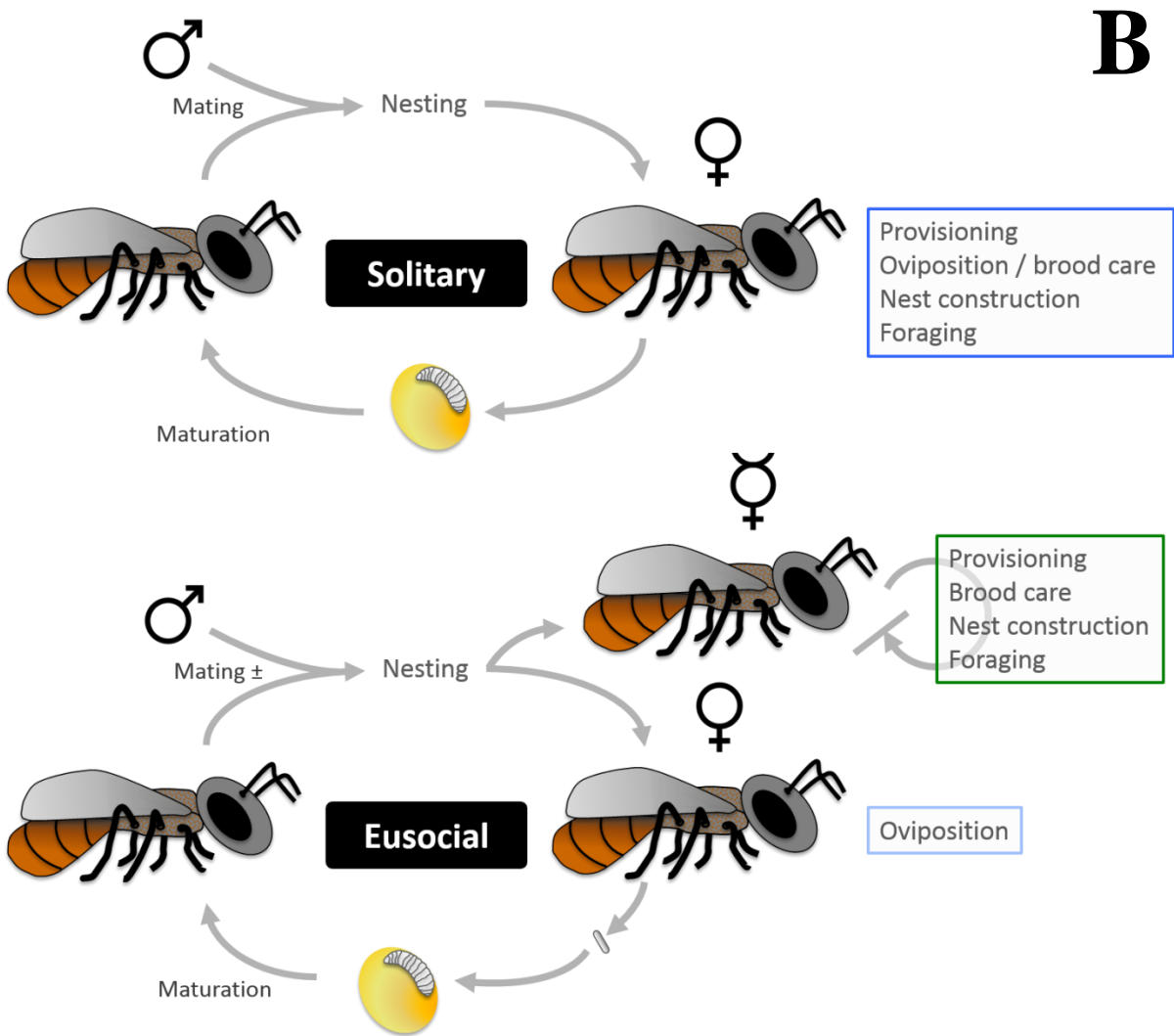
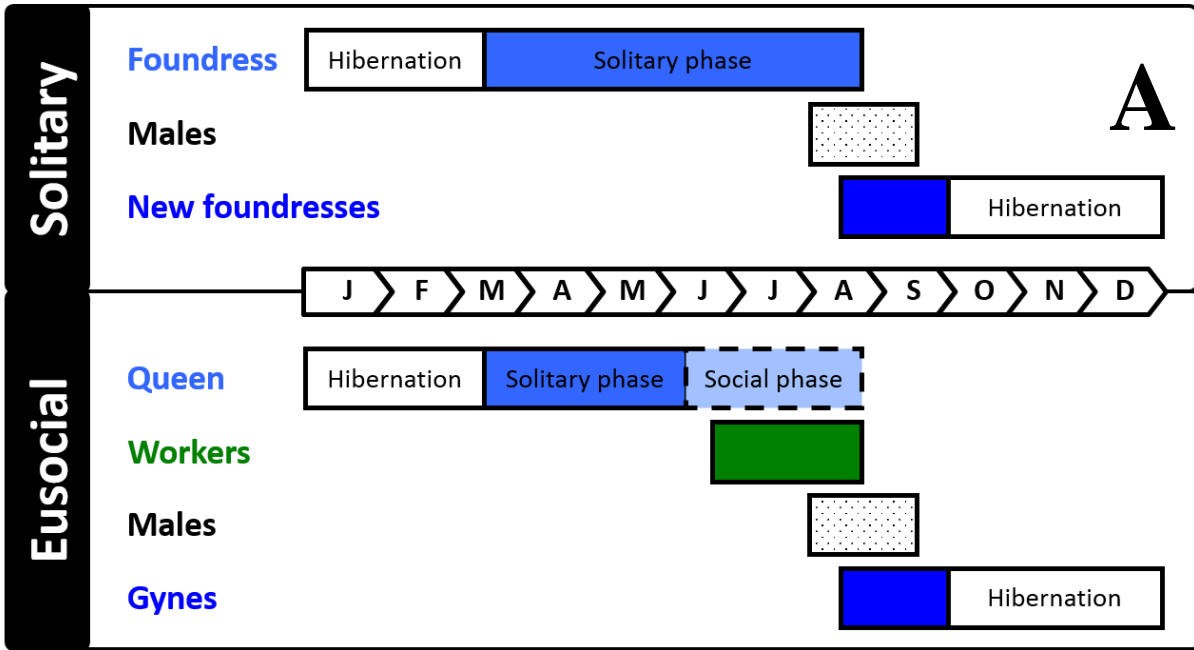




Figure 1: Life cycle and reproductive division of labor in a facultative eusocial aculeate hymenopteran.

(A) Life cycle of a facultative eusocial aculeate hymenopteran in the temperate Northern Hemisphere. In the solitary cycle (*e.g.* northern populations of *Halictus rubicundus*), the foundress raises a single brood generation of males and females. At the end of the activity period, new females overwinter, then found a new nest the following spring. In the eusocial cycle (*e.g.* southern populations of *Halictus rubicundus*, see Field *et al.* 2010), a first brood of largely non-reproductive females is raised by the queen, which remain in the maternal nest and help the foundress to rear a second or more broods, the last one(s) of which contains sexuals (new males and gynes).

(B) De-coupling of egg-laying from provisioning of offspring in a facultative eusocial aculeate hymenopteran. In the solitary life cycle, egg-laying and provisioning of the next generation are linked: solitary females have to alternate between reproductive (oviposition) and non-reproductive (provisioning the brood, nest construction and maintenance) tasks. In the eusocial life cycle, reproductive division of labor occurs after raising the first brood; the foundress specializes in egg-laying whereas the (non-reproductive) workers specialize in non-reproductive tasks. Mating may be a proximate mechanism determining the developmental trajectory of an adult female, with early mating leading to the foundress behavioral caste and lack of, or delayed, mating leading to specialization in worker behavior.

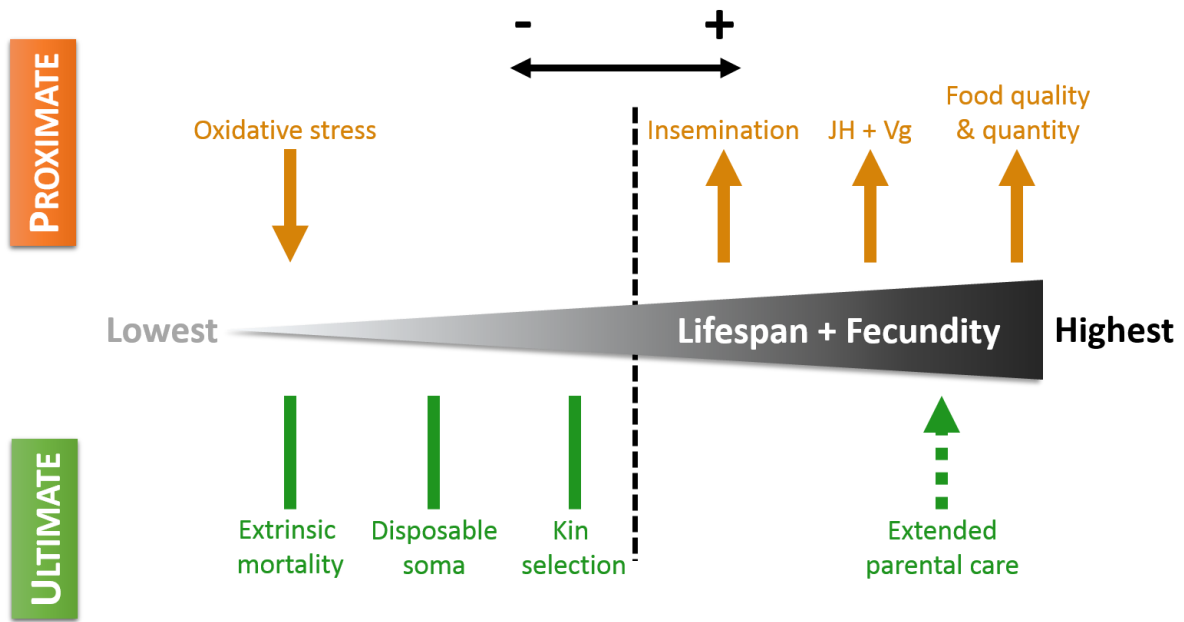


Figure 2: Gradient representing the positive association between lifespan and fecundity in facultative eusocial insects.

Some of the proximate factors contributing to an individual's position on this gradient (oxidative stress, insemination, juvenile hormone (JH) and vitellogenin (Vg) titres) are represented on top, and some of the ultimate factors (extrinsic mortality rate, disposable soma theory, kin selection, and extended parental care) are represented below the gradient. Arrows on the right of the dotted line contribute to increasing lifespan and fecundity, whereas that on the left leads to their decrease. In complex social systems, the queen is relieved of most or all brood care activities, minimizing the role of 'extended brood care' in explaining the extraordinary lifespan of highly eusocial queens. Modified from Gadau (2015).

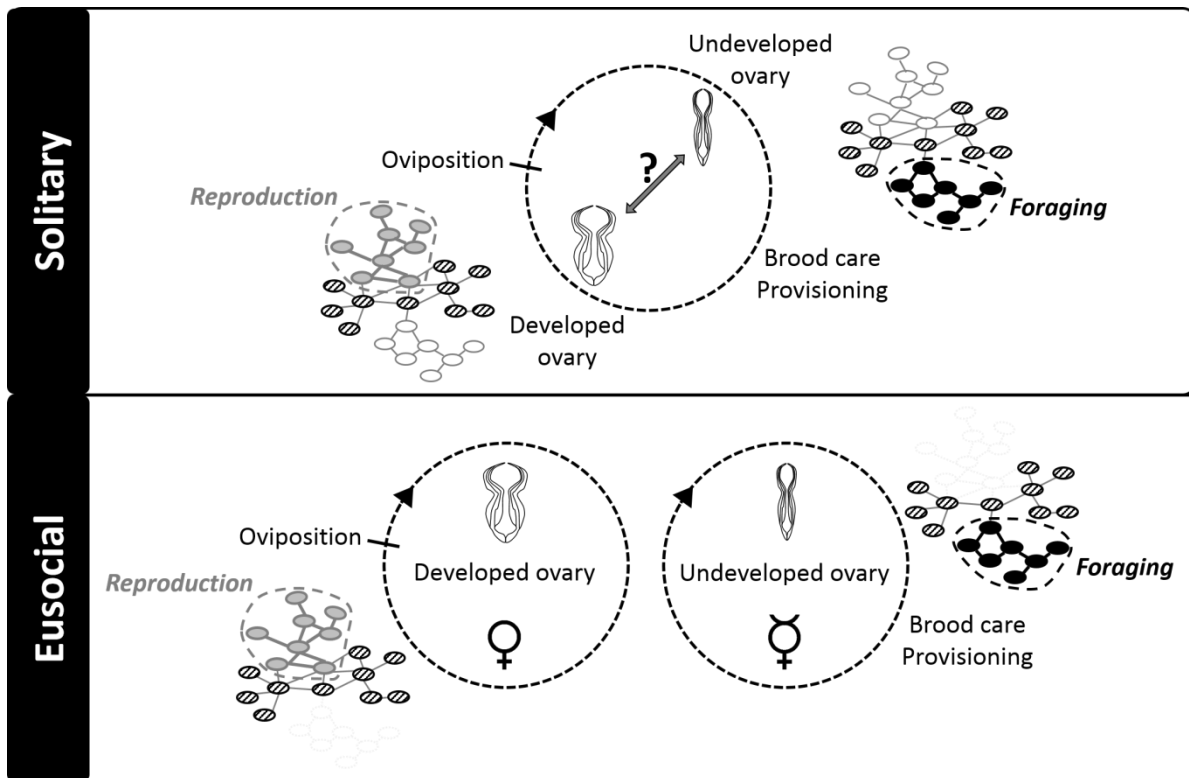


Figure 3: The reproductive (ovarian) ground plan hypothesis (RGPH).

(A) In solitary insects, females may alternate between the life history phases of foraging and reproduction (as shown) or may exhibit synchronous foraging and reproduction. In both cases, the foraging and reproductive phases are tightly coupled. (B) In eusocial insects, in contrast, reproductive division of labor leads to partitioning of foraging and oviposition, which are undertaken by workers and queens respectively. The reproductive (ovarian) ground plan hypothesis posits that gene networks (shown as an inter-connected group of ovals) regulating foraging (*Foraging*) and reproduction (*Reproduction*) in solitary insects became decoupled in eusocial insects; workers display the foraging phase whereas queens are ‘stuck’ in the reproductive phase (actively transcribed gene networks are enclosed by a dashed line). Analysis of facultative eusocial species can shed light on the RGPH and whether decoupling of gene networks underpins eusocial behavior.



Transcriptomic signatures of ageing vary in solitary and social forms of an orchid bee

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Abstract

Eusocial insect queens are remarkable in their ability to maximise both fecundity and longevity, thus escaping the typical trade-off between these two traits. In species exhibiting complex eusocial behaviour, several mechanisms have been proposed to underlie the remoulding of the trade-off, such as reshaping of the juvenile hormone pathway, or caste-specific susceptibility to oxidative stress. However, it remains a challenge to disentangle the molecular mechanisms underlying the remoulding of the trade-off in eusocial insects from caste-specific physiological attributes that have subsequently arisen due to their different life histories. Socially plastic species such as the orchid bee *Euglossa viridissima* represent excellent models to address the role of sociality *per se* in longevity as they allow direct comparisons of solitary and social individuals within a common genetic background. We present data on gene expression and juvenile hormone levels from young and old bees, from both solitary and social nests. We found 940 genes to be differentially expressed with age in solitary females, *versus* only 14 genes in social dominant females, and seven genes in subordinate females. We performed a weighted gene co-expression network analysis to further highlight candidate genes related to ageing in this species. Primary “ageing gene” candidates were related to protein synthesis, gene expression, immunity and venom production. Remarkably, juvenile hormone titres did not vary with age or social status. These results represent an important step in understanding the proximate mechanisms underlying the remodeling of the fecundity/longevity trade-off that accompanies the evolutionary transition from solitary life to eusociality.

Key words: gene expression, juvenile hormone, ageing, Hymenoptera, *Euglossa*, facultative sociality



Introduction

Ageing and the costs of reproduction

There is longstanding empirical support for the costs of reproduction, and more specifically for the trade-off between fecundity and longevity (Maynard-Smith 1958). Typically, investment in the production and care of offspring has a negative effect on survival (Edward & Chapman 2011). Evidence for such a trade-off has been found in organisms ranging from zebra finches (Alonso-Alvarez *et al.* 2004) to Columbian ground squirrels (Festa-Bianchet & King 1991), with much support found in model systems such as *Drosophila melanogaster* (Flatt 2011).

Previous studies have helped identify some of the proximate mechanisms underlying this widespread negative correlation between reproduction and lifespan. Endocrine pathways may play a role for instance, with juvenile hormone (JH) correlating positively with reproduction but negatively with lifespan in *Drosophila* (Flatt *et al.* 2005), in line with the antagonistic pleiotropy theory of ageing (Medawar 1952; Williams 1957). Additionally, oxidative stress, a molecular marker of senescence according to the free radical theory of ageing (Harman 1956), has been proposed as a mechanism mediating the trade-off between reproduction and lifespan, with reproduction leading to a decrease in antioxidant defences in zebra-finches (Alonso-Alvarez *et al.* 2004), and increased egg production inducing increased susceptibility to oxidative stress in *Drosophila melanogaster* (Wang *et al.* 2001). Another widespread life history mediator is the nutrient-sensing pathway, more specifically the insulin/insulin-like signalling pathway (IIS) and the target of rapamycin (TOR) pathway (Kapahi *et al.* 2010). Indeed, these pathways mediate the link between reproductive investment and lifespan in many organisms, including *D. melanogaster* (Flatt *et al.* 2008), *Caenorhabditis elegans* (Kenyon 2010), and humans (Blagosklonny 2010).

While evidence for a trade-off between reproduction and longevity is abundant, controversy remains. Germ-line ablation does not cause any difference in lifespan in *D. melanogaster* males, and even slightly decreases lifespan in females (Barnes *et al.* 2006). The insulin/IGF1 pathway, previously thought



central in mediating the fecundity/longevity trade-off across animals, has been shown to control longevity and fecundity independently of each other in *C. elegans* (Dillin *et al.* 2002).

Remoulding of the fecundity/longevity trade-off in eusocial insects

Adding to this controversy, a major challenge to the existence of a universal fecundity/longevity trade-off is the remarkable case of eusocial insects, where queens are often the only reproductively active individuals in their colony, yet also live up to 30 times longer than their worker counterparts (Carey 2001). Reproductive individuals in species exhibiting complex eusocial behaviour such as honeybees and ants, but also hemimetabolous insects like termites, thus do not exhibit a negative correlation between reproduction and lifespan (Page & Peng 2001). This remoulding of the expected longevity/fecundity trade-off in eusocial insects has also been supported experimentally. In the ant *Cardiocondyla obscurior*, mating appears to incur no cost in terms of longevity, as mated queens live longer than virgin queens (Schrempf *et al.* 2005), and enforced changes in egg-laying rate do not affect the longevity of queens (Schrempf *et al.* 2017). Moreover, in honeybees, workers which develop under queenless conditions have higher reproductive potential and also live longer than workers developing in queenright colonies, thus seemingly circumventing the trade-off between fecundity and longevity (Kuszevska *et al.* 2017).

Several molecular mechanisms have been suggested to underlie the apparent remoulding of the fecundity/longevity trade-off in insects exhibiting complex eusocial behaviour compared to solitary insects and other solitary organisms. For instance, caste-specific differences in somatic maintenance have been observed in ants, with *Lasius niger* queens exhibiting higher expression of somatic repair genes than workers (Lucas *et al.* 2016). Evidence suggests that a rewiring of central endocrine pathways may underlie the remoulding of the trade-off. Specifically, titres of JH and vitellogenin (a yolk precursor) are both positively correlated with reproduction at the expense of longevity in solitary insects, but the JH/vitellogenin network connectivity varies in social insects (Rodrigues & Flatt 2016). In *Apis mellifera* for instance, JH and vitellogenin are not connected in queens, as JH titres are low throughout their adult life, while vitellogenin levels increase rapidly as they start to lay eggs and remain at high levels as they age (Hartfelder & Engels 1998). In workers, JH and vitellogenin are even



negatively connected, and the increase in JH levels once workers become foragers actually sets a limit on their adult lifespan by promoting immunosenescence (Amdam *et al.* 2005). Hence, in *A. mellifera* queens and workers, vitellogenin is seemingly positively related to somatic maintenance and thus longevity, with high vitellogenin levels making them more resistant to oxidative stress (Seehus *et al.* 2006; Corona *et al.* 2007). By circumventing the positive correlation between JH and vitellogenin, and given the positive impacts of vitellogenin on longevity in this species, honeybee queens are thus able to maintain high reproductive function without sacrificing longevity.

Recently, de-regulation of transposable elements (TEs), which has previously been linked to ageing in many species (de Cecco *et al.* 2013; Li *et al.* 2013), has also been suggested as a mechanism allowing a positive correlation between reproduction and lifespan in other eusocial insect lineages: in the termite *Macrotermes bellicosus*, TE-silencing pathways are differentially expressed between sterile and reproductive castes, possibly underlying the striking differences in lifespan (Elsner *et al.* 2018). Although a multitude of mechanisms that promote queen longevity have been proposed in eusocial insects, we still lack a clear understanding of when and how this apparent remoulding of the fecundity/longevity trade-off evolved (Toth *et al.* 2016). At least within Hymenoptera, eusociality along with a remoulding of a fecundity/longevity trade-off has been suggested to have evolved independently at least eight times (Peters *et al.* 2017).

Investigating the remoulding of the fecundity/longevity trade-off in the socially polymorphic orchid bee *Euglossa viridissima*

Studies have investigated the fecundity/longevity trade-off in insect species across the sociality gradient, in particular primitively social and socially polymorphic species. The reversal of the trade-off is evident in primitively social wasps (Toth *et al.* 2016), shedding light on the mechanisms at play during early stages of social evolution within wasps, such as caste-specific modulation of reproduction by JH (Kapheim 2017). Socially polymorphic species such as the hover wasp *Parischnogaster alternata* (Bolton *et al.* 2006), the sweat bee *Megalopta genalis* (Kapheim *et al.* 2013), or the orchid bee *Euglossa viridissima* represent excellent study organisms for such investigations; the behavioural plasticity known for these species allows a direct comparison of solitary and social individuals within the same



species, or even within the same population. Such comparisons allow us to identify molecular mechanisms underlying the remoulding of the trade-off in early stages of social evolution, thus distinguishing them from mechanisms which maintain or reinforce this remoulding in complex insect societies (Séguret *et al.* 2016; Shell & Rehan 2018).

The Neotropical orchid bee *E. viridissima* Friese, 1899, is facultatively eusocial, with solitary and social nests co-occurring within the same population (Cocom Pech *et al.* 2008; May Itzá *et al.* 2014). All nests established by a “foundress” female are initially solitary until the first brood emerges approximately two months after founding. Nests are commonly reused for a second brood, and can be reactivated as a single-female nest (solitary) or a multi-female nest in which daughters from the first brood remain in the natal nest and share in care of future broods (social nests). In such multi-female nests, a dominance hierarchy is established through aggressive behaviour, with a reproductive skew in favour of the mother (Cocom Pech *et al.* 2008).

In this study we aimed to determine how social organisation and reproductive status (caste) influence lifespan in *E. viridissima* females. Specifically, we investigated changes in gene expression and JH titre with age in reproductive individuals from solitary nests, and compared these to observed changes with age in dominant and subordinate females from social nests. In particular, we aimed to identify candidate genes and gene networks that are linked to differential ageing in relation to reproductive and social status, *i.e.* genes potentially connected with fecundity and longevity. Such gene candidates identified within a socially polymorphic species are central to our understanding of the reversal of the fecundity/longevity trade-off in eusocial insects.

Materials and Methods

Experimental setup and sample collection

All *E. viridissima* samples were collected at the Department of Apiculture of the Campus of Biological Sciences and Animal Husbandry, Autonomous University of Yucatán in Xmatkuil, Mexico (89.37°W, 20.52°N, sample collection permit n° 41593). Wooden boxes (7 × 3 × 3 cm) with an inner coating of



beeswax and stingless bee cerumen were placed around the campus, with a glass cover between the box and wooden lid to facilitate observations. Females from the wild observed constructing cells in or bringing back provisions to a nest box were individually marked on the thorax with a diamond tipped pen. Nests were checked three times weekly from February 2016 until June 2018 to record the presence of marked females. In multifemale nests, the hierarchy of the females (dominant, subordinate) was determined by observing nests until individuals could be classified based on behaviour (Supplementary Table S1). For multifemale nests with only two females, observations continued until a female was observed returning to the nest with pollen, which characterised this female as the subordinate. In multifemale nests with three or more females, the individual spending most time in the nest, on the brood cells, and exhibiting the largest number of aggressive behaviours towards other females was characterised as dominant (Boff *et al.* 2015).

For females from solitary nests, and dominant females from social nests, both young (< 1.5 months since marking) and old (> 1.5 months since marking) individuals were collected. The establishment of a threshold of 1.5 months was based on personal observations in the field and on general life history: we estimated lifespan in *E. viridissima* to be of 2-4 months based on anecdotal observations of *E. viridissima* during previous studies of this species at the same campus sites and on what is known for the socially polymorphic *Euglossa melanotricha* (Andrade-Silva & Nascimento 2015). Given that all nests are first established as solitary, only becoming social during the founding female's second brood phase if her daughters from the first brood remained in the nest (Cocom Pech *et al.* 2008), dominant females were, by definition, older than the age threshold set in this study, even during early stages of social life. However, in some cases, dominant females died or left the nest, and one of their subordinate daughters took over as the dominant female. These females were below the set age threshold and were therefore sampled here as young dominant females. Since "worker" (subordinate female) lifespan was unknown for this species at the time of sampling, but is generally much shorter than queen lifespan in other eusocial hymenopteran species (Carey 2001; Séguret *et al.* 2016), we used three weeks as a threshold for distinguishing "young" vs "old" workers based on previous personal observations of this species at the same sites, and supported post-hoc by comparison of transcriptomes (see Results, Figure 1). Collection continued until at least five individuals were collected for each



combination of age and social status, except for old subordinate females which were rarely found in the field.

Upon collection, each individual was weighed and its thorax width (intertegular distance) was measured using a Canon EOS 60D camera and the imaging software Motic Images Multifocus Pro 1.0. Next, a haemolymph sample (1.5 - 4 μ L) was collected from the abdomen using microcapillaries for JH measurements. Each haemolymph sample was transferred to Teflon capped GC-vials containing 500 μ L acetonitrile that was then stored at -80°C . Whole bees were then kept overnight at -80°C , and once frozen, the abdomen was removed and transferred to RNAlater[®] (Sigma-Aldrich) for storage at -80°C . Altogether, 34 individuals were used for molecular analyses (Supplementary Table S2).

RNA extraction and sequencing

Total RNA was extracted from each individual (whole abdomen) using the RNeasy[®] Plus Mini kit, including DNase I digestion, according to manufacturer's instructions, using 350 μ l starting material (Qiagen, Hilden, Germany). RNA concentration, purity and integrity were measured using an Agilent 4200 TapeStation and 6 RNA ScreenTapes. Thirty-two samples which passed our quality criteria ($260/280 = 2.1 \pm 0.1$, RINe > 9 , total RNA mass $> 1 \mu\text{g}$) were used for RNA sequencing (Supplementary Table S2). Library preparation and transcriptome sequencing were undertaken at Beijing Genomics Institute (BGI, Shenzhen). For each sample, a cDNA library was prepared with the TruSeq RNA Library Preparation kit (Illumina) and SuperScript II Reverse Transcriptase (Thermo Fisher Scientific). Libraries were sequenced on an Illumina HiSeq4000 platform (11 samples per lane) to generate 100 bp paired-end reads, with ~ 4 GB of raw data per sample, *i.e.* 29 million read pairs per sample on average (range 24-35 million).

Raw reads were quality-checked with FastQC v0.11.5 (Andrews 2010), then filtered and trimmed using Skewer v0.2.2 (Jiang *et al.* 2014) to remove low-quality bases and reads, adapter contamination and reads shorter than 70 bp.



Exclusion of individuals from a sister species

Individuals belonging to the cryptic sister species *Euglossa dilemma* were identified through sequence comparison of the olfactory receptor gene *or41*, which has been described as a means of differentiating between *E. viridissima* and *E. dilemma* (Brand *et al.* 2020) (Supplementary Table S3). Six *E. dilemma* individuals were identified and subsequently excluded from further analyses in order to avoid any bias, although overall patterns in the data did not change when including these individuals (Supplementary Figure S4 and Tables S6, S7).

Differential gene expression analysis

Transcriptome assembly, genome annotation, read mapping and quantification

For full details on the transcriptome assembly and genome annotation, as well as software references, see Supplementary File S8. The transcriptomic analyses are based on the previously published draft genome sequence assembly for *E. dilemma* GCA_002201625.1 (Brand *et al.* 2017). Repeats were soft-masked (35.30% of the total genome assembly length) using bedtools v2.27.1 based on repeat annotations from Tandem Repeats Finder v4.09 and RepeatMasker. To improve the previously published annotation of this genome, which was based solely on gene predictions and homology to *Apis mellifera* proteins (Brand *et al.* 2017), we used Funannotate v1.5.1 with the previous gene annotation (edil.1.0.annotations.gff), novel experimental evidence derived from our RNAseq data generated in this study, namely: (i) RNAseq paired-end reads aligned to the sister species *E. dilemma* genome with HiSat2 v2.1.0, (ii) a transcriptome assembly with binpacker v1.1 from one sample, and (iii) a genome-guided transcriptome assembly from four samples with Stringtie2 v1.3.3b, as well as data from published studies such as (iv) an RNAseq data-based transcriptome assembly of *E. dilemma* using Trinity v1.5.1, and (v) transcripts of the closely related orchid bee *Eufriesea mexicana* (GCF_001483705.1). Further, protein sequences from five related bee species (*Bombus impatiens*: GCF_000188095.2; *B. terrestris*: GCF_000214255.1; *Apis mellifera*: GCF_003254395.2; *Melipona quadrifasciata*: GCA_001276565.1; *Eufriesea mexicana*: GCF_001483705.1) and Uniprot (sprot) were used for homology-based evidence. Additionally, genes were predicted from the *E. dilemma* genome



using SNAP v2006-07-28 based on SNAP's *Apis mellifera* HMM dataset, Genemark-ET, Augustus v3.3 based on BUSCO3 v3.0.2 training, and the PASA pipeline v2.3.3.

In brief, PASA was used to train gene predictions which were then performed using Genemark-ES, PASA, Augustus v.3.3, and CodingQuarry v2.0. Predictions as well as additional, previous annotations were then integrated in Evidence Modeler v.1.1.1. Gene models which were too short, gap-spanning or repeat-overlapping were removed and tRNA genes were detected with tRNAscan SE v2.0.0. Previous gene models were then updated with PASA and Transdecoder v5.5.0 based on RNAseq evidence and expression levels of models at each locus. Genes were functionally annotated using PFAM v32.0, the UniProt database v2018_11, EggNog Annotations (eggnog_4.5/hmmdatabases: Arthropoda, Insecta, Hymenoptera, *Drosophila*), MEROPS v12.0, CAZymes in dbCAN v7.0, BUSCO Hymenoptera models v3.0.2, Hymenoptera odb9, SignalP v4.1, and InterProScan5 v5.33-72.0. After processing and quality filtering, gene models were compared to the previous *E. dilemma* annotation (v1.0). Non-duplicated gene models from Edil1.0 which were missing in the Funannotate annotations (largely due to missing stop codons (n=268), gap spanning (n=16), or other (n=13)) were appended. The final annotation contained 24,991 gene models (including 126 tRNA genes), and was estimated to be 80.6% complete (BUSCO3: 3,560 were found out of 4,415 single copy protein coding orthologs conserved across Hymenoptera, and 89 (2.0%) of these were duplicated).

The set of 24,991 gene models was then used for subsequent transcriptome analyses. Trimmed and filtered RNAseq reads were pseudo-aligned to these gene models and were simultaneously quantified using Kallisto v0.46.2 (Bray *et al.* 2016).

Differential expression analyses

In order to analyse differential gene expression between young and old individuals within each group (see below), we used the tximport package v1.12.3 (Soneson *et al.* 2015) in R v3.6.1 (R Development Core Team 2019) to import transcript-level read quantification data, and convert these to gene-level quantification values (Supplementary Table S9). We then ran DESeq2 v1.18.1 (Love *et al.* 2014) with default settings (adjusted *p*-value calculated to correct for multiple testing following the BH method (Benjamini & Hochberg 1995)) to perform pairwise comparisons of gene expression profiles between



young and old individuals of a given social status (*i.e.*, solitary, social dominant or social subordinate), and between individuals of different social status for a given age. Differentially expressed genes (DEGs) were identified as those with a *p*-adjusted value below 0.05. After plotting gene expression profiles of all individuals, excluding *E. dilemma* individuals, in a Principal Component Analysis plot (PCA of variance stabilised read counts, with genes as columns and samples as rows in the PCA matrix, Supplementary Figure S5), individual eug6 was identified as an outlier and was excluded from gene expression analyses. All PCA figures were produced using the R package ggbiplot v0.55 (Vu 2011), with ellipse probabilities (if ellipses shown) set to 95% confidence level (ellipse.prob=0.95). The final dataset comprised 25 *E. viridissima* transcriptomes.

To identify the genes for which expression levels were affected by the interaction between age and social status, we performed a likelihood ratio test to contrast the following models in DESeq2: Full model (age + social_status + age:social_status), Reduced model (age + social_status). This contrast identified genes with a significant change in expression associated with the interaction of the two factors, age and social status.

Weighted gene co-expression network analysis

Network construction and correlation of modules with phenotypic traits of interest

Weighted gene co-expression network analysis was carried out using the R package WGCNA (Langfelder & Horvath 2008). The aims of this analysis were to (a) identify sets of co-expressed genes (modules), (b) calculate module eigengenes (*i.e.* values representative of the gene expression profile in a module) and correlate these with phenotypes of interest (here, social status and age), and finally (c) identify “hub genes”, *i.e.* genes that were most highly connected, within modules which were significantly associated with the phenotypic trait of interest (here, age). Additionally, the network analysis was performed to identify age-related hub genes overlapping with those differentially expressed between young and old individuals. For this analysis, genes with read counts lower than 10 in 90% of the samples were removed, and raw read counts were transformed using the varianceStabilizingTransformation function in DESeq2, as recommended by Langfelder and Horvath



(2008). The input table thus consisted of a matrix of 25 individuals across all social types and ages and 12,902 gene expression values (Supplementary Table S10). Modules of co-expressed genes were defined using average linkage hierarchical clustering with the topological overlap-based dissimilarity measure, with parameters set according to recommendations of Zhang and Horvath (2005) and Langfelder and Horvath (2008) (soft thresholding power set to 14 as the lowest value for which scale-free topology fit index reached 0.9; minimum module size set to 30 genes; modules of highly co-expressed genes merged using a cut-off value of 0.2).

Hub genes correlated with age and social status

Module eigengenes were correlated with two phenotypic traits: social status and age. Hub genes from each module associated with age, *i.e.* genes which were most highly connected within those modules (intramodular connectivity > 0.75) were extracted and concatenated to create a list of hub genes associated with age.

Overlap of age-related genes from two independent analyses

Genes found to be common to the list of hub genes associated with age in the WGCNA and the list of genes found to be differentially expressed with age were considered to be of particular interest. The functions of these overlapping genes were further investigated through orthology analyses (see below).

Functional annotation

Selection of “top genes” from gene lists of interest

Functional annotation was done for top genes from each gene list of interest. For lists of significant DEGs, the top ten genes, *i.e.* genes with the highest absolute log₂ fold change, were extracted from the lists of 1) genes up-regulated in young compared to old, and 2) genes up-regulated in old compared to young, separately for a) solitary, b) dominant and c) subordinate females (six lists in total). The top ten genes whose expression was affected by the interaction between age and social status were defined as genes with the highest absolute log₂ fold change value in the model contrast (likelihood ratio test). For



hub genes from the modules associated with age in the network analysis, the top ten genes were defined for each module as those with the highest intramodular connectivity.

Finally, all genes found to be associated with age in both the differential expression analysis and the network analysis were considered of particular interest and were functionally annotated.

Assigning gene functions based on orthology with A. mellifera and Bombus spp.

To assign functions to genes highlighted as relevant with regards to ageing, we searched for the known function of their closest ortholog. OrthoFinder v2.3.7 (Emms & Kelly 2015) was run with default settings on protein level using the genome annotation we produced for *E. dilemma* and protein sets of three closely related species with well-annotated genomes: *A. mellifera* (GCF_003254395.2), *Bombus terrestris* (GCF_000214255.1), and *Bombus impatiens* (GCF_000188095.3). For all top gene lists, ortholog protein names were extracted from *A. mellifera* annotations if available as this is the most extensively annotated genome, or from the annotations in *Bombus* spp. if no *Apis* ortholog was found. For the remaining 19% of *Euglossa* top genes which did not have an ortholog in any of these three species (see Results), nucleotide sequences were searched against the NCBI nucleotide database (NCBI, database downloaded on 07.01.2020) using BLAST v2.7.1 (Altschul *et al.* 1990). The closest orthologous gene was then determined based on its best hit according to its lowest e-value.

Ortholog protein names were then searched manually in UniProt for existing functional annotations in the species in which the ortholog was found, or in a closely related species if available. Based on the functional annotations found, each gene was then assigned to one of the following functional categories: energy metabolism, stress response, growth/trophic factors, cell cycle, signalling, venom, protein turnover, gene regulation or TE suppression.

Deposition of genome annotation files and raw sequence reads

All raw sequence reads for each sample, including *E. dilemma* individuals, have been deposited in the NCBI Sequence Read Archive under Bioproject ID PRJNA636137. The updated genome annotation for *E. dilemma* has been deposited in Dryad (see Data Availability Statement).



Juvenile hormone quantification and analysis

The same individuals used for the transcriptomic analyses were also used to assess juvenile hormone (JH) levels in the haemolymph by a radioimmunoassay. The protocols for JH extraction from the haemolymph samples in acetonitrile and preparation of the samples in the radioimmunoassay (RIA) followed the detailed description for JH quantification in the honeybee (Hartfelder *et al.* 2013) and wasps (Kelstrup *et al.* 2018), using a JH-specific antiserum, tritiated [$10\text{-}^3\text{H(N)}$]-JH III (specific activity 19.4 Ci/nmol, Perkin Elmer Life Sciences, Waltham, MA, USA), and synthetic juvenile hormone-III (Fluka, Munich, Germany) as the non-radioactive competitor. For the haemolymph JH titre calculations, we used a non-linear four-parameter regression. After exclusion of *E. dilemma* individuals, 21 *E. viridissima* individuals were included in the analysis (Supplementary File S2).

All statistical analyses were performed in R (v3.6.1). To investigate the effect of age and social status on JH titres, we performed a generalised linear mixed model on our log-transformed data using the package lme4 v1.1-21 (Bates *et al.* 2015). A stepwise Akaike Information Criterion (AIC) method for variable selection was applied, with the best-fitting model including age, social status and the interaction between age and social status as fixed factors, and nest ID and date of collection as random factors. An ANOVA was then used to test for significant effects.

Results

RNA sequencing

After trimming and filtering, sequenced libraries contained on average 29 million read pairs (range \pm SD = 24-35 million reads \pm 5 million read pairs), of which 79% (range 66-79 \pm 19% SD) could be mapped to the *E. dilemma* genome annotation.

Gene expression changes with age in solitary but not in social females

A total of 19,136 genes had at least one read count, representing 77% of annotated genes. The PCA of variance-stabilised read counts across genes which were differentially expressed between young and



old individuals, cumulative across all social types (Figure 1; for a PCA across all these genes, not just those which were differentially expressed, see supplementary Figure S12) showed a clear distinction between the gene expression profiles of young and old solitary females across both PC1 (explaining 37% of the variance) and PC2 (explaining 21% of the variance). Young and old subordinate females from social nests also exhibited distinct gene expression profiles, although to a lesser extent than solitary females. In dominant females, however, gene expression profiles did not show a clear pattern with respect to age; data points from young and old individuals overlapped (Figure 1).

Differential gene expression analyses revealed 940 genes to be significantly differentially expressed with age in solitary females, versus only 14 genes differentially expressed with age in dominant females, and seven in subordinate females (adjusted $p < 0.05$, Supplementary File S14 and Figure 2a). Additionally, solitary and dominant females exhibited increasingly divergent expression profiles with age, with 213 DEGs between young solitary and young dominant females, versus 2,601 DEGs between old solitary and old dominant females. Subordinate females from social nests showed gene expression profiles which were intermediate between those of solitary and dominant females, with 38 DEGs between young solitary and young subordinate females, 53 DEGs between old solitary and old subordinate females, 212 DEGs between young subordinate and young dominant females, and 38 DEGs between old subordinate and old dominant females (for a list of differentially expressed genes in each comparison, see Supplementary File S14). Finally, old dominant females exhibited considerably more differences to young subordinate females compared to young dominant females (1,007 and 14 DEGs, respectively), despite young dominant females in our study having been born as subordinate females which switched to the dominant position upon their mother's absence from the nest (see Methods).

Functional annotation of the top ten genes upregulated in young compared to old solitary females revealed several genes related to metabolic pathways (*e.g.* alpha-glucosidase precursor, generally involved in the break-down of starch molecules into glucose), as well as genes involved in stress response and in growth and trophic factors (Figure 2b; for a full table of functional annotations see Supplementary File S15). Top genes upregulated in old compared to young solitary females were mainly involved in the stress response (*e.g.* a gene from the cytochrome P450 superfamily, which plays a role in protection against reactive oxygen species in *Apis cerana cerana* (Zhu *et al.* 2016)) and in the



cell cycle, as well as in growth and trophic factors (Figure 2f). Top genes upregulated in young *vs* old dominant females were involved in a range of pathways, from signalling (*e.g.* metabotropic glutamate receptor 1, a G-protein coupled receptor thought to play a role in the action of glutamate in the central nervous system of *Mus musculus* (Charette *et al.* 2016)) to energy metabolism (*e.g.* unconventional myosin-XVIIIa, putatively involved in motor activity and ATP binding (Redowicz 2007)) (Figure 2c). Genes upregulated in old compared to young dominant females were involved in gene regulation (*e.g.* zinc finger protein 567-like, likely involved in the regulation of transcription (Fasken *et al.* 2019)) and response to stress (Figure 2g). Finally, genes upregulated in young compared to old subordinate females were mainly uncharacterised, but one was linked to growth and trophic factors (nesprin-1, potentially involved in subcellular spatial organisation (Zhang *et al.* 2001)) (Figure 2e). Genes upregulated in old compared to young subordinate females related to the silencing of transposable elements (mitochondrial cardiolipin hydrolase (Todeschini *et al.* 2010)), energy metabolism and venom production (Figure 2h). The one gene which was upregulated in young compared to old individuals in both solitary and dominant females is to date uncharacterised (Gene ID FUN_020310). The two genes that were convergently upregulated with age in solitary and subordinate females were related to TE suppression and energy metabolism (Gene IDs FUN_003022 and FUN_017407, Supplementary File S15).

By contrasting two models in DESeq2 (with *versus* without the interaction between age and social status), we detected 314 genes exhibiting different changes in expression with age according to social status (likelihood-ratio test adjusted $p < 0.05$, Supplementary File S14). Functional annotation of these genes revealed an overlap with functions of genes differentially expressed between young and old solitary and subordinate females, including links to the stress response, metabolic pathways, growth and trophic factors, and the cell cycle (Supplementary File S15).

Modules of co-expressed genes correlated with age

Independent identification of genes for which expression patterns correlate with age was performed using the same gene expression dataset, following the Weighted Gene Correlation Network Analysis approach (WGCNA (Langfelder & Horvath 2008)). A total of 10,446 genes (81% of the 12,902 expressed genes used in this analysis, see Methods and Supplementary File S10) were assigned to



26 modules forming a co-expression network. Correlation of each module's eigengene with age and social status revealed that four modules of co-expressed genes were significantly associated with age, 10 modules were associated with the solitary phenotype regardless of age, 13 modules were associated with the dominant phenotype (seven of which overlapped with those associated with solitary phenotype, Supplementary File S13 and Supplementary Figure S16), and three modules were associated with the subordinate phenotype, again irrespective of age. These three latter modules were also associated with the dominant phenotype (Supplementary Figure S16).

For the four modules significantly associated with age, we extracted hub genes, *i.e.* genes with an intramodular connectivity > 0.75 (intramodular connectivity range 0-1). This resulted in a total of 35 genes (out of 2,053 genes in total belonging to the four age-correlated modules) revealed to be significantly related to age (Figure 3a; for the full list of genes see Supplementary File S13, and for functional annotation of the top ten hub genes in each module see Supplementary File S15). Functions of hub genes from the four age-related modules mainly related to protein synthesis, energy metabolism, fatty acid biosynthesis, and response to stress (Figure 3).

Overlap of age-related genes between the differential gene expression and the network analyses

Of the 35 genes for which expression was significantly correlated with age in the WGCNA, ten were also found to be significantly differentially expressed between young and old solitary females (Figure 3b, Figure 4). There was no overlap of age-related genes between the two analyses for dominant or subordinate females. The overlap for solitary females was significantly higher than expected by chance (hypergeometric test, $p < 0.001$). Functional annotation of these overlapping genes revealed that these included genes related to immunity and stress response (*e.g.* serine protease inhibitor genes 27A and 88Ea, which negatively regulate the melanisation cascade and the Toll signalling pathway in *Drosophila melanogaster* (Tang *et al.* 2006)), as well as to gene translation and protein turnover (*e.g.* 60S ribosomal protein L34 is potentially involved in translation in *Apis cerana*, and kynurenine/alpha-aminoadipate aminotransferase is involved in the synthesis of amino acids), and to venom production (venom acid phosphatase Acph-1) (Figure 4b).



No change in juvenile hormone titres with age or social status

After controlling for nest of origin and sampling date, there were no significant differences in juvenile hormone titre in relation to age, social status, or the interaction between the two factors (ANOVA, age: $\chi^2(1,21) = 0.01$, $p = 0.92$; social status: $\chi^2(2,21) = 4.75$, $p = 0.09$; age \times social status: $\chi^2(1,21) = 0.68$, $p = 0.41$; Supplementary Figures S17, S18).

Discussion

Differential gene expression with age depends on social status

Solitary females exhibit extensive changes in gene expression profiles with age

The difference in gene expression profiles of young and old solitary females, and the high number of differentially expressed genes between these two groups (940 genes) strongly suggest that solitary females undergo substantial physiological changes with age. Functional annotation of these 940 DEGs revealed that metabolic pathways likely play a major role in (or are affected by) these changes. Genes relating to metabolic activities appear to be upregulated in young rather than old solitary females, suggesting that high metabolic activity early in life may increase senescence, as predicted by the free radical theory of ageing (Harman 1982). In the cryptic sister species *Euglossa dilemma*, young foundress females indeed spend more time foraging for brood provisions, a metabolically costly task, compared to when they later enter the “guard phase”, during which they stay in the nest, guarding their brood cells (Saleh & Ramírez 2019). Interestingly, genes directly related to the oxidative metabolism of various substrates, notably lipids (cytochrome P450, cytochrome b5) are upregulated with age in solitary females. In mammals, cytochrome P450 genes are controlled through tight regulation of gene transcription, and a dysregulation of transcription with age can therefore lead to an accumulation of reactive oxygen species and increased oxidative stress (Zangar *et al.* 2004).

The relationship between high metabolic activity and ageing has long been suspected, as the accumulation of reactive oxygen species, an inevitable by-product of metabolic activity, is thought to accelerate senescence, therefore making it a potential hallmark of biological ageing (Alonso-Alvarez



et al. 2004; Monaghan 2014). In solitary insects such as *Drosophila*, gene expression changes during ageing overlap with those induced by increased exposure to oxidative stress, including an increase in cytochrome P450 genes (Landis *et al.* 2004). The *Drosophila* mutant line *methuselah* and mutants of the *age-1* gene in *C. elegans* are both longer-lived and more resistant to oxidative stress (Larsen 1993; Lin *et al.* 1998), reinforcing the idea of oxidative stress as a cause of senescence. However, multiple studies have called this idea into question (Pérez *et al.* 2009; López-Otín *et al.* 2013), suggesting that the link between metabolism and biological ageing is not so straightforward. Still, the upregulation of metabolic pathways early in life and of genes related to the oxidative stress response later in life are likely signs of senescence in solitary females.

It should be noted that half of the top ten genes upregulated in young compared to old solitary females do not yet have functional annotations; the functional interpretation of genes differentially expressed with age in solitary females should therefore be interpreted with caution.

Social females undergo very little transcriptomic change with age

Although very few genes were differentially expressed with age in social compared to solitary females, those may provide insight into ageing patterns associated with the social phenotype in *E. viridissima*.

a. Metabolic pathways: down in old dominant, up in old subordinate

While genes linked to energy metabolism were upregulated in young compared to old dominant females, the opposite trend was observed in subordinate females (although it is worth noting that two of the three genes upregulated in young subordinate females are to date still uncharacterised). This discrepancy may be explained by the different life histories exhibited by dominant and subordinate females in this species. Indeed, while subordinate females may perform more costly tasks later in their life as they must provision for an increasingly large brood, dominant females may be subject to more metabolically costly tasks at a younger age, as they produce the brood and establish their dominance through aggressive interactions with other females in the nest early in the nesting cycle. Although *E. viridissima* has been reported to be remarkably tolerant towards daughters in the nest (Cocom Pech *et al.* 2008), we observed multiple cases of agonistic behaviours from the dominant towards subordinate



females, as described in several primitively social species as a way of establishing dominance (Freiria *et al.* 2017; Boff *et al.* 2017).

Interestingly, a similar gene expression pattern has been found in honeybees, in which genes linked to oxido-reductase activity were upregulated with age in workers (Aumer *et al.* 2018); in the ant *Temnothorax rugatulus*, where the oxidation-reduction process was the only significantly enriched GO term for genes downregulated with age in queen brains (Negroni *et al.* 2019); and in the termite *Cryptotermes secundus*, where multiple transcripts related to oxidative stress were upregulated in young compared to old queens and kings. Workers showed the opposite trend, with such transcripts being upregulated in older individuals (Monroy Kuhn *et al.* 2019).

b. Silencing of transposable elements

The gene coding for mitochondrial cardiolipin hydrolase, which was one of the two genes upregulated in old subordinate females in our study, is required for the activity of the piRNA pathway in *Drosophila melanogaster* (Todeschini *et al.* 2010). This pathway is known for its role in silencing transposable elements (TEs). Recently, TEs have been highlighted as a potentially major factor underpinning differential ageing between the queen/king and worker caste in the “higher” termite *Macrotermes bellicosus* (Elsner *et al.* 2018), and in the “lower” termite *Cryptotermes secundus* (Monroy Kuhn *et al.* 2019). Given the high repetitive proportion of the *E. viridissima* genome (Brand *et al.* 2017; annotation Edilemma_v2.2 presented here), TE silencing may play an important role in *Euglossa* bees too, in contrast to highly eusocial bees which have very few active TEs (Kapheim *et al.* 2015).

c. Gene regulation: an underestimated hallmark of ageing?

Functional annotation of genes differentially expressed between young and old dominant females also suggests that the regulation of gene expression may play a role in ageing. A meta-analysis of age-related gene expression profiles using 27 datasets from mice, rats and humans revealed that the “negative regulation of transcription” was indeed one of the GO categories overrepresented in age-related transcriptional profiles, making it a common signature of ageing across those organisms (de Magalhães *et al.* 2009). Frenk and Houseley (2018) also propose dysregulation of gene expression and mRNA



processing with age as a candidate “gene expression hallmark” of cellular ageing. Empirical studies have supported this theory: for example, gene expression correlation decreases with age in mice (Southworth *et al.* 2009), which can be interpreted as transcriptional noise increasing with age (Bahar *et al.* 2006). Experimental exposure to oxidative stress increases cell-to-cell variation in gene expression, suggesting that dysregulation of gene expression is a possible mechanism linking DNA damage with ageing, cellular degeneration and death. In our study, upregulation of a gene linked to the regulation of transcription (zinc finger protein 567 (Fasken *et al.* 2019)) with age in dominant females, but not in solitary females, suggests that dominant females may actively counter the dysregulation of gene expression with age, thus supporting differential gene regulation as a potential mechanism for the reduced signs of ageing in social dominant females.

Does sociality prevent ageing?

The considerable age-related differences in gene expression in solitary females but not in the social females observed in this study (neither dominant nor subordinate) suggest that solitary females undergo more extensive physiological changes with age, particularly when compared to dominant females in social nests. The divergence in observed age-related gene expression changes between solitary and social dominant females despite them belonging to comparable age groups are worth noting and support the idea of the co-evolution of extended longevity and eusociality in its early evolutionary stages (Carey 2001). Further studies on other molecular markers related to ageing (López-Otín *et al.* 2013) in species along the sociality gradient may help deepen our understanding of this issue.

As subordinate females perform costly tasks such as brood provisioning, and given that workers of obligate eusocial species exhibit considerably shorter lifespan than their queen counterparts (Carey 2001), the absence of major changes in gene expression with age in subordinate *E. viridissima* is somewhat surprising. However, the two genes which were convergently upregulated with age in both solitary and subordinate females provide some insight into the costs of the tasks performed by both solitary and subordinate females in *E. viridissima*. These genes were related to TE suppression and energy metabolism, and are thus potentially related to known ageing pathways (Elsner *et al.* 2018).



Experimental studies in which putatively costly behaviours, such as brood provisioning, are manipulated may further our understanding of the actual costs of such tasks.

An alternative explanation for the differences in gene expression with age observed in the solitary, but not observed in the social females is a potential shift in behaviour with age in the solitary females. It has been proposed for the sister species *E. dilemma* that transcriptomic shifts throughout the solitary phases of the life cycle may be attributed to a shift in behaviour, as the female goes from an actively foraging foundress to a relatively inactive guard, caring for her brood (Saleh & Ramírez 2019). Similar to the differences observed between young and old solitary females in our study species *E. viridissima*, Saleh and Ramírez (2019) found that metabolic pathways were upregulated in foundresses compared to females in the guarding phase. Additional information on the life cycle and associated shifts in behaviour in *E. viridissima* would therefore allow us to better account for this potential factor, and determine whether the differences observed in our study are due to ageing *per se* as opposed to behavioural repertoire. Another factor which may explain the lack of ageing signs in dominant females is the ingestion of subordinate-laid eggs by dominant females. Video recordings of *E. dilemma* nests revealed that oophagy, whereby the dominant female ingests eggs laid by subordinate females in her nest to replace them with her own, occurs commonly in this species (Saleh and Ramírez 2019). If this behaviour also occurs in the closely related *E. viridissima*, this could constitute a notable nutritional source for dominant females, thereby reducing any trade-off generated by the allocation of limited resources.

Gene co-expression networks as a tool to further highlight “ageing gene” candidates

Using network hub genes to independently identify ageing pathways in E. viridissima

Our gene co-expression network analysis was performed in part to identify age-related genes overlapping with those differentially expressed with age, thus narrowing down the list of genes that potentially play a role in the ageing process. The results from the co-expression network analysis highlighted modules of genes for which expression was correlated with age, independent of social status. Functional annotation of these genes indeed revealed that they were related to well-established age-related pathways such as metabolic processes and the stress response (Landis *et al.* 2004). Protein



synthesis was the main function associated with one of these age-related modules (black module in Figure 4). The expression of ribosomal proteins increases with ageing; however, as proteasome levels decrease with age, they are accompanied by a loss of protein homeostasis, a common feature of ageing (García-Velázquez & Arias 2020). Finally, the top ten hub genes of one of the age-related modules (blue module in Figure 4) were related, among other things, to fatty acid biosynthesis (very-long-chain 3-oxoacyl-CoA reductase and fatty acyl-CoA reductase), a pathway which has been linked to ageing in several species (Shmookler Reis *et al.* 2011; Proshkina *et al.* 2015). Thus, hub genes from age-related modules in the co-expression network presented here may help to disentangle the effects of ageing *versus* the effects of a shift in behaviour, especially within solitary nests.

Genes highlighted both in differential expression analyses and the co-expression network represent particularly strong candidates for ageing research

The ten genes in solitary females convergently linked to ageing in both DEG and WGCN analyses are particularly good candidates to be further tested for their role in ageing processes. They were linked to immunity, venom production, gene expression and protein turnover, as well as fatty acid biosynthesis. Firstly, the increased expression of Toll-pathway inhibitors with age (*i.e.* the decreased activity of this immune pathway with age) suggests a negative relationship between age and immune defences in solitary females. This may indicate a trade-off between reproduction and immunity in solitary females, as observed in many organisms and in line with the resource allocation model (van Noordwijk & de Jong 1986; Harshman & Zera 2007). Secondly, the higher expression of a gene related to venom production, *i.e.* venom acid phosphatase *Acph-1*, in young compared to old solitary females may indicate increased investment in defense early in life as females go through the nest-founding phase and must exit the nest to actively forage for their first brood. Again, following the resource allocation model, this investment may trade off with other life history traits in solitary females, such as somatic repair and longevity. Social dominant females may circumvent this trade-off, as in obligate eusocial species in which reproduction does not trade off against maintenance as a consequence of the abundant resources provided to the queen (Kramer *et al.* 2015). Further investigations would be necessary to confirm whether this is also the case in primitively eusocial species such as *E. viridissima*, but the pronounced



transcriptomic signs of ageing in solitary compared to social individuals in this study seem to support this hypothesis. Thirdly, we found that a gene related to fatty acid biosynthesis (elongation of very long chain fatty acids) was linked to ageing in both of our analyses and was upregulated in young solitary females. Such genes have been associated with reduced lifespan in *C. elegans*, as higher expression of elongases is found in wild-type strains compared to long-lived mutants (Shmookler Reis *et al.* 2011). Thus high expression levels of this gene in young solitary females may come at a cost for longevity. Finally, genes related to mRNA translation and protein turnover increased with age in solitary *E. viridissima* females. Although overall protein turnover rates are generally found to decrease with age in animals, leading to the accumulation of damaged proteins as organisms senesce (Ryazanov & Nefsky 2002), the dysregulation of certain transcription factors with age may lead to a “loss of silencing” and increased expression of particular genes (Frenk & Houseley 2018).

No apparent role for juvenile hormone in *E. viridissima* ageing or social status

We report here the first data on JH titre levels in an orchid bee species. Strikingly, we found no significant difference in the haemolymph JH titres between young *vs* old and dominant *vs* subordinate females (Figure S17). So far, we cannot say much about the solitary *vs* social females due to the low sample number of solitary individuals (Figure S18).

On first sight, these findings apparently stand in contrast to the marked increase in JH levels in the honeybee workers, related with ageing and the transition from nursing to foraging tasks (Huang & Robinson 1996) and the equally strong positive correlation in JH levels with social status in the bumblebee *Bombus terrestris* (Bloch *et al.* 2000; Shpigler *et al.* 2014). In the stingless bee *Melipona quadrifasciata*, however, JH haemolymph titres in foragers are lower than those in workers (Cardoso-Júnior *et al.* 2017). These evident discrepancies in the role of JH among representatives of the three social tribes of the monophyletic corbiculate bees, and now also a facultatively social euglossine, indicate that in corbiculate bees, which is the only clade within the bees that comprises members of all social complexities, there is no strong link between JH and reproduction, *i.e.* social status. Rather, each tribe appears to have moulded this ancient gonadotropic insect hormone (Santos *et al.* 2019) according



to its idiosyncratic mode of social biology. Such idiosyncrasies are not unique to the family Apidae (comprising the honeybees, bumblebees, orchid bees and stingless bees), but are also found in wasps. For instance, in the solitary progressively provisioning eumenine wasp *Synagris cornuta* there is also no correlation between JH levels and age or reproductive status (Kelstrup *et al.* 2018). Further, two sympatric species of the same genus of *Polistes* paper wasps showed a divergent patterns of JH titre with respect to age/task and social status (Kelstrup *et al.* 2015). Hence, the lack of a link between JH and age or social status in *E. viridissima* is not such a surprising result.

Conclusions and future directions

The extensive changes in gene expression with age in solitary *Euglossa* females, which stand in stark contrast to the absence of gene expression changes with age in social *Euglossa* females, provide insight into the molecular pathways underlying ageing in solitary organisms, and how these may be modified in eusocial individuals. Our gene co-expression network further highlighted several hub genes correlated with age that were related to metabolic pathways, fatty acid biosynthesis and protein synthesis. The significant overlap of genes for which expression levels are associated with ageing across differential expression and co-expression network analyses revealed that a subset of genes related to immunity, venom production, protein production and fatty acid biosynthesis represent particularly relevant candidates for future ageing studies, both in solitary and social individuals. Ultimately, these results in a socially polymorphic species represent a tentative step in understanding the genetic mechanisms allowing the reversal of the fecundity/longevity trade-off which seemingly accompanies the evolutionary transition to eusociality (Carey 2001). Our study, based on expression differences inferred from transcriptome data, represents a useful starting point in the search for genetic markers and proximate mechanisms of ageing in the transition from solitary life to eusociality. A necessary next step in future studies would be to experimentally test the function of candidate genes identified here in order to eliminate possible confounding factors such as shifts in behaviour with age, for instance through knock-down experiments to corroborate or refute the role of these genes in relation to senescence. Finally, in order to determine the relevance of these genes in the context of the fecundity/longevity trade-off, additional life history data are needed, such as information on the lifespan and reproductive



output of individuals, to determine how these relate to expression of the candidate genes. Due to the invasive nature of RNA sampling from such small organisms like bees, this is difficult to achieve. Emerging technologies may make this an accessible goal in the future, or studies in species for which larger sample sizes are available may allow parallel sampling of a subset of individuals alongside life history observations in the same or similar individuals.

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Data Availability Statement

The data underlying this article are available in the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA), and will be accessible under Bioproject PRJNA636137, BioSample accession numbers SAMN15065573-SAMN15065604 upon publication of this manuscript. The genome annotation produced for *E. dilemma* (v2.2) will be available in the Dryad Digital Repository (doi:10.5061/dryad.2547d7wnh) upon publication of this manuscript.

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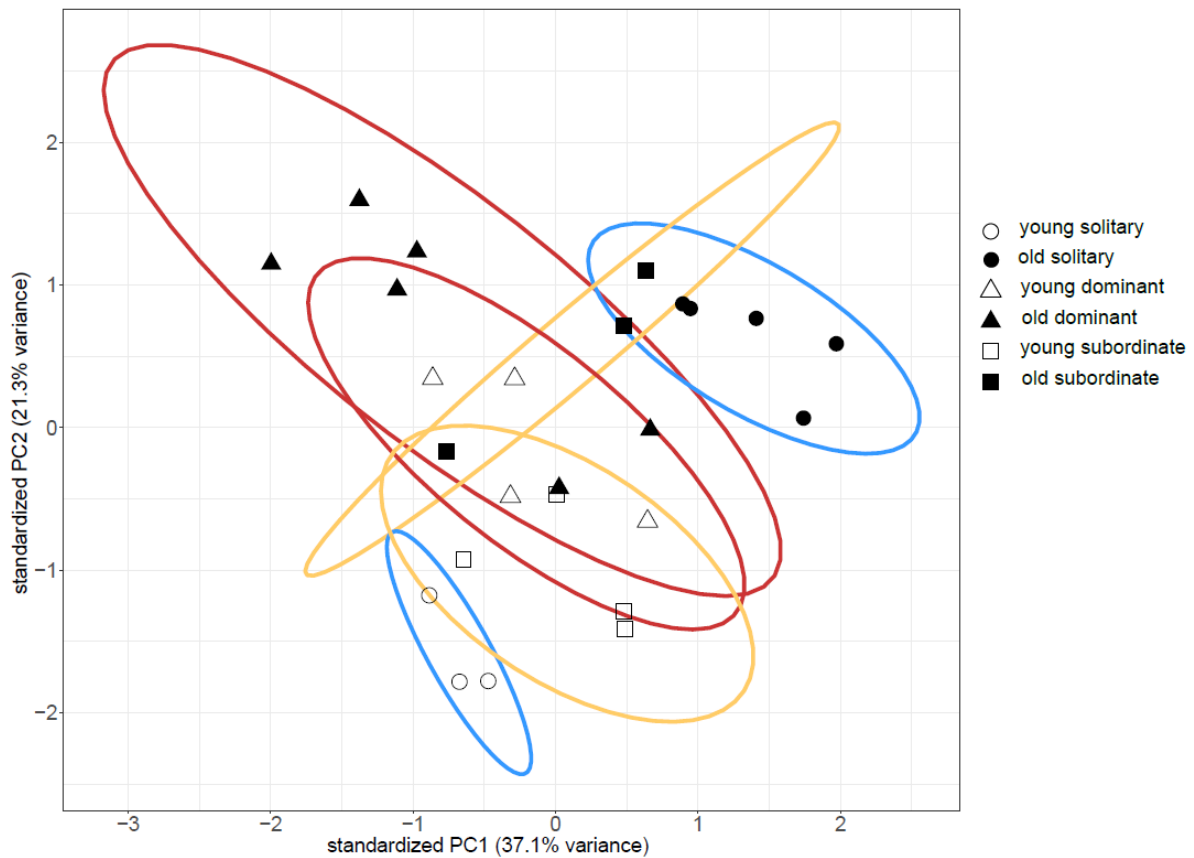


Figure 1. Principal component analysis (PCA) of variance-stabilised RNA read counts of young and old females from solitary and social nests. Each point represents the expression profile of one individual across the 958 genes which were differentially expressed between young and old individuals, cumulative across all social types (solitary: 940 DEGs, dominant: 14 DEGs, subordinate: 7 DEGs, with 2 DEGs shared between solitary and subordinate, and 1 DEG shared between solitary and dominant, see Fig. 2). Axis labels indicate the amount of variance in gene expression explained by the first two principal components (PC1 and PC2). Ellipses represent 95% confidence levels, and further illustrate the social type (blue=solitary; red=dominant; yellow=subordinate) of individuals.

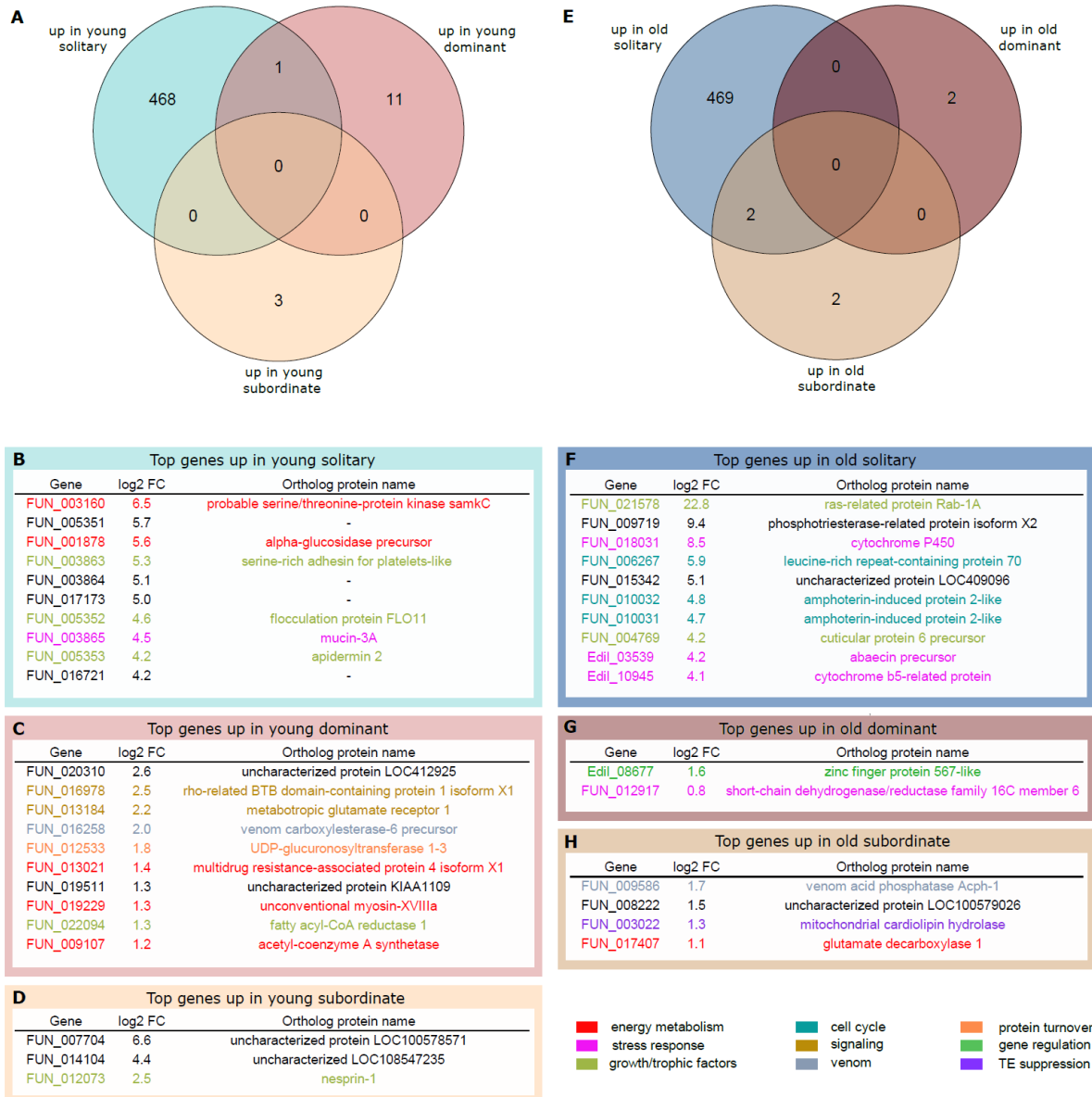


Figure 2. Venn diagrams and ortholog protein names of genes differentially expressed with age in solitary, dominant and subordinate females. The Venn diagram and tables on the left (A-D) represent genes upregulated in young compared to old individuals. The Venn diagram and tables on the right (E-H) represent genes upregulated in old compared to young individuals. Venn diagrams and table frames are colour-coded according to comparison as in Figure 1. Tables B-D and F-H show, for each comparison, the list of top genes (highest absolute log₂ fold-change) as well as the protein names of their closest found orthologs. The listed genes are colour-coded according to putative functional category based on annotations in UniProt (see legend, bottom-right). Genes left in black were not assigned.

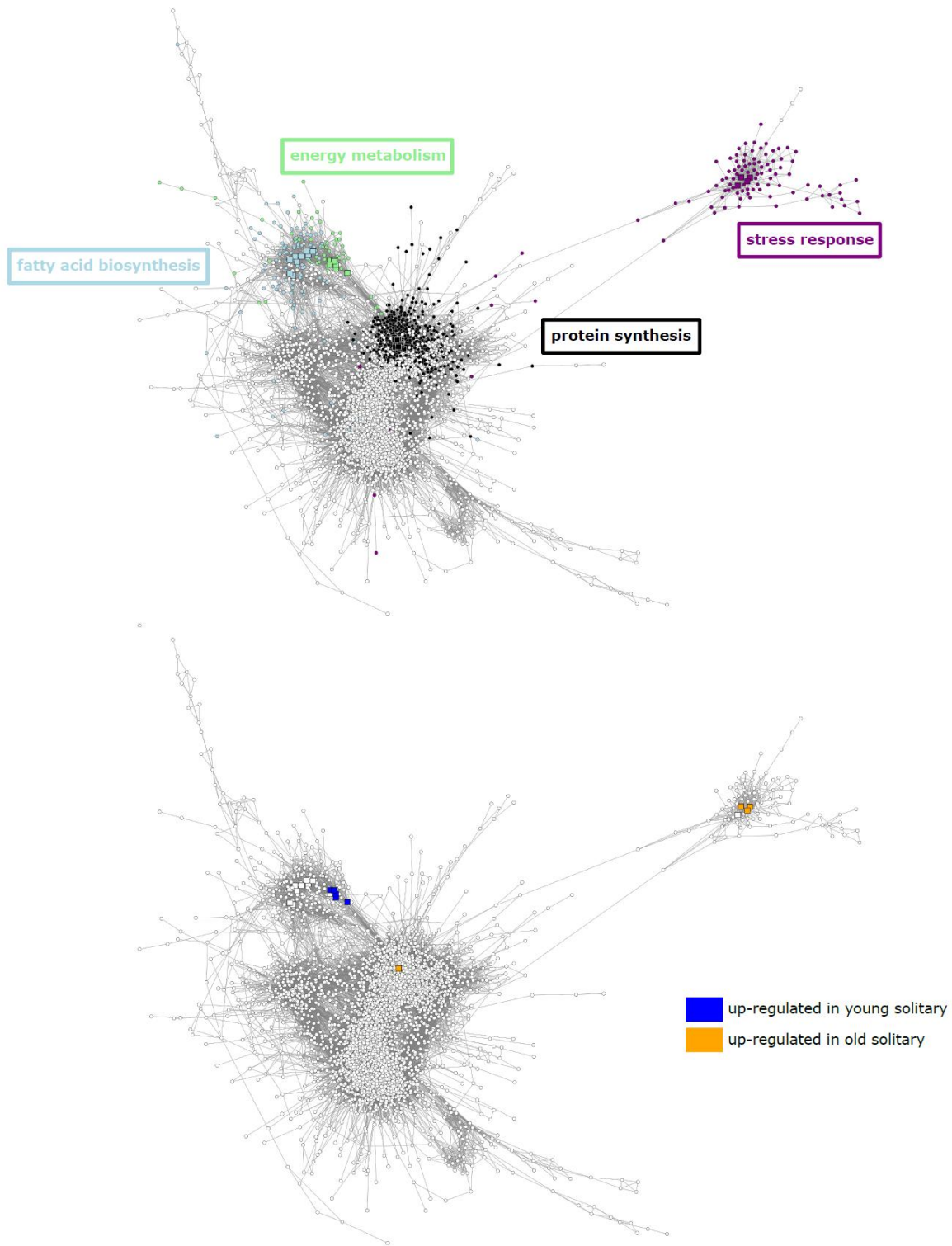


Figure 3. Weighted gene co-expression networks across all expressed genes. Coloured nodes in A represent all genes from the four age-associated modules (purple, black, blue, green), with each colour representing a different module. Square nodes represent hub genes (intramodular connectivity > 0.75). Framed words indicate the top functions of the top ten hub (most highly connected) genes in each module. In B, coloured nodes represent genes from modules in A which are differentially expressed



between young and old solitary females (blue = up-regulated in young, orange = upregulated in old). Coloured nodes in network B thus highlight the overlap between the genes found to be significantly associated with age in each independent analysis, *i.e.* the coexpression network analysis and differential gene expression analysis. A reduced version of the network is presented here for visualisation purposes, with 6,105 nodes and *circa* 500,000 edges.

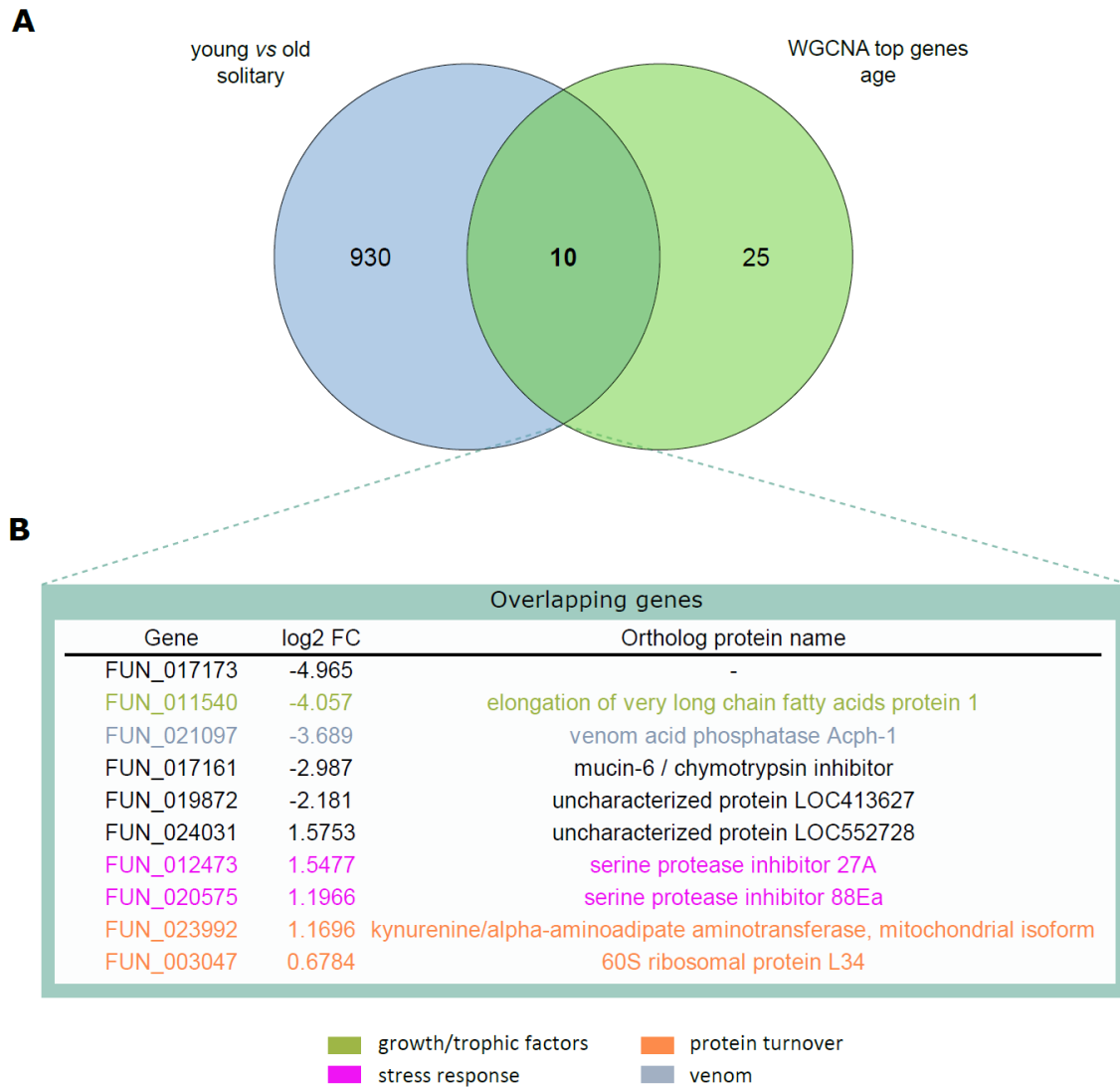


Figure 4. Venn diagram (A) and functional annotation (B) for genes significantly associated with age in solitary females in the WGCNA network and that are differentially expressed between young and old solitary females. The green circle in A represents the cumulative 35 hub genes (intramodular connectivity > 0.75) across all four age-related modules in the WGCNA. The blue circle shows the number of DEGs between young and old solitary females. Table B shows the functional annotation of the ten genes shown in bold (intersection in A), which were found to be significantly associated with age in solitary females in both the DEG and the WGCN analyses. Genes with a negative log2 fold change were up-regulated in young compared to old solitary females; genes with a positive log2 fold change were up-regulated in old compared to young solitary females. The listed genes are colour-coded according to functional category.



Additional files:

All Supplementary Material associated with Chapter 2 has been uploaded to BioRxiv and is available at the following link:

<https://www.biorxiv.org/content/10.1101/2020.07.30.228304v1.supplementary-material>

These supplementary materials are not included in the present thesis due to space limitations.



Sociality is associated with a lower cost of brood provisioning in the orchid bee *Euglossa viridissima*: a transcriptome analysis

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In prep.



Abstract

Reproduction is costly. As a consequence, most species in the wild experience a trade-off between fecundity and other life history traits such as longevity. Eusocial insects constitute a major exception to this pattern, as eusocial queens are seemingly able to maximise fecundity at no apparent cost. However, the mechanisms enabling them to escape the costs of reproduction remain poorly understood. Here, we manipulated brood provisioning effort, a potentially costly maternal behaviour, in the facultative eusocial orchid bee *Euglossa viridissima*. We then investigated transcriptomic signatures of increased effort in solitary and social nests within a same population, allowing us to evaluate the impact of sociality on the costs of brood provisioning. Solitary females exhibited more pronounced changes in gene expression, both in the brain (25 differentially expressed genes) and in the abdomen (47 differentially expressed genes), in response to increased brood provisioning effort when compared to females from social nests (10 differentially expressed genes or less, irrespective of tissue or social hierarchy). These results support the hypothesis that queens from social nests (referred to as “dominant” in this study) somehow forego the costs of reproductive investment, yet they go against the expectation that workers (here referred to as “subordinate”) bear the bulk of these costs in social nests. We highlight valuable candidate genes potentially underlying the remoulding of life histories in eusocial insects. Similar pathways exhibited changes in gene expression in both solitary and social females, such as those involved in immunity and protein turnover. Our study helps shed light on the mechanisms underlying the costs of reproduction in a species at the cusp of sociality, thus furthering our understanding of the remoulding of the fecundity/longevity trade-off which seemingly accompanied the transition from solitary to eusocial living.

Key words: facultative eusociality, reproduction, costs, gene expression, *Euglossa viridissima*



Introduction

Costs of reproduction

The cost of reproduction is a central component of life history evolution, as increased reproductive effort often trades off with traits such as immunity and longevity (Harshman & Zera 2007; Edward & Chapman 2011; Flatt 2011). Increased mating effort leads to reduced immune ability and longevity in crickets (Fedorka *et al.* 2004). In the mealworm beetle, mating negatively affects phenoloxidase activity (a major effector of the humoral immune response in insects) and is thought to induce the release of immunosuppressive signals (Rolff & Siva-Jothy 2002; for a review of the trade-offs between reproduction and immunity in insects see Schwenke *et al.* 2016). Aside from being associated with a compromised immune system, experimental evidence suggests that reproduction leads to increased vulnerability to environmental stress (reviewed in Harshman & Zera 2007). For instance, treatments to increase egg production lead to reduced resistance to oxidative stress in *Drosophila melanogaster* (Salmon *et al.* 2001; Wang *et al.* 2001) and in zebra-finches (Alonso-Alvarez *et al.* 2004). More generally, reproduction has been shown to trade off with longevity in many species (reviewed in Reznick 1985; Flatt *et al.* 2013). In *D. melanogaster*, high rates of mating experimentally reduce female lifespan (Fowler & Partridge 1989), and removal of the germline in *Caenorhabditis elegans* extends lifespan (Kenyon 2010), providing support for reproduction being costly to longevity.

Several hypotheses have been put forward regarding the proximate mechanisms mediating the cost of reproduction. The classic view put forward by van Noordwijk and de Jong (1986) posits that such costs are the product of a trade-off in the allocation of limited resources. According to this view, reproduction incurs a cost to the organism as it diverts resources away from somatic functions such as immunity and defence against oxidative stress. This hypothesis has received some empirical support (*e.g.* Alonso-Alvarez *et al.* 2008; King *et al.* 2010). An alternative view proposed by Leroi (2001) suggests that apparent life history trade-offs are not caused by reproduction diverting resources away from somatic maintenance, but rather by molecular signals emanating from the germ line which have a negative impact on survival. Indeed, molecular pathways such as the insulin/insulin-like growth factor (IGF)



signalling pathway are thought to act as upstream mediators regulating both fecundity and longevity (Hsin & Kenyon 1999; Flatt *et al.* 2008). The pleiotropic role of hormones such as juvenile hormone (JH) and testosterone has also been well-documented in various species, as these often have opposite effects on fecundity and other traits such as immunity and longevity, thus potentially mediating the cost of reproduction (Wingfield *et al.* 2001; Amdam *et al.* 2005; reviewed in Harshman & Zera 2007).

Redistribution of costs in social insects

A notable exception to these widespread costs of reproduction is the case of eusocial insects. In eusocial insect societies, the most reproductively active individuals (queens, and in the case of termites, also kings) seemingly do not bear the costs of high reproductive output, as they exhibit remarkably long lifespans compared to their worker counterparts (Wilson 1971; Keller & Genoud 1997; Carey 2001). This positive correlation between reproduction and lifespan in eusocial insects has also been supported experimentally, for instance in *Cardiocondyla obscurior*, where mated ant queens live longer than virgin queens (Schrempf *et al.* 2005) and experimentally increased egg-laying rates do not affect longevity of queens (Schrempf *et al.* 2017). Reproduction also does not always trade off with immunity in such systems; in *Lasius niger* queens, mating even seems to promote immune priming and pathogen resistance (Galvez & Chapuisat 2014).

Over the last decades, experimental studies have helped shed light on the molecular mechanisms underlying the apparent remoulding of life history trade-offs in eusocial insects (Corona *et al.* 2007; Lucas *et al.* 2016), but to this day these mechanisms still remain poorly understood. One theory which has gained empirical support is the maternal heterochrony hypothesis (Linksvayer & Wade 2005), which suggests that although queens and workers within a species share the same genome, the transition to eusociality was accompanied by a shift in the timing of the expression of pathways underlying maternal care, such that maternal care behaviours are expressed pre-reproductively towards siblings, rather than post-reproductively towards offspring. This may help explain life history differences between queens and workers, despite their common genetic background, and has been supported by data in the primitively eusocial wasp *Polistes metricus* (Toth *et al.* 2007) and the subsocial carpenter bee *Ceratina calcarata* (Rehan *et al.* 2014).



Our system and study aim

Socially polymorphic species offer unique opportunities for investigating the apparent remoulding of life history trade-offs with eusociality as they allow direct comparison of solitary and social phenotypes within a same species, or even within the same population, thus removing the confounding effects of genetic background (Séguret *et al.* 2016). Here, we use transcriptomic analyses of the socially polymorphic bee *Euglossa viridissima* Friese, 1899, to investigate changes in gene expression in response to experimental manipulation of brood provisioning effort, a reproduction-related behaviour.

Euglossa viridissima is a Neotropical orchid bee, with solitary and social nests co-occurring within the same population (Cocom Pech *et al.* 2008; May-Itzá *et al.* 2014). In this species, all nests are first established by a solitary “foundress” female. Upon emergence of the foundress female’s first brood, one or several daughters may remain in the natal nest, thus initiating the transition to a multi-female (social) matrilineal nest structure in which daughters share in care of future broods (Boff *et al.* 2015). In such multi-female nests, aggressive behaviours lead to the establishment of a dominance hierarchy, with a reproductive skew in favour of the mother (Cocom Pech *et al.* 2008). *E. viridissima* is a mass-provisioning species (Skov & Wiley 2005); females carrying out the task of brood provisioning use resin to construct barrel-shaped brood cells, then fill these cells with sufficient provisions (nectar, pollen) to sustain the offspring to adulthood, before laying an egg on top of these provisions and capping the cell with resin. In multi-female nests, foraging for resin to construct brood cells and brood provisions is carried out by one of the daughters, thus effectively redistributing this costly task away from the mother (Boff *et al.* 2015).

By manipulating brood provisioning effort in solitary and in social nests, our aim was to identify genetic pathways which were up- or down-regulated in response to a reproduction-related behaviour, and to shed light on the remoulding of fecundity-induced trade-offs which seemingly accompanies the transition to social behaviour. Given that queens in obligate eusocial insect species apparently forego the costs of reproduction, we expected social dominant *E. viridissima* females to exhibit fewer changes in gene expression in response to nest-wide increased brood provisioning compared to solitary females and subordinate females in social nests. In addition, we expected either 1) different molecular pathways



to be affected in solitary compared to social females, suggesting a complete remoulding of the molecular costs of reproduction during the transition to social living, or 2) pathways affected in solitary females to be distributed among social dominant and social subordinate females, suggesting a redistribution of the costs of reproduction in social females, as predicted by the maternal heterochrony hypothesis.

Methods

Experimental setup and sample collection

Collection site

All samples were collected at the Department of Apiculture of the Campus of Biological Sciences and Animal Husbandry, Autonomous University of Yucatán in Xmatkuil, Mexico (89.37°W, 20.52°N, sample collection permit n° 41593). Wooden nesting boxes (7 x 3 x 3 cm) with a glass plate under the lid (to facilitate observations) and an inner coating of a mixture (1:1) of honey bee wax and stingless bee cerumen were placed around campus. Nests were monitored three times a week from May 2017 to May 2018 to determine nest occupancy and detect the time when a female started building a brood cell.

Behavioural observations

Euglossa viridissima females which started bringing back brood cell provisions or which commenced building a first brood cell in experimental nesting boxes were marked with a diamond-tipped pen on the thorax using symbols for individual identification. In *Euglossa viridissima*, all nests are first established by a solitary foundress female. Upon emergence of the foundress' first brood, one or two of her female offspring may remain in the nest and help with the provisioning of a second set of brood cells, thus initiating the shift to a social nest. In a social nest, the mother is typically dominant, laying eggs and displaying aggression towards nestmates whilst the daughter(s) are subordinate, carrying out provisioning and brood care tasks (Cocom Pech *et al.* 2008; May-Itzá *et al.* 2014). We focused on social nests with only one subordinate female to facilitate interpretation of the effects of our experimental manipulation. In such multifemale nests, the social status of each female (dominant *versus* subordinate) was determined through behavioural observations; the female returning to the nest with provisions was



characterised as subordinate, while the other female in a nest was characterised as dominant (Séguret *et al.* submitted).

Experimental manipulations and sampling

Newly established nests were randomly assigned to treatment or control groups. Nests in the treatment group were manipulated to experimentally increase nest-wide brood provisioning effort. For this, food (brood cell provisions) was experimentally removed from the second brood cell built in each nest, either from the first brood phase (solitary nests) or second brood phase *i.e.* in social nests, as follows. When the cell contained provisions, but before an egg had been laid in it, nests were brought into a laboratory directly adjacent to the collection site, and provisions were carefully removed from the filled, uncapped cell using a flat-ended probe (between 104 and 341 mg of provisions were removed from each treatment nest, Supplementary Table S1). The amount of provisions removed relative to body weight did not differ according to female social type (types compared: solitary, dominant, subordinate, Kruskal-Wallis test, $\chi^2=0.67$, $df = 2$, $p = 0.72$). The purpose of the manipulation was to induce female(s) to make additional foraging trips to fill the cell with provisions again until it was ready to receive an egg and be sealed. In control nests, a sham manipulation was performed whereby provisions were removed from the second brood cell following the same protocol as for treatment nests and immediately placed in the cell again. In both treatment and control nests, the female(s) were collected once an egg had been laid in the manipulated cell and it had been sealed.

Individuals were collected from a total of 8 control nests (4 solitary, 4 social) and 10 treatment nests (5 solitary, 5 social). Upon collection, each individual was weighed and its thorax width (intertegular distance) was measured using a Canon EOS 60D camera and the imaging software Motic Images Multifocus Pro 1.0. Whole bees were then kept overnight at -80°C , then transferred to RNAlater® (Sigma-Aldrich) for storage at -80°C . In total, 27 individuals were used for molecular analyses (Suppl. Table S1).



RNA sample preparation, sequencing & read processing

RNA isolation and sequencing

As gene expression profiles have been shown to vary considerably according to tissue (Saleh & Ramirez 2019, Negroni *et al.* 2019), RNA was isolated from two tissues (brain and whole abdomen) in order to better characterise transcriptomic changes according to treatment and social status. Brains were dissected in RNAlater® by removing the compound eyes, the cuticle around the frons- and post-occiput, and the tracheal sacs. Total RNA was extracted separately from the brain and the whole abdomen of 27 individuals (*i.e.* yielding a total of 54 RNA samples) using the RNeasy® Plus Mini kit, including DNase I digestion, according to manufacturer's instructions, using 350 µl starting material (Qiagen, Hilden, Germany). RNA concentration and purity were measured using an Epoch® microplate spectrophotometer (BioTek) (brains: RNA concentration range = 9-40 ng/µl, total RNA range = 0.25-1.12 µg, 260/280 range = 1.7-2.6; abdomen: RNA concentration range = 20-415 ng/µl, total RNA range = 0.5-11.2 µg, 260/280 range = 1.9-2.1).

Library preparation and transcriptome sequencing were undertaken at the Beijing Genomics Institute (BGI, Shenzhen). For each sample, a cDNA library was prepared using the TruSeq RNA Library Preparation kit (Illumina) and SuperScript II Reverse Transcriptase (Thermo Fisher Scientific). Libraries were sequenced on an Illumina HiSeq4000 platform (11 samples per lane) to generate 100 bp paired-end reads, with ~4 GB of raw data per sample, *i.e.* 60 million read pairs per sample on average (range = 43-116 million read pairs).

Trimming and filtering of raw reads

Raw RNA reads were checked for quality using FastQC v0.11.5 (Andrews 2010), then filtered and trimmed using Skewer v0.2.2 (Jiang *et al.* 2014) to remove low quality bases and reads, adapter contamination and reads shorter than 70 bp.

Exclusion of individuals from a cryptic sister species

Individuals belonging to the cryptic sister species *Euglossa dilemma* were identified by sequence comparison at the olfactory receptor locus *or41* (Brand *et al.* 2020). For each individual, DNA was



isolated from one front leg using Chelex® 100. The resulting DNA was amplified at the *or41* locus and commercially Sanger sequenced (see Suppl. Text S2 for details). Genotyping individuals at this locus allowed us unambiguously to characterise them all as *E. viridissima* or *E. dilemma* (Suppl. Table S1). Nine individuals were characterised as *E. dilemma* and were excluded from analysis.

Differential expression analyses

Read mapping and quantification

Trimmed and filtered reads were pseudo-aligned to the *E. dilemma* genome v2.2 (GCA_002201625.1, Brand et al. 2017; annotation v2.2 available at DRYAD digital repository, doi:10.5061/dryad.2547d7wnh) and quantified using Kallisto v0.46.2 (Bray et al. 2006).

Differential gene expression analysis

Read counts on the transcript level were imported and converted to gene-level counts using the package tximport v1.16.1 (Soneson, Love and Robinson 2015). To determine which genes were differentially expressed in response to increased brood provisioning effort, a differential expression analysis was performed separately for each sample type (solitary, social dominant, social subordinate) and for each tissue (brain, abdomen) using the package DESeq2 v1.28.1 (Love, Huber and Anders 2014) in R v4.0.2 (R Development Core Team 2020), contrasting the control individuals to those undergoing experimental increase in brood provisioning effort.

Functional assignment of differentially expressed genes

For *E. viridissima* genes which were differentially expressed in response to experimental increase in brood provisioning effort, we searched for the known function of their closest ortholog. OrthoFinder v2.3.7 (Emms and Kelly 2015) was run with default settings on the protein level using the protein sets of the following genomes: *E. dilemma* (annotation v2.2), *A. mellifera* (GCF_003254395.2), *Bombus terrestris* (GCF_000214255.1), and *Bombus impatiens* (GCF_000188095.3). Ortholog protein names were extracted from *A. mellifera* annotations, if available, or from *Bombus* spp. if no *A. mellifera* ortholog was found. When multiple ortholog copies were found in the species of interest, the first one listed was selected. Multiple copies represented isoforms of the same gene in 24 out of 25 cases, thus



this selection did not affect functional assignment. For remaining genes for which no ortholog was found in *A. mellifera* or *Bombus* spp., we used BlastP (Altschul 1990) implemented in the BLAST+ suite v2.7.1 (Camacho et al. 2009) to identify putative orthologs based on the best hit to the UniProt Arthropoda database (downloaded 27.04.2018). Each orthologous protein was then searched manually in the UniProt online database (www.uniprot.org) for functional annotations.

Results

RNA sequencing

For the brain samples, after filtering and trimming, sequenced libraries contained on average 34 million read pairs (SD = 4 million; range = 27-40 million), of which 60% (SD = 4%; range 47-66%) pseudo-aligned to the genome annotation *E. dilemma* v2.2. For the abdominal samples, filtered and trimmed libraries contained on average 33 million read pairs (SD = 10 million; range = 24-61 million), of which 75% (SD = 4%; range = 65-82%) pseudo-aligned to the genome.

Stronger transcriptomic signatures of increased provisioning effort in solitary compared to social females

Solitary females

Fifteen genes were upregulated in the brains of solitary females undergoing experimentally increased brood provisioning, in comparison to expression levels in the brains of solitary females from control nests, while ten genes were downregulated with experimental treatment. In the abdomens of solitary females, 14 genes were upregulated with increased brood provisioning, while 33 genes were downregulated with increased brood provisioning (Figure 1, for a full table of the number of differentially expressed genes (DEGs) for each pairwise comparison, see Suppl. Table S3).

Social females

In contrast, an experimental increase in brood provisioning effort in social nests led to a change in expression of only three genes in the brains of dominant females, all of which were upregulated with



treatment. In the abdomens of dominant females, four genes were upregulated with increased brood provisioning, and six genes were downregulated. Similarly, in the brains of subordinate females from social nests, only three genes were upregulated with increased brood provisioning and three genes were downregulated. Finally, in the abdomens of subordinate females, only six genes were upregulated with increased brood provisioning and no genes were downregulated (Figure 2, Supp. Table S3).

Functional assignment of differentially expressed genes

Solitary females

In solitary females, functional annotation of proteins encoded by DEGs based on orthology in *A. mellifera* and *Bombus* spp. revealed that genes upregulated in the brain with increased brood provisioning effort were seemingly mainly related to energy metabolism (*e.g.* glucose oxidase, cytochrome b5; for full details on functional annotation results, see Supp. Material S4) as well as gene regulation (*e.g.* zinc finger protein 771-like) and protein turnover (*e.g.* sodium-dependent nutrient amino acid transporter 1). In the abdomens of solitary females, genes upregulated with increased provisioning were involved in lipid metabolism (elongation of very long chain fatty acids protein), nutrient sensing (sarcoplasmic reticulum histidine-rich calcium binding), gene regulation (paired mesoderm homeobox protein 2-like), protein turnover (F-box only protein 33) and reproduction (broad-complex core protein).

Genes downregulated in the brain with increased brood provisioning effort were seemingly related to DNA repair and regulation (protein arginine N-methyltransferase 3), control of DNA replication (N-acetyltransferase ESCO1) and potentially energy metabolism (*e.g.* sugar transporter SWEET1). Genes downregulated in the abdomen with increased brood provisioning were also mainly involved in energy metabolism (*e.g.* cytochrome b5-related protein, two genes from the cytochrome P450 superfamily, sarcosine dehydrogenase), as well as immunity (*e.g.* phenoloxidase-activating factor 2, serine protease inhibitor 27A, peptidoglycan-recognition protein SA precursor), cell cycle (*e.g.* protein Skeletor, organic cation transporter protein isoform X2) and inhibition of gene transcription (SET domain-containing protein SmydA-8).



Social females

In dominant females from social nests, genes which were upregulated in the brain with increased brood provisioning were potentially related to cellular functions (basic proline-rich protein; Supp. Material S4) and metabolism or detoxification (solute carrier family 22 member 3), while the only annotated gene upregulated in the brains of subordinate females in response to brood provisioning was related to protein turnover (proteasome subunit alpha type-6). One gene was upregulated in response to brood provisioning in the brains of both dominant and subordinate females, but this gene is currently uncharacterised (FUN_016599; Figure 1). In the abdomens of dominant females, genes upregulated with increased brood provisioning were seemingly involved in protein turnover (protein saal1), and potentially also in cellular functions (annexin B9) and sensory perception or signalling (nicotinic acetylcholine receptor alpha9 subunit precursor). Genes upregulated with treatment in the abdomens of subordinate females were also seemingly related to protein turnover (*e.g.* conserved oligomeric Golgi complex subunit 8, proteasome endopeptidase complex) and maintenance or cellular functions (*e.g.* annexin B9, leucine-rich repeat-containing protein 15). Two genes were upregulated with increased brood provisioning in the abdomens of both dominant and subordinate females, one seemingly involved in protein turnover and the other in cellular functions (Figure 2; Supp. Material S4).

In dominant females, no gene was downregulated in the brain in response to increased brood provisioning. The only gene downregulated in the brains of subordinate females with increased brood provisioning was seemingly related to development and cellular functions (neither inactivation nor afterpotential protein G-like). In the abdomens of dominant females, genes downregulated in treatment compared to control nests were mainly uncharacterised, but some were potentially involved in protein turnover (kelch protein) and in regulatory pathways (probable serine/threonine-protein kinase sakC). No genes were downregulated in the abdomens of subordinate females in response to treatment.

No overlap of DEGs between different tissues

Within each social type (solitary, social dominant, social subordinate), there was no overlap of genes differentially expressed with increased brood provisioning between the brain and the abdomen. In other



words, for solitary, dominant and subordinate females, all DEGs between control and treatment nests were tissue-specific.

Discussion

Solitary females showed a subtle response in terms of differential gene expression to increased brood provisioning effort whilst social females (either dominant or subordinate) showed hardly any response, suggesting that sociality may buffer bees from the costs of this reproduction-related behaviour. Though the same pattern of differential expression of genes was seen in two tissue types (more genes differentially expressed in solitary *versus* social individuals in response to increased brood provisioning effort), those genes that were differentially expressed differed across tissue types.

Tissue-specific responses

The finding that no DEGs overlapped between tissues (brain and abdomen) within each social type highlights the importance of tissue-specific measurements for gene expression studies. This is in line with results from previous studies, such as that by Negroni *et al.* (2019) who, upon examining transcriptome-wide gene expression patterns in tissues similar to those in our study (brain and fat body) in *Temnothorax rugatulus*, also found very little overlap in age related DEGs between tissues. In their study, only 11 out of 1,755 DEGs were differentially expressed with age in both the brain and the fat body, representing less than 1% of differentially expressed genes.

Stronger effects in solitary females

We found more genes to be differentially expressed in response to increased brood provisioning in solitary females compared to social dominant females, following our hypothesis that social dominant females exhibit lower costs of reproduction than solitary females. This may indicate that social “queens” forego some of the costs of reproduction, even in facultative eusocial species. Interestingly, subordinate females from social nests in our study also exhibited weaker changes in gene expression in response to increased brood-provisioning effort compared to solitary females. As similar pathways experienced



changes in regulation in both dominant and subordinate females from social nests (immunity, protein turnover, cellular functions...), this suggests that costs are not redistributed in a way that certain pathways underpin the costs in dominant females and different pathways underpin those in subordinate females, as may be expected under the maternal heterochrony hypothesis (Linksvayer & Wade 2005). Rather, it appears that costs may have been redistributed in social nests so that dominant and subordinate females experience the same type of cost, but to a lower extent than females from solitary nests. In other words, they may share the burden of reproductive costs. Moreover, social females may exhibit a nest-wide net reduction of costs as, even when combined, dominant and subordinate females from within a nest exhibit fewer DEGs than solitary females in response to increased brood provisioning effort.

Pathways affected by increase in brood provisioning

The genetic pathways which we found to be affected by increased brood provisioning effort are those which have previously been linked to the physiological costs of reproduction, such as metabolism and oxidative stress, immunity, nutrient sensing, gene regulation and protein turnover (Wang *et al.* 2001; Schwenke *et al.* 2016; Templeman & Murphy 2018; Moltshaniwskyj & Carter 2012). Based on our results, it is difficult to determine exactly how changes in the expression of genes within these pathways ultimately affects individual phenotype, as genes may be positively or negatively linked to a phenotypic trait (*e.g.* effectiveness of the immune response). Also, as *E. viridissima* is a non-model species, functional annotations of specific genes highlighted as relevant are based on functions identified in *Drosophila melanogaster*, or in some cases more distantly-related species, and such functions may not be conserved in *E. viridissima*. Indeed, single-copy orthologs between *D. melanogaster* and the bee *A. mellifera* show only 53% protein identity on average (Zdonov & Bork 2007).

However, our study does provide strong candidates for knock-down experiments, as we identify sets of genes which appear to play a role in underpinning the costs of reproduction in solitary and in social insects, with changes in expression either in the brain or in the abdomen. Knock-down studies would help elucidate the function of such candidate genes in more closely related species, allowing more robust pathway-wide conclusions, and would help determine their relative importance in underpinning the costs of reproduction.



Interestingly, genes involved in the nutrient sensing and lipid metabolism pathway were differentially expressed in response to increased brood provisioning effort in solitary females, but not in social females. This may indicate that the nutrient sensing pathway regulates the costs of reproduction in solitary females, as observed in other solitary species such as *D. melanogaster* and *C. elegans* (Flatt *et al.* 2008; Wang *et al.* 2008), but that such regulatory underpinnings do not exist or have been remodelled in social females, as has been proposed in complex eusocial species (Rodrigues & Flatt 2016).

Conclusions and future directions

To conclude, our study identifies specific pathways and candidate genes associated with investment in brood provisioning, a reproduction-related behaviour, ultimately shedding light on the mechanisms underlying the costs of reproduction in a species on the brink of sociality. Our results suggest a lower cost of this behaviour in social females, both dominant and subordinate, compared to solitary females, suggesting there is a reshaping of the fecundity/longevity trade-off in incipient stages of sociality. The identification of candidate genes potentially underlying the costs of reproduction in socially polymorphic species paves the way for knock-down experiments in closely related non-model species, which could allow us to elucidate the role of specific genes within the identified pathways, and to identify phenotypic effects (*e.g.* changes in immune capacities, effects on longevity...) induced by changes in the expression of such candidate genes, ultimately expanding our understanding of their role in remoulding the fecundity/longevity trade-off which seemingly accompanied the transition from solitary to social living.

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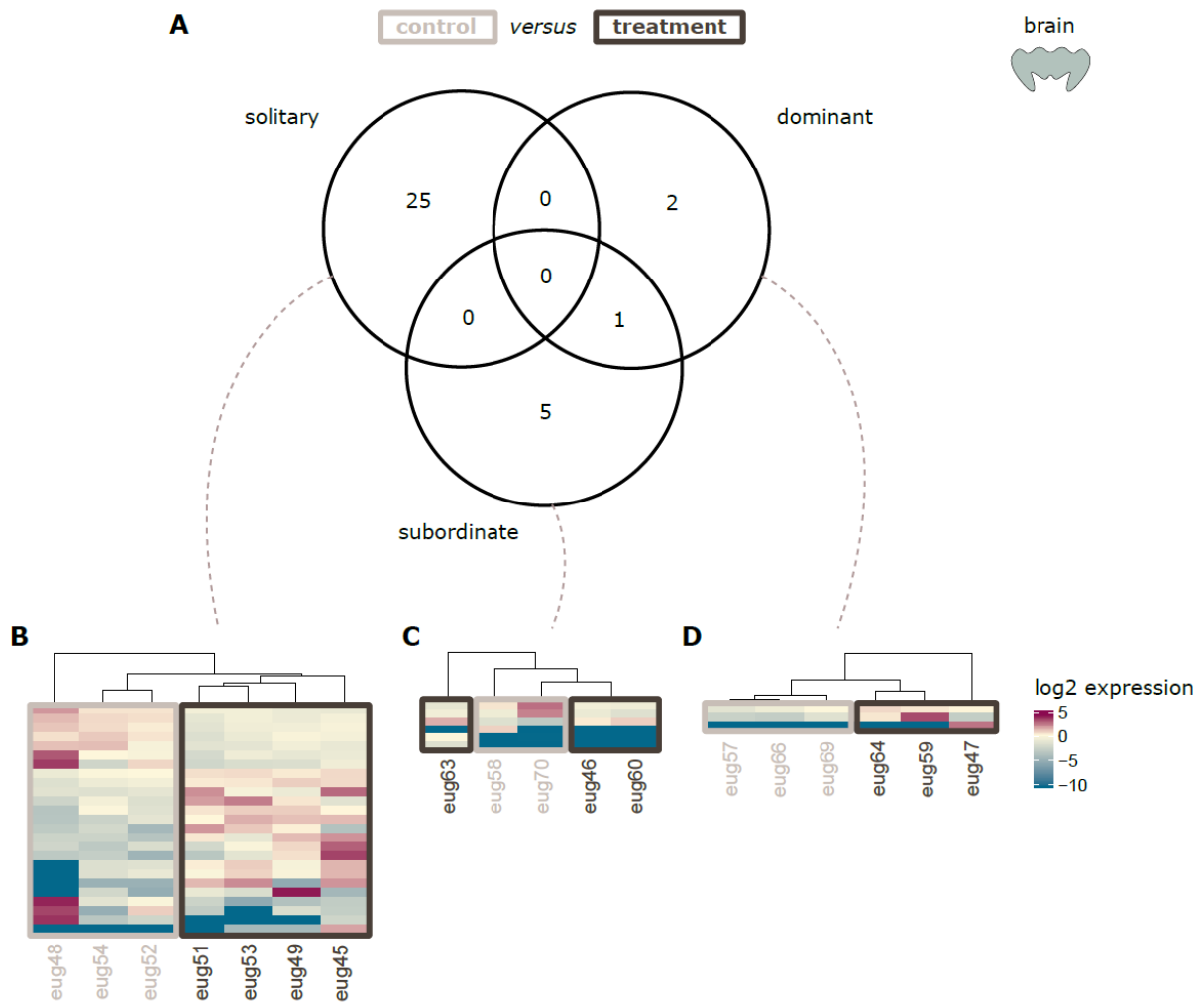


Figure 1. Gene expression patterns in the brains of control and treatment females. The Venn diagram (A) shows the numbers of differentially expressed genes (DEGs) between females undergoing experimentally increased brood provisioning (treatment) and females undergoing a sham manipulation (control). The DEG analysis was run separately for solitary, dominant and subordinate females. Heatmaps show gene expression patterns for DEGs across control and treatment females for the solitary (B), subordinate (C) and dominant phenotype (D). The heatmap key shows log₂ scaled expression level relative to the mean value across all individuals for each gene. Light grey frames in the heatmaps highlight gene expression patterns of control females, and dark grey frames highlight gene expression patterns of treatment females.

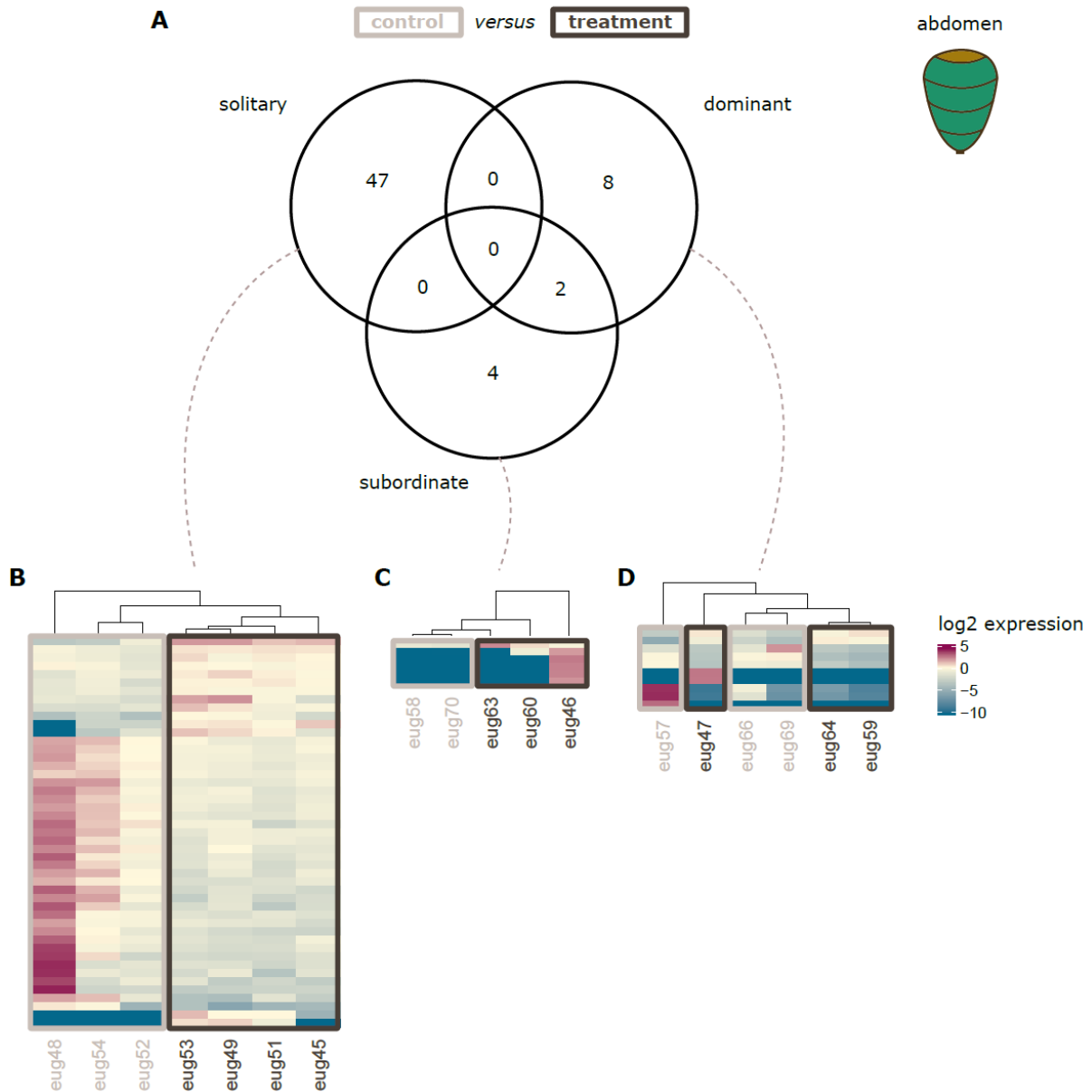


Figure 2. Gene expression patterns in the abdomens of control and treatment females. The Venn diagram (A) shows the numbers of differentially expressed genes (DEGs) between females undergoing experimentally increased brood provisioning (treatment) and females undergoing a sham manipulation (control). The DEG analysis was run separately for solitary, dominant and subordinate females. Heatmaps show gene expression patterns for DEGs across control and treatment females for the solitary (B), subordinate (C) and dominant phenotype (D). The heatmap key shows log₂ scaled expression level relative to the mean value across all individuals for each gene. Light grey frames in the heatmaps highlight gene expression patterns of control females, and dark grey frames highlight gene expression patterns of treatment females.



Additional files:

Supplementary Table S1: Sample information	p. 93
Supplementary Text S2: Species assignment of samples to <i>E. viridissima</i> or <i>E. dilemma</i>	p. 94
Supplementary Table S3: Number of differentially expressed genes (DEGs) for each comparison in DESeq2, excluding <i>E. dilemma</i> individuals	p. 95
Supplementary Material S4: Orthology results for genes differentially expressed between control and treatment nests (experimentally increased brood provisioning). Includes Supplementary Tables SI-SXII.	pp. 96-103



5. General conclusion

This thesis sought to explore the molecular mechanisms underpinning the remoulding of the fecundity/longevity trade-off which seemingly accompanies the transition from solitary to eusocial living in insects. Comparative analyses across a sociality gradient suggest that this remoulding is already apparent in primitively and facultatively social insects (Toth *et al.* 2016), making facultatively social species excellent systems for investigating the mechanisms underlying the reshaping of the trade-off through direct comparisons between solitary and social individuals of the same species (Séguret *et al.* 2016; Shell & Rehan 2018).

My results support a reshaping of the fecundity/longevity trade-off in social compared to solitary individuals of *Euglossa viridissima*, a facultatively eusocial orchid bee. First, the results from the analyses of transcriptomic data in young and old bees from solitary and social nests support the hypothesis that sociality is associated with a remoulding of ageing trajectories (Chapter 2). These results highlight candidate genetic pathways potentially underpinning such a remoulding. Second, the results from an experimental manipulation of brood provisioning effort suggest that such an energetic (costly) task has a higher impact on gene expression in solitary compared to social individuals (Chapter 3). The pathways highlighted in this study may further our understanding of how social individuals may “escape” the costs of reproduction experienced by solitary individuals, with implications for the remoulding of the fecundity/longevity trade-off in eusocial insects.

5.1. Support for a remoulding of ageing trajectories with sociality in *Euglossa viridissima*

Using my nest observation data collected three times a week in the field from February 2016 to March 2018 in which I noted the presence or absence of marked females in all nests at the collection site (described in Chapter 2, Methods), I was able to further estimate the lifespan of solitary and social females which were not sampled for RNAseq analyses. Although these are potentially under-estimates, as the sudden absence of a female from a nest could indicate either death or relocation of the individual, these data support our initial assumption, based on the literature (Toth *et al.* 2016), that despite being



5. General conclusion

morphologically identical, social dominant females exhibit a longer lifespan than social subordinate females, with solitary females having an intermediate lifespan (Table 1, below).

Table 1. Estimated lifespan of individuals observed in the field which were not sampled for RNAseq analyses. Females were individually marked and their presence in experimental nests was monitored three times per week. Age was calculated for each individual based on date of first and last observations, which may represent an underestimate of actual total lifespan in the field.

	Average lifespan \pm SD (days)	Minimum lifespan (days)	Maximum lifespan (days)
Solitary (n = 23)	71 \pm 29	11	137
Dominant (n = 14)	129 \pm 34	63	184
Subordinate (n = 5)	28 \pm 12	12	46

Accordingly, in Chapter 2, I sought to shed light on the mechanisms which may have enabled such a remoulding of ageing trajectories with sociality, both on the level of genetic pathways (comparing gene expression levels) and on the endocrine level (comparing titres of juvenile hormone). Firstly, the remarkable difference between solitary and social females in the number of genes which show a change in expression levels with age provides support on the molecular level that social females of this facultatively eusocial bee exhibit reduced transcriptomic signatures of ageing compared to conspecific solitary females. Secondly, different genes exhibit changes in expression with age in solitary *versus* social females. Indeed, my results show that genes, some of which are involved in pathways such as metabolism, growth and development, and immunity or response to stress, show a shift in expression with age in solitary, but not in social females. This is demonstrated by the lack (or negligible amount) of overlap between solitary and social females in terms of which genes are differentially expressed with age (Chapter 2, Figure 2). Finally, the fact that juvenile hormone titres did not differ according to age and social status suggests that JH does not underlie the remoulding of life history traits with sociality in this facultative eusocial species. My results add to the growing body of evidence suggesting that JH is perhaps not the best marker to reflect the reshaping of life histories in eusocial insects, as its role



appears to be species-specific, making it potentially unsuitable for cross-species comparisons (see references in Chapter 2, Discussion).

5.2. Reduced costs of reproduction in social compared to solitary females

In Chapter 3, I went a step further, experimentally investigating the costs of reproduction by looking into changes in gene expression following an increase in brood provisioning effort. By doing so in solitary and in social nests, I was able to quantitatively (*how many* genes show changes in expression levels) and qualitatively (*which* genes show changes in expression levels) evaluate the cost of such a reproductive investment-related behaviour with regard to sociality.

Again, the lack of overlap between solitary and social females in terms of which genes show changes in expression with increased brood provisioning effort (Chapter 3, Figures 1 and 2) indicates that different genes underpin the costs of such a reproduction-related behaviour in social compared to solitary individuals. The changes in gene expression specific to solitary females in response to brood provisioning, and those specific to social females therefore highlight important candidate genes underlying potential changes in the cost of reproduction with sociality. These genes belong to pathways previously suggested to underlie the negative link between reproduction and lifespan, such as lipid metabolism and immunity (Hansen *et al.* 2013; Schwenke *et al.* 2016). These candidates may ultimately shed light on the remoulding of the fecundity/longevity trade-off in eusocial insects.

5.3. Next steps and open questions

This work was carried out in the context of the DFG Research Unit FOR2281, Sociality and the reversal of the fecundity/longevity trade-off (www.so-long.org). The aim of this collaborative project was to generate life history and transcriptomic data, as well as data for certain molecular markers of ageing (juvenile hormone, oxidative damage) in insect species across the sociality gradient, ranging from the solitary *Drosophila melanogaster*, to the facultatively social bee which is the focus of this thesis (*E. viridissima*), all the way to complex eusocial bees (*Apis mellifera capensis*, Aumer *et al.* 2018), ants (*Temnothorax rugatulus*, Negroni *et al.* 2019) and termites (*Cryptotermes secundus*, Monroy Kuhn *et al.* 2019; *Macrotermes bellicosus*, Elsner *et al.* 2018). These data are currently being utilised for



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comparative studies across species to shed light on the molecular mechanisms underpinning the remoulding of the fecundity/longevity trade-off in eusocial insects. *Euglossa viridissima* represents the transitional stage between solitary and eusocial, and as such constitutes a crucial element of these comparative studies. Two manuscripts are currently under review, in which we investigate 1) comparative patterns of gene expression with sociality (Korb *et al.* submitted) and 2) comparative patterns of oxidative damage with ageing in species across the social gradient (Kramer *et al.* submitted). Candidate genes identified in individual species such as those presented in this thesis, or highlighted through comparative analyses across species, serve as a starting point for experimental work to confirm (or refute) their importance in shaping the remoulding of life histories with sociality. Knock-down experiments of selected candidate genes would allow us to better understand their role in regulating the fecundity/longevity trade-off, as well as the magnitude of their effect and their interactions with other genes. Unfortunately, as *E. viridissima* is sensitive to manipulation and can so far only be observed in the field, this makes it an unsuitable study system for such experiments.

However, other molecular mechanisms potentially underlying the remoulding of the fecundity/longevity trade-off could still be investigated in this species. For instance, gene methylation has been suggested as a physiological marker of ageing (López-Otín *et al.* 2013). It would thus be of interest to investigate patterns of methylation with ageing in relation to sociality in *E. viridissima*, for instance through whole genome bisulfite sequencing (Lewis *et al.* 2020). Additionally, studies in the termite *M. bellicosus* highlight that transposable elements may underlie differences in ageing trajectories in eusocial reproductives vs non-reproductives (Elsner *et al.* 2018). Long-read genomic data for *E. viridissima* would allow one to produce a more complete transposable element annotation for the species, thus enabling one to explore this question using the transcriptomic data presented in this thesis. Finally, comparative analyses across the datasets generated by the So-Long research unit could look into the position of candidate genes in co-expression networks, to elucidate how their connectivity with other genes changes with sociality. These additional investigations, in combination with the genetic candidates identified through our studies, could help further our understanding of the complex physiological changes underlying the reshaping of life histories with sociality.



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Appendix A: references of Table 1

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Supplementary Table S1. Sample information

Sample ID	Date collected	Estimated age (days)	Nest ID	Intertegular width (mm)	Weight (mg)	Social status	Experimental group	Weight of provisions removed (mg)	Cryptic species assignment	Included in gene expression analyses
eug44	20170522	96	F11	3.558	114	solitary	treatment	104	<i>E. dilemma</i>	no
eug45	20170522	12	F17	3.558	234	solitary	treatment	234	<i>E. viridissima</i>	yes
eug46	20170612	26	F21	3.7	124	subordinate	treatment	341	<i>E. viridissima</i>	yes
eug47	20170612	26	F21	3.566	86	dominant	treatment	341	<i>E. viridissima</i>	yes
eug48	20170619	15	10E	3.429	90	solitary	control	-	<i>E. viridissima</i>	yes
eug49	20170619	16	4	3.743	114	solitary	treatment	215	<i>E. viridissima</i>	yes
eug50	20170619	28	A3	3.55	116	solitary	control	-	<i>E. dilemma</i>	no
eug51	20170626	21	4E	3.526	115	solitary	treatment	298	<i>E. viridissima</i>	yes
eug52	20170717	9	5	3.864	106	solitary	control	-	<i>E. viridissima</i>	yes
eug53	20170724	11	A18	3.622	108	solitary	treatment	140	<i>E. viridissima</i>	yes
eug54	20170725	12	21E	3.31	101	solitary	control	-	<i>E. viridissima</i>	yes
eug55	20171003	25	F19	3.543	113	dominant	treatment	111	<i>E. dilemma</i>	no
eug56	20171003	25	F19	3.179	85	subordinate	treatment	111	<i>E. dilemma</i>	no
eug57	20171023	74	F6	3.471	113	dominant	control	-	<i>E. viridissima</i>	yes
eug58	20171023	26	F6	3.349	100	subordinate	control	-	<i>E. viridissima</i>	yes
eug59	20171029	65	4	3.707	145	dominant	treatment	202	<i>E. viridissima</i>	yes
eug60	20171029	11	4	3.535	113	subordinate	treatment	202	<i>E. viridissima</i>	yes
eug61	20171107	144	A5	3.327	108	dominant	control	-	<i>E. dilemma</i>	no
eug62	20171107	18	A5	3.272	86	subordinate	control	-	<i>E. dilemma</i>	no
eug63	20171124	14	31E	3.54	125	subordinate	treatment	125	<i>E. viridissima</i>	yes
eug64	20171124	87	31E	3.597	71	dominant	treatment	125	<i>E. viridissima</i>	yes
eug65	20180115	26	9E	3.443	108	subordinate	control	-	<i>E. dilemma</i>	no
eug66	20180115	116	9E	3.266	98	dominant	control	-	<i>E. viridissima</i>	yes
eug67	20180226	66	2E	3.419	116	dominant	treatment	209	<i>E. dilemma</i>	no
eug68	20180226	49	2E	3.348	107	subordinate	treatment	209	<i>E. dilemma</i>	no
eug69	20180511	87	A25	3.559	117	dominant	control	-	<i>E. viridissima</i>	yes
eug70	20180511	30	A25	3.575	115	subordinate	control	-	<i>E. viridissima</i>	yes



Supplementary Text S2

Species assignment of samples to *Euglossa viridissima* or *Euglossa dilemma*

1. DNA extraction

DNA was isolated from a front leg, separately for each individual (for a list of all individuals, see Supplementary Table 1) using a standard Chelex 100® method for DNA extraction. For this, each leg was transferred to a tube with 100 µl Chelex® (Sigma-Aldrich) and 5 µl proteinase K (Thermo Scientific™) and extracted using the following steps: 55°C for one hour, 99°C for 15 minutes, 37°C for 1 min, 99°C for 15 min.

2. Amplification at the *or41* locus

The assignment to cryptic sister species was based on single nucleotide polymorphisms at the *or41* locus (Brand *et al.* 2020). Amplification at this locus was done using the following PCR cycles: 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 49.4°C for 30 sec and 72°C for 1 min, and a final step of 72°C for 2 min.

3. Sequencing and species assignment

All amplified products were sent to Eurofins for LightRun GATC sequencing using standard in-house protocols. All individuals in our study were homozygous across all six single nucleotide polymorphisms known to differentiate the two species in males, making the assignment of our diploid female samples unambiguous. For species assignment results of samples used in this study, see Supplementary Table 1.

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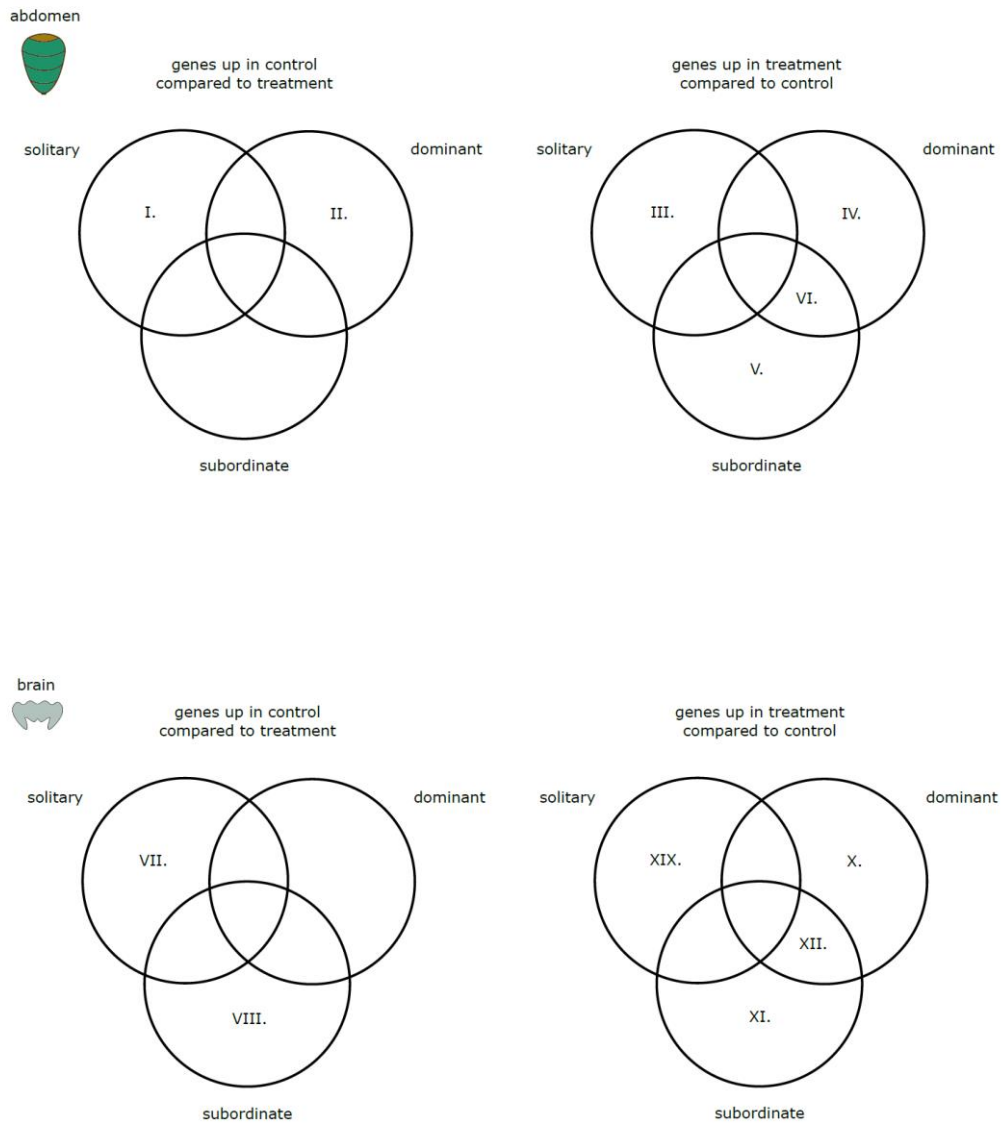
Supplementary Table S3. Number of differentially expressed genes (DEGs) for each comparison in DESeq2, excluding *E. dilemma* individuals. Q = queen (referred to as dominant in the text), W = worker (referred to as subordinate in the text), S = solitary, C = control, T = treatment.

Comparison	Number DEGs brain	Number DEGs abdomen
Q vs W	3	2
Q vs S	142	258
S vs W	5	11
T vs C	2	3
CS vs TS	25	47
CQ vs TQ	3	10
CW vs TW	6	6
CS vs CQ	11	531
TS vs TQ	192	70
CS vs CW	14	219
TS vs TW	16	0
CQ vs CW	0	1
TQ vs TW	8	2
Up CS vs TS	10	33
Up TS vs CS	15	14
Up CQ vs TQ	0	6
Up TQ vs CQ	3	4
Up CW vs TW	3	0
Up TW vs CW	3	6



Supplementary Material S4

Orthology results for genes differentially expressed between control and treatment nests (experimentally increased brood provisioning). The following tables are numbered according to the sections in these Venn diagrams.





Supplementary Table I. Expression fold change and protein orthology information for genes upregulated in the abdomen of solitary females from control nests in comparison to solitary females from treatment nests (experimentally increased brood provisioning).

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_021228*	4.1	<i>Eufriesea mexicana</i>	uncharacterized LOC108555290
FUN_009464	3.4	<i>Apis mellifera</i>	uncharacterized protein LOC102656088
FUN_023104	3.3	<i>Apis mellifera</i>	hexamerin 70a precursor
FUN_012238	3.3	<i>Apis mellifera</i>	uncharacterized protein LOC100577043
FUN_014970	3.2	<i>Apis mellifera</i>	ankyrin repeat and ELMO domain-containing protein D isoform X2
FUN_017297	3.2	<i>Apis mellifera</i>	protein Skeletor, isoforms B/C
FUN_016587	3.0	<i>Apis mellifera</i>	fructose-1,6-bisphosphatase 1
FUN_007520	2.9	<i>Apis mellifera</i>	esterase E4
FUN_011348	2.9	<i>Apis mellifera</i>	uncharacterized protein LOC410167
FUN_020411	2.9	<i>Apis mellifera</i>	cytochrome b5-related protein
FUN_018407	2.8	<i>Apis mellifera</i>	uncharacterized protein LOC551533
FUN_006200	2.4	<i>Bombus terrestris</i>	lipid storage droplets surface-binding protein 1
FUN_001074	2.3	<i>Bombus terrestris</i>	organic cation transporter protein isoform X2
FUN_011477	2.2	<i>Apis mellifera</i>	cytochrome P450 6AQ1
FUN_018312	2.2	<i>Apis mellifera</i>	phenoloxidase-activating factor 2
FUN_000184	2.2	<i>Apis mellifera</i>	uncharacterized protein LOC726803
FUN_011281	2.0	<i>Apis mellifera</i>	uncharacterized protein LOC100578618
FUN_020809	2.0	<i>Bombus terrestris</i>	gamma-glutamyl hydrolase A
FUN_020725	1.9	<i>Apis mellifera</i>	matrix metalloproteinase-24-like
FUN_015551	1.8	<i>Bombus terrestris</i>	sarcosine dehydrogenase, mitochondrial
FUN_011305	1.8	<i>Apis mellifera</i>	flavin-containing monooxygenase FMO GS-OX-like 4
FUN_009609	1.8	<i>Apis mellifera</i>	peptidoglycan-recognition protein SA precursor
FUN_017454	1.7	<i>Apis mellifera</i>	E3 ubiquitin-protein ligase MARCH3-like
FUN_008791*	1.7	-	-
Edil_07053*	1.7	<i>Habropoda laboriosa</i>	TWiK family of potassium channels protein
FUN_012907	1.7	<i>Apis mellifera</i>	uncharacterized protein LOC408320
FUN_002978	1.5	<i>Apis mellifera</i>	uncharacterized protein LOC409139
FUN_012473	1.5	<i>Apis mellifera</i>	serine protease inhibitor 27A



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FUN_002014	1.2	<i>Apis mellifera</i>	SET domain-containing protein SmydA-8
FUN_021581	1.2	<i>Apis mellifera</i>	cytochrome P450
FUN_000415	1.2	<i>Apis mellifera</i>	glutathione S-transferase S4
FUN_000212	1.0	<i>Apis mellifera</i>	uncharacterized protein LOC100579054
FUN_008044	0.8	<i>Apis mellifera</i>	lysosomal Pro-X carboxypeptidase

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.
For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).

Supplementary Table II. Expression fold change and protein orthology information for genes upregulated in the abdomen of dominant females from control nests in comparison to dominant females from treatment nests (experimentally increased brood provisioning).

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_021707	21.5	<i>Bombus terrestris</i>	uncharacterized protein LOC110120091
FUN_015106	10.2	<i>Apis mellifera</i>	probable serine/threonine-protein kinase samkC
FUN_015107	10.1	<i>Apis mellifera</i>	probable serine/threonine-protein kinase samkC
FUN_023996*	3.7	<i>Apis cerana cerana</i>	kelch protein
FUN_008627*	3.7	-	-
FUN_002179*	2.9	-	-

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.
For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).



Supplementary Table III. Expression fold change and protein orthology information for genes upregulated in the abdomen of solitary females from treatment nests (experimentally increased brood provisioning), in comparison to solitary females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_023913	-10.1	<i>Apis mellifera</i>	glutamate dehydrogenase, mitochondrial
FUN_017671*	-9.0	<i>Pararge aegeria</i>	uncharacterized protein
FUN_022208*	-3.7	-	-
FUN_015070	-3.2	<i>Apis mellifera</i>	paired mesoderm homeobox protein 2-like
FUN_005594*	-3.0	-	-
FUN_014513*	-2.9	<i>Lasius niger</i>	sarcoplasmic reticulum histidine-rich calcium-binding
FUN_016555	-2.6	<i>Apis mellifera</i>	heparan sulfate 2-O-sulfotransferase pipe
FUN_001308	-2.0	<i>Apis mellifera</i>	elongation of very long chain fatty acids protein AAEL008004
FUN_002870	-1.7	<i>Apis mellifera</i>	uncharacterized protein LOC410515
FUN_003020	-1.4	<i>Apis mellifera</i>	serine/threonine-protein kinase meng-po
FUN_009600	-1.4	<i>Apis mellifera</i>	broad-complex core protein isoforms 1/2/3/4/5
Edil_07298*	-1.3	-	-
FUN_001786	-1.2	<i>Apis mellifera</i>	F-box only protein 33
FUN_015756	-0.7	<i>Apis mellifera</i>	serine/threonine-protein phosphatase 4 regulatory subunit 4

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.

For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).

**Supplementary Table IV.** Expression fold change and protein orthology information for genes upregulated in the abdomen of dominant females from treatment nests (experimentally increased brood provisioning), in comparison to dominant females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_021101	-21.7	<i>Apis mellifera</i>	annexin B9
FUN_009362	-19.1	<i>Bombus terrestris</i>	protein saal1
FUN_008263	-3.3	<i>Apis mellifera</i>	nicotinic acetylcholine receptor alpha9 subunit precursor
FUN_010130	-2.7	<i>Apis mellifera</i>	uncharacterized protein LOC107964975

Supplementary Table V. Expression fold change and protein orthology information for genes upregulated in the abdomen of subordinate females from treatment nests (experimentally increased brood provisioning), in comparison to subordinate females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_009362	-46.3	<i>Bombus terrestris</i>	protein saal1
FUN_023692	-43.3	<i>Apis mellifera</i>	uncharacterized protein LOC102656541
FUN_023466	-41.7	<i>Apis mellifera</i>	conserved oligomeric Golgi complex subunit 8
FUN_021101	-32.3	<i>Apis mellifera</i>	annexin B9
FUN_009369*	-23.9	<i>Melipona quadrifasciata</i>	Proteasome endopeptidase complex
FUN_002172	-2.5	<i>Apis mellifera</i>	leucine-rich repeat-containing protein 15

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.

For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).



Supplementary Table VI. Protein orthology information for genes upregulated in the abdomen of both dominant and subordinate females from treatment nests (experimentally increased brood provisioning) in comparison to dominant and subordinate females from control nests.

<i>E. dilemma</i> Gene ID	Ortholog Species	Ortholog Protein Name
FUN_009362	<i>Bombus terrestris</i>	protein saal1
FUN_021101	<i>Apis mellifera</i>	annexin B9

Supplementary Table VII. Expression fold change and protein orthology information for genes upregulated in the brain of solitary females from control nests in comparison to solitary females from treatment nests (experimentally increased brood provisioning).

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_003183*	6.3	-	-
FUN_021981*	5.4	-	-
FUN_004082*	4.7	-	-
FUN_012322*	3.5	-	-
FUN_004310	2.7	<i>Apis mellifera</i>	protein arginine N-methyltransferase 3
Edil_04218	2.5	<i>Apis mellifera</i>	sugar transporter SWEET1
FUN_008231	1.6	<i>Apis mellifera</i>	N-acetyltransferase ESCO1
FUN_009153	1.4	<i>Apis mellifera</i>	uncharacterized protein LOC100576157
FUN_001789	1.2	<i>Apis mellifera</i>	basigin
FUN_004857	1.2	<i>Apis mellifera</i>	probable serine/threonine-protein kinase DDB_G0283337

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.

For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).



Supplementary Table VIII. Expression fold change and protein orthology information for genes upregulated in the brain of subordinate females from control nests in comparison to subordinate females from treatment nests (experimentally increased brood provisioning).

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_021707	30.4	<i>Bombus terrestris</i>	uncharacterized protein LOC110120091
FUN_004870	3.0	<i>Bombus terrestris</i>	uncharacterized protein LOC110119507
FUN_004930	2.9	<i>Apis mellifera</i>	neither inactivation nor afterpotential protein G-like

Supplementary Table IX. Expression fold change and protein orthology information for genes upregulated in the brain of solitary females from treatment nests (experimentally increased brood provisioning), in comparison to solitary females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_006876*	-20.4	<i>Apis mellifera</i>	uncharacterized protein
FUN_018568*	-6.9	-	-
FUN_016735*	-5.9	<i>Apis mellifera</i>	-
FUN_014795	-4.9	<i>Apis mellifera</i>	glucose oxidase
FUN_001703	-3.9	<i>Apis mellifera</i>	uncharacterized protein LOC409163
FUN_003952	-3.8	<i>Bombus impatiens</i>	zinc finger protein 771-like
FUN_017172	-3.6	<i>Bombus terrestris</i>	uncharacterized protein LOC100644783
FUN_007358	-3.2	<i>Apis mellifera</i>	sodium-dependent nutrient amino acid transporter 1
FUN_002550	-3.1	<i>Apis mellifera</i>	leucine-rich repeat-containing protein 74B
FUN_017243*	-3.0	-	-
FUN_015273	-2.9	<i>Apis mellifera</i>	probable phospholipid-transporting ATPase IF
FUN_017242	-2.5	<i>Apis mellifera</i>	tubulin alpha-1C chain
FUN_013369	-2.0	<i>Apis mellifera</i>	cytochrome b5
FUN_023265	-1.6	<i>Bombus terrestris</i>	15-hydroxyprostaglandin dehydrogenase [NAD(+)]
FUN_013857	-1.0	<i>Apis mellifera</i>	estradiol 17-beta-dehydrogenase 2

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.

For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).



Supplementary Table X. Expression fold change and protein orthology information for genes upregulated in the brain of dominant females from treatment nests (experimentally increased brood provisioning), in comparison to dominant females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_016599	-20.4	<i>Apis mellifera</i>	uncharacterized protein LOC552484
FUN_002144	-4.2	<i>Apis mellifera</i>	basic proline-rich protein isoform X2
FUN_011673*	-1.4	<i>Habropoda laboriosa</i>	solute carrier family 22 member 3

*Genes for which no ortholog was found in *Apis mellifera*, *Bombus terrestris* or *Bombus impatiens*.

For these genes, putative orthology was inferred through protein sequence similarity (Blastp to UniProt database).

Supplementary Table XI. Expression fold change and protein orthology information for genes upregulated in the brain of subordinate females from treatment nests (experimentally increased brood provisioning), in comparison to subordinate females from control nests.

<i>E. dilemma</i> Gene ID	Log ₂ Fold Change	Ortholog Species	Ortholog Protein Name
FUN_011997	-33.6	<i>Apis mellifera</i>	proteasome subunit alpha type-6
FUN_016599	-27.8	<i>Apis mellifera</i>	uncharacterized protein LOC552484
FUN_019775	-2.8	<i>Apis mellifera</i>	uncharacterized protein LOC113219280

Supplementary Table XII. Protein orthology information for genes upregulated in the brain of both dominant and subordinate females from treatment nests (experimentally increased brood provisioning) in comparison to dominant and subordinate females from control nests.

<i>E. dilemma</i> Gene ID	Ortholog Species	Ortholog Protein Name
FUN_016599	<i>Apis mellifera</i>	uncharacterized protein LOC552484



CV

Personal information

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Date of birth: 18.05.1992
Birthplace: Pithiviers, Frankreich
Nationality: French, United States citizen
Sex: Female

University education

- 2019- **Research associate** – Molecular Evolution and Bioinformatics, Institute for Evolution and Biodiversity, University of Münster, Germany
- 2015- **Doctor rerum naturalium (Dr rer. nat.)** – General Zoology, Department of Zoology, Institute for Biology, Martin-Luther-University, Germany.
Thesis title: “Ageing and the costs of reproduction: insights from *Euglossa viridissima*, an orchid bee at the cusp of sociality”
Supervised by: Prof. Robert J. Paxton
- 2014-2015 **Master of Science (M.Sc.), second year, passed with distinction** – Ecophysiology and Ethology, University of Strasbourg, France
Thesis title: “European hamster personality, reaction to predation pressure and behaviour of individuals released in the wild”
Supervised by: Prof. Yves Handrich, Prof. Odile Petit and Dr. Mathilde Tissier
- 2013-2014 **Master of Science (M.Sc.), first year, passed with distinction** – Functional, Behavioural and Evolutionary Ecology, University of Rennes, France
Thesis title: “Perceptual range and dispersal capacities of the brown garden snail: effects of habitat heterogeneity”
Supervised by: Prof. Luc Madec, Prof. Armelle Ansart and Dr. Maxime Dahirel
- 2010-2013 **Bachelor of Science (B.Sc.) with honours** – Conservation Biology and Ecology, University of Exeter, UK
Thesis title: “The influence of parasite pressure on mating success in *Plodia interpunctella*”
Supervised by: Prof. Mike Boots

Scientific conferences and workshops

- July 2020 Arthropods Genomics Symposium, virtual conference (poster)
- August 2019 European Society for Evolutionary Biology (ESEB) International Meeting - Turku, Finland (talk)
- March 2019 International Union for the Study of Social Insects (IUSI) Central European Meeting - Vienna, Austria (poster)
- February 2019 Akyut Kence Evolutionary Biology Conference – Ankara, Turkey (invited talk)
- August 2018 Joint Congress on Evolutionary Biology - Montpellier, France (poster)
- August 2018 IUSI International Meeting - Guarujá, Brazil (poster)
- July 2018 Minerva School: Sociobiology and Sociogenomics - University of Haifa, Israel
- March 2018 Deutsche Zoologische Gesellschaft Research Meeting, Freiburg (talk)
- August 2017 ESEB European Meeting - Groningen, Netherlands (poster)
- April 2017 Workshop: Transcriptome analyses and gene orthologs, University of Münster
- October 2016 Workshop: Transcriptomics, University of Freiburg
- August 2016 IUSI European Meeting - Helsinki, Finland (poster)
- February 2016 Workshop: Biocomputing in Linux and Python, University of Münster
- February 2016 Workshop: Life history modelling, University of Groningen, Netherlands



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Séguet, A., Bernadou, A., Paxton, R. J. (2016) Facultative social insects can provide insights into the reversal of the longevity/fecundity trade-off across the eusocial insects. *Current Opinion in Insect Science*. 16, 95-103.

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Papers close to submission, submitted or in revision

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Eidesstattliche Erklärung

Münster, den 02.12.2020

Hiermit erkläre ich an Eides statt, dass diese Arbeit, in der gegenwärtigen bzw. in einer anderen Fassung, von mir bisher weder an der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch an einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre weiterhin, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich, dass ich diese Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht worden.

Alice C. Séguret



Declaration of own contribution to the original articles presented in this thesis

- I. **Séguret, A.**, Bernadou, A., Paxton, R. J. (2016) Facultative social insects can provide insights into the reversal of the longevity/fecundity trade-off across the eusocial insects. *Current Opinion in Insect Science*. 16, 95-103.
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Literature review: 50%
Writing the paper: 50%

- II. **Séguret, A. C.**, Stolle, E., Fleites-Ayil, F. A., Quezada-Euán, J. J. G., Hartfelder, K., Meusemann, K., Harrison, M., Soro, A., Paxton, R. J. Transcriptomic signatures of ageing vary in solitary and social forms of an orchid bee. Submitted to *Genome Biology and Evolution*. Manuscript in review.

Design of the project: 80%
Collection of field data: 50%
Laboratory work: 100%
Data analysis: 90%
Writing of the paper: 80%

- III. **Séguret, A.**, Stolle, E., Teixeira, A., Fleites-Ayil, F. A., Quezada-Euán, J. J. G., Paxton, R. J. Sociality is associated with a lower cost of brood provisioning in the orchid bee *Euglossa viridissima*. Manuscript in preparation.

Design of the project: 80%
Collection of field data: 50%
Laboratory work: 50%
Data analysis: 100%
Writing of the paper: 90%