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Rapid juvenile hormone downregulation in subordinate wasp queens facilitates stable cooperation

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In many cooperatively breeding animals, subordinate group members have lower reproductive capacity than dominant group members. Theory suggests subordinates may downregulate their reproductive capacity because dominants punish subordinates who maintain high fertility. However, there is little direct experimental evidence that dominants cause physiological suppression in subordinates. Here, we experimentally test how social interactions influence subordinate reproductive hormones in *Polistes dominula* paper wasps. *Polistes dominula* queens commonly found nests in cooperative groups where the dominant queen is more fertile than the subordinate queen. In this study, we randomly assigned wasps to cooperative groups, assessed dominance behaviour during group formation, then measured levels of juvenile hormone (JH), a hormone that mediates *Polistes* fertility. Within three hours, lowest ranking subordinates had less JH than dominants or solitary controls, indicating that group formation caused rapid JH reduction in low-ranking subordinates. In a second experiment, we measured the behavioural consequences of experimentally increasing subordinate JH. Subordinates with high JH-titres received significantly more aggression than control subordinates or subordinates from groups where the dominant's JH was increased. These results suggest that dominants aggressively punished subordinates who attempted to maintain high fertility. Low-ranked subordinates may rapidly downregulate reproductive capacity to reduce costly social interactions with dominants. Rapid modulation of subordinate reproductive physiology may be an important adaptation to facilitate the formation of stable, cooperative groups.

1. Introduction

In many animal social groups, subordinates obtain a smaller share of reproduction than dominants. Reduced subordinate reproduction is often associated with 'physiological suppression', in which subordinates downregulate one or more hormones involved in the reproductive endocrine axis relative to dominants [1]. Physiological suppression of subordinates occurs across a broad range of cooperatively breeding taxa, including fishes, mammals, birds and insects [2–6].

Downregulation of subordinate physiology is typically called 'physiological suppression', but some argue 'physiological restraint' is a more appropriate term [7,8]. Suppression implies dominants control subordinate reproduction, while restraint implies subordinates limit their own reproduction. Here, we use 'physiological suppression' as a general term for downregulation of subordinate physiology without implying that dominants actively suppress subordinates.

Although physiological suppression of subordinates is common, the factors that cause hormonal differences between dominants and subordinates are less clear. Hormonal differences could be caused by: (i) behaviour of dominants towards subordinates, (ii) aspects of group membership not directly related to dominant

behaviour, or (iii) be unrelated to group membership [4]. A large body of behavioural-endocrine research has tested the three hypotheses and all three have received some support. However, fewer experimental studies have clearly differentiated between the alternatives by measuring the causes and consequences of hormone variation during group formation [1,3,4].

First, the behaviour of dominant group members may favour subordinate physiological suppression. Theory suggests dominants may suppress subordinate reproduction by 'punishing' (*sensu* [9], retaliatory infliction of fitness reduction) subordinates who have high reproductive capacity [10]. For example, dominants may kill subordinate offspring via infanticide or egg destruction [11–14]. Dominants may also evict subordinates who are a reproductive threat or who attempt to breed [15–17]. In addition to behaviours that are clearly costly for subordinates like infanticide and eviction, dominants could also suppress subordinates via aggression or more subtle behaviours such threats or socially induced stress [8,18]. These behaviours may cause subordinate reproductive suppression because agonistic dominant behaviour is costly and disfavours subordinates who attempt to reproduce. Although dominants often direct apparently costly behaviour towards subordinates, we lack experiments that explicitly test whether dominant behaviour is a direct response to subordinate reproductive capacity or whether subordinates reduce their reproductive capacity in response to dominant behaviour [4,10].

Second, reproductive capacity of subordinate group members may be reduced because of aspects of group living not directly related to the behaviour of dominants. For example, subordinates perform energetically expensive tasks like foraging more frequently than dominants [19,20] and also obtain smaller shares of food than dominants [21,22]. Given the energetic costs of breeding, disparities in work rates, feeding, and physical condition may alter the relative costs and benefits of maintaining fertility and favour reproductive suppression of subordinate group members [23].

Third, differences in dominant and subordinate reproductive capacity may be owing to correlated variation in other traits rather than a direct consequence of group living. Subordinates are often younger, smaller, and in poorer physical condition than dominants and often lack unrelated breeding partners [21]. All these traits influence reproduction, so they may contribute to reproductive differences between dominants and subordinates. For example, subordinate meerkats appear physiologically suppressed compared with dominants, but after controlling for factors like body weight and access to breeding partners, dominants and subordinates have similar levels of reproductive hormones [24]. Further, physiological suppression of subordinates sometimes persists after dominants are removed, suggesting that at least some subordinate suppression is unrelated to group membership [25].

Group membership and reproductive capacity are difficult to experimentally manipulate in cooperative breeders, so few studies have experimentally differentiated between the alternative hypotheses for subordinate reproductive suppression. A strong test of whether group formation is responsible for subordinate reproductive suppression is to experimentally create cooperative groups, then measure subordinate fertility after group formation. If reproductive suppression is unrelated to group membership (hypothesis (iii)), obtaining subordinate rank in experimentally created groups will not influence subordinate reproductive capacity. If aspects of group membership like foraging or food availability influence subordinate

reproductive capacity (hypothesis (ii)), subordinate reproduction is expected to change slowly (days or weeks) rather than rapidly (hours) after group formation because factors like nutrition slowly alter fertility. If dominant behaviour causes subordinate reproductive suppression (hypothesis (i)), subordinate reproductive capacity could either change rapidly (within hours of group formation) or slowly (days to weeks). Notably, hypothesis (i) is the only hypothesis predicted to cause rapid subordinate suppression after group formation. Experimental group formation rapidly suppresses subordinate reproduction in marmosets and cichlid fishes [3,26], but experiments in other taxa are lacking.

Whether dominant behaviour favours subordinate suppression (hypothesis (i)) can also be tested by measuring how dominants respond to experimentally increased subordinate reproductive capacity. If dominants behave more aggressively to subordinates with increased reproductive hormones than to subordinates with lower reproductive hormones, this suggests that dominant behaviour favours subordinate suppression. This experimental approach has been proposed [4,18], but, to our knowledge, such experiments have not been performed.

Polistes dominula provide a good model for studying physiological suppression because they are a cooperatively breeding species where subordinates have lower fertility than dominants [27]. *Polistes dominula* nests can be founded by a solitary nest-founding queen (foundress) or a group of cooperating foundresses. When multiple foundresses cooperate, they form a linear dominance hierarchy where dominants lay most of the eggs and subordinates do most of the work [28]. Cooperation has fitness benefits for subordinates because dominants and subordinates are often related and subordinates inherit the nest if the dominant dies [29,30]. Previous work in stable groups of foundresses has shown that subordinates are physiologically suppressed; subordinates have lower ovarian development than dominants [19]. Subordinates also have reduced activity of the gland that produces juvenile hormone (JH) [28], a key hormone that influences paper wasp fertility. JH is positively correlated with *P. dominula* foundress fertility [31] and application of the JH analogue methoprene increases foundress fertility [32].

JH is a key insect hormone that provides a good model for insect behavioural endocrinology. JHs are a class of sesquiterpenoid lipid-like hormones found in invertebrates. JH influences multiple aspects of insect behaviour and physiology including metamorphosis, diapause, sexual behaviour and caste development [33]. Although JH and androgens have different structures, divergent evolutionary history, and work in different physiological backgrounds, JH in insects provides a good comparison to androgens in vertebrates because JH and androgens have similar effects [34,35]. Specifically, both JH and androgens are associated with fecundity versus lifespan life-history trade-offs. High JH and androgens are often associated with benefits, such as increased fertility and dominance, as well as costs, such as reduced survival and immune function [36,37].

In this study, we analyse how social group formation influences reproductive suppression in *P. dominula* foundresses. It is currently unclear whether subordinate reproductive suppression is caused by dominant behaviour, group formation, or correlated variation in traits unrelated to group membership. First, we measure dominance behaviour and JH in experimentally created groups of *P. dominula* foundresses. If group formation causes subordinate physiological suppression, we expect foundresses who obtain subordinate rank in

experimental groups will have lower JH than dominants or solitary controls. If the JH change happens rapidly (within hours), this is consistent with dominant behaviour causing suppression. If the JH change happens over days or weeks, either behavioural or non-behavioural mechanisms could contribute to subordinate suppression. Second, we test whether dominant behaviour favours subordinate suppression by experimentally increasing subordinate JH in newly formed groups and measuring the behavioural consequences. If dominants 'punish' subordinates who are not reproductively suppressed, we expect dominants to be more aggressive towards subordinates with high JH than subordinates with low JH.

2. Methods

Polistes dominula paper wasp foundresses were collected from sites around Ann Arbor, Michigan in the early spring, within 3 weeks of nest foundation. After collection, foundresses were marked with unique paint marks using Testor's enamel paint, held in individual containers with their nest and given water, rock candy, and *Galleria mellonella* caterpillars. All foundresses used in these experiments were collected as single foundresses to ensure foundresses were not physiologically suppressed prior to experimentation. In the early spring, nesting strategy in *Polistes* is flexible such that wasps move between nests and can change from solitary to cooperative nesting [19,27,38].

(a) Experiment 1: how does group formation influence juvenile hormone titres?

Hormonal consequences of experimental group formation were tested by randomly assigning wasps to one of two treatments. (1) *Social* ($n = 27$ individuals). In the social treatment, three foundresses that had not previously met were placed together in an $8 \times 8 \times 2$ cm plastic container and videotaped as they interacted for 3 h. A small nest containing eggs that belonged to a different foundress was also placed in the container. (2) *Solitary* ($n = 32$ individuals). In the solitary treatment, a solitary foundress was placed in an $8 \times 8 \times 2$ cm plastic container with a small nest containing eggs from a different foundress. Social and solitary trials were run simultaneously between 10.00 and 16.00, when wasps are most active. All wasps were marked with enamel paint on the thorax so they could be individually distinguished. Both social and solitary experimental treatments mimic natural situations experienced by foundresses. During this period, wasps commonly start nests alone or with other foundresses, join established foundress groups, or attempt to usurp established nests [19,27,38].

Wasps from solitary and social groups were a similar weight ($t_{57} = 0.35$, $p = 0.72$, mean ± s.d. solitary $= 0.109$ g ± 0.018, social $= 0.108$ g ± 0.016). Wasps were also a similar age, approximately nine months old. In Michigan, nest-founding females eclose from pupation in August, mate, overwinter, and emerge from hibernation to start nests the following May.

Wasps from the social and solitary treatments remained in the containers for 3 h, then were bled for analysis of JH titres. Three hours was chosen because previous work demonstrated that *P. dominula* JH titres can change within 3 h of social interactions [39]. Further, 3 h is a short period of time compared with the four months that cofoundresses cooperate. Bleeding is fatal in wasps because most of the haemolymph is removed. JH III titres in haemolymph were assessed using established radioimmunoassay methods developed by Huang & Robinson [40,41], and previously validated in *P. dominula* [31].

Wasps were dissected to measure ovarian development. Then, the number of mature oocytes (i.e. oocytes greater than

1 mm in length) was recorded [31]. Owing to errors in storage, the ovarian development of 10 wasps was not measured. These wasps are included in analyses of JH and rank, but excluded from ovarian development analyses.

Videos of the groups in the social treatment were watched by E.A.T. to determine dominance rank. At the time of video analysis, E.A.T. had no knowledge of JH titres. Dominance was determined by mounting behaviour. Mounting is a stereotyped dominance behaviour in *Polistes* colonies. During a mount, the dominant wasp positions herself above the subordinate and drums antennae on the subordinate, while the subordinate lowers her antennae and remains stationary. Dominants regularly mount subordinates, and subordinates never mount dominants [19]. There were no mounts in three of the social trials, so the wasps in these trials were not included in the analyses.

(b) Experiment 2: do dominants punish subordinates who are not reproductively suppressed?

We tested whether dominant behaviour favours subordinate physiological suppression by experimentally increasing subordinate JH in newly formed groups and measuring the behavioural consequences. Aggression was compared across three treatment groups: (1) subordinate treated with 25 µg methoprene, (2) dominant treated with 25 µg methoprene, and (3) neither wasp treated with methoprene. If dominant behaviour favours subordinate physiological suppression, we predict dominants will be more aggressive to subordinates in treatment 1 than subordinates in other treatment groups. Comparing dominant aggression towards subordinates in treatments 1 and 2 is a particularly critical test of the hypothesis because one individual is treated with methoprene in both treatments. Therefore, if dominants are most aggressive to subordinates in treatment 1, we can conclude that aggression is caused by subordinates being treated with methoprene rather than methoprene causing a generalized increased in group aggression.

First, two unfamiliar foundresses were allowed to interact and establish dominance. Unfamiliar foundresses were collected from sites at least 5 km apart, placed in an $8 \times 8 \times 2$ cm plastic container, and videotaped as they interacted for 3 h. After the initial 3 h interaction, one individual in each pair was randomly chosen and topically treated on the abdomen with 25 µg of methoprene in 1 µl acetone. The other individual in each pair was topically treated on the abdomen with 1 µl acetone alone. Within the hymenoptera, methoprene has behavioural and physiological effects similar to those of JH [42–45]. Further, methoprene acts in ways similar to JH at the cellular level [46–48]. In *P. dominula*, previous work in this population has shown that foundress JH is correlated with high dominance rank and ovarian development [31]. Additionally, treatment with methoprene increases foundress ovarian development and dominance (among unfamiliar foundresses) [32]. After hormone treatment, wasps were isolated for 15 min, then returned to their original partner and videotaped as they interacted.

A no treatment control was also performed. The no treatment control was similar to the other treatments except neither wasp was treated with methoprene or acetone. Interactions between the no treatment pairs were videotaped for 4 h continuously. ($n = 13$ pairs subordinate JH treatment, 18 pairs dominant JH treatment, 18 pairs no treatment control).

Later, aggressive behaviour in videos was scored by undergraduate assistants who were blind to experimental treatment and predictions at the time of behavioural analysis. During the first 3 h of observation (prior to hormone treatment in the treated groups), students recorded mounting behaviour. Mounts provide the most effective method to determine rank. Trials where dominance was not established prior to hormone treatment were excluded from further analysis. In the final hour of interaction (after hormone treatment in the treated groups) all aggression

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between foundresses was recorded, including bites, mounts and grapples. Bites and mounts are directional aggression, so the individual who initiated versus received the aggression was recorded. Grapples are bidirectional, so both individuals were scored as participating in grapples.

(c) Statistical analysis

(i) Experiment 1: how does group formation influence juvenile hormone titres?

Analyses were performed in SPSS v. 24. In all analyses, JH titres were log $(x + 1)$ transformed to improve model fit. Trial was included as a subject variable in all analyses with multiple wasps in the same trial to control for any similarity within trials.

The relationship between JH titres and rank was assessed using mixed linear models with log JH titres as the dependent variable and rank as the categorical independent variable (categories = rank 1, rank 2, rank 3, solitary). Pairwise least significant difference (LSD) *post hoc* comparisons were performed to assess which categories were significantly different.

JH titres could vary with rank because either JH influences rank or rank influences JH. In other words, subordinates could have lower JH than dominants because individuals with low JH attain subordinate rank, subordinate rank causes JH suppression, or a combination of the two. JH measurement is fatal in small insects, so it is not possible to measure JH before and after social interactions. As a result, we differentiated between these alternatives with three analyses that test how JH differed across the social and solitary treatments. If social interactions alter JH titres, we expect there will be JH differences across wasps experimentally assigned to social versus solitary treatment groups.

First, the relationship between JH and ovarian development was compared in solitary and social treatments using a mixed linear model. Previous work has shown that JH mediates ovarian development in a range of insects [36], including *P. dominula* [31,49]. JH influences ovarian development over days, not hours, so short-term JH changes will not immediately alter ovarian development. Therefore, different relationships between JH and ovarian development in solitary wasps and wasps assigned to social groups suggests that JH changed recently in the social wasps owing to the social treatment. In this analysis, JH titre was the dependent variable. The independent variables were number of developed eggs (continuous), social treatment (categorical: social or solitary), and the interaction between number of eggs and social treatment. The interaction term was significant, so we followed-up the initial analysis by splitting the data into social and solitary groups. In each group, we tested the relationship between JH and ovarian development. A mixed linear model was used to analyse the social treatment, with trial included as a subject variable in the model. A linear regression was used to test the relationship between JH and ovarian development in the solitary treatment.

Second, we compared variance in JH titres across social and solitary wasps using Levene's test. If social behaviour causes subordinate JH suppression, we expect greater variance in JH titres in social wasps than solitary wasps.

Third, we created a simulation that randomly sampled three solitary wasps from the experiment 10 000 times. The simulation was carried out in the statistical program R v. 3.3. Within each randomly selected solitary group, wasps were ranked as having the highest JH (1) to lowest JH (3). Then, we compared the JH titres of social wasps which naturally attained rank 1 with solitary wasps with the highest JH (1), social wasps who naturally attained rank 2 with solitary wasps intermediate JH (2), and social wasps who naturally attained rank 3 with solitary wasps with the lowest JH (3). Comparisons were performed using two-tailed *t*-tests. If differences in JH titres across ranks are entirely owing to initial differences in JH titres, there should be no difference between social and solitary wasps at a given rank. If obtaining a
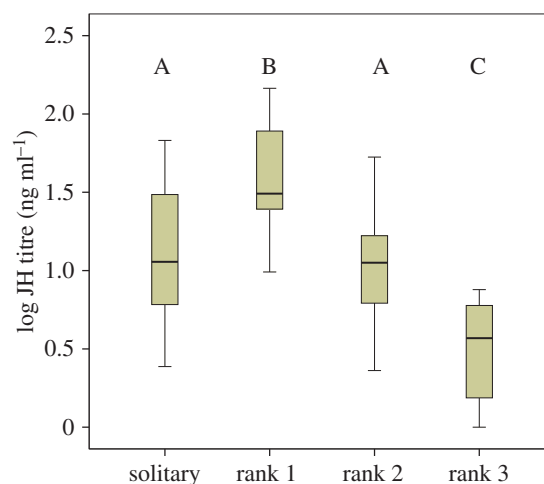
Figure 1. JH titres in wasps experimentally assigned to the solitary treatment and those that obtained rank 1, 2 or 3 after being assigned to the social treatment. The box reflects the 1st quartile, median and 3rd quartile. The whiskers denote minimum and maximum values. Letters denote significant differences ($p < 0.05$). (Online version in colour.)

particular rank causes a change in JH titres, there will be differences between social and solitary wasps at a given rank. This comparison is a conservative estimate of how rank influences hormones because solitary wasps were ranked based on JH titres alone, while social wasps were ranked based on behaviour. Hormones are not perfectly correlated with behaviour, so social wasps that attain a subordinate rank are unlikely to naturally always have lower JH than dominant wasps.

### (ii) Experiment 2: do dominants punish subordinates who are not reproductively suppressed?

General linear models were used to compare aggression across treatments. The dependent variable was total aggressive acts (log $(x + 1)$ transformed to improve model fit). The independent variables were hormone treatment (categorical: dominant received JH treatment, subordinate received JH treatment, no hormone treatment), and year (categorical: 2013, 2015). The interaction between hormone treatment and year is reported in the results, but excluded from the final model because it was not statistically significant. Separate analyses were performed to assess subordinate aggression and dominant aggression.

3. Results

(a) Experiment 1: how does group formation influence juvenile hormone titres?

JH titres after contests were significantly associated with dominance rank attained in social groups (figure 1; $F_{2,16} = 24.8, p < 0.001$). Pairwise *post hoc* analysis shows that JH titres differed significantly across all ranks, with rank 1 having the highest JH and rank 3 having the lowest JH (all $p < 0.005$). There were also significant differences between wasps in the social and solitary treatments (figure 1; $F_{3,22} = 16, p < 0.001$). Pairwise *post hoc* analysis shows that solitary wasps had less JH than dominant (rank 1) wasps ($p = 0.001$) and more JH than rank 3 wasps ($p < 0.001$), but similar JH to rank 2 wasps ($p = 0.58$).

The different JH titres of solitary wasps and social wasps that attain rank 1 and rank 3 could arise because social interactions influence JH, because JH titres influence ranks, or a
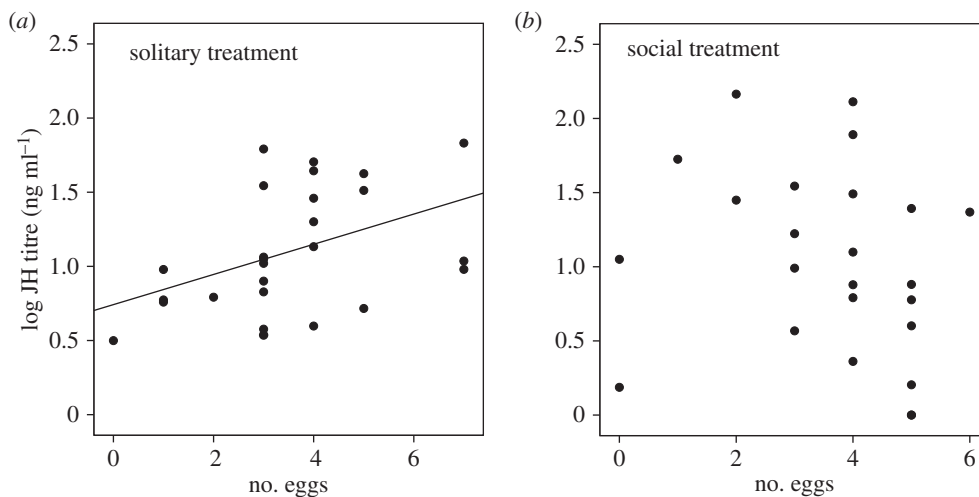
Figure 2. Relationship between JH titres and ovarian development (measured in number of developed eggs) in wasps experimentally assigned to (a) solitary ($p = 0.025$) and (b) social treatment groups ($p = 0.34$).

combination of the two. We show that social interactions influence JH titres with three analyses.

First, the social treatment altered the relationship between JH titres and ovarian development, consistent with social interactions altering JH titres. JH titres were significantly linked with the interaction between ovarian development and social experience ($F_{1,46} = 4.25$, $p = 0.045$), but not ovarian development alone ($F_{1,46} = 0.6$, $p = 0.8$) or social experience alone ($F_{1,46} = 2.76$, $p = 0.10$). To further explore how the social treatment alters the relationship between JH titres and ovarian development, we split the data into social and non-social treatment groups. Within the solitary group, there was a positive relationship between JH titres and ovarian development (figure 2a; $F_{1,24} = 0.57$, $p = 0.025$). Within the social group, there was no relationship between JH titres and ovarian development (figure 2b; $F_{1,22} = 0.95$, $p = 0.34$).

Second, there was higher variance in the JH titres of social wasps than solitary wasps ($F_{57} = 4.8$, $p = 0.032$; variance (social) = 1437, variance (solitary) = 338). The higher variation in JH titres of social wasps is consistent with social interactions altering JH titres.

Third, as a conservative test of whether subsampling could account for JH differences between solitary wasps and social wasps of different ranks, solitary wasps were randomly placed into groups of three and ranked 1 to 3 based on their JH titres (details in statistical methods). Social wasps that obtained rank 3 in the social groups tended to have lower JH titres than solitary wasps with the lowest JH titres (figure 3; $t_8 = -2.5$, $p = 0.034$). This suggests that losing a contest causes JH downregulation in subordinates. There was no difference in the JH titres of dominant wasps and solitary wasps with the highest JH titres ($t_8 = 1.1$, $p = 0.29$) or rank 2 social wasps and solitary wasps with intermediate JH titres ($t_8 = -0.6$, $p = 0.60$).

### (b) Experiment 2: do dominants punish subordinates who are not reproductively suppressed?

Experimentally increasing subordinate JH increased dominant aggression towards subordinates. There were differences in aggression across the three treatments (figure 4; $F_{1,45} = 5.4$, $p = 0.005$). LSD post hoc analysis shows dominants were significantly more aggressive when subordinates were treated with
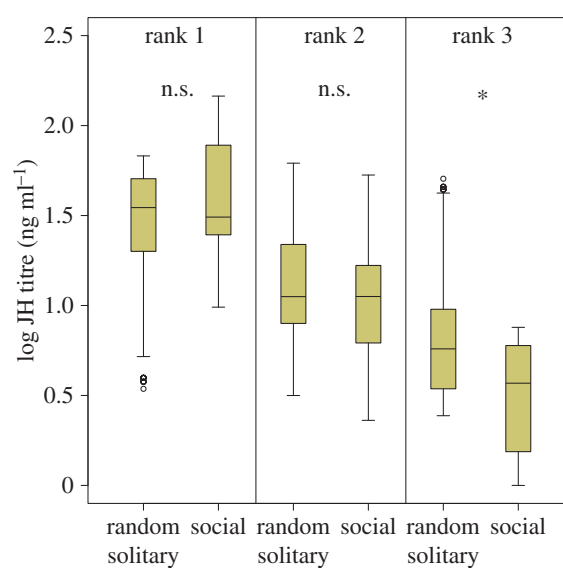
Figure 3. JH titres of wasps experimentally assigned to solitary and social treatments. Solitary wasps were randomly placed into groups of three and ranked 1 (highest) to 3 (lowest) based on JH titres (detailed in statistical methods). Social wasps were randomly placed into groups of 3 for behavioural observations and ranked 1–3 based on dominance behaviour during social interactions. Statistical comparisons are between solitary wasps with the most JH and rank 1 social wasps, solitary wasps with intermediate JH and rank 2 social wasps, and solitary wasps with the least JH and rank 3 social wasps. The box reflects the 1st quartile, median and 3rd quartile. The whiskers denote minimum and maximum values. Dots reflect outliers greater than 1.5 interquartile range. (n.s. = no significant difference, *$p < 0.05$). (Online version in colour.)

JH than when the dominant's own JH was increased or neither wasp was treated with hormones (subordinate JH versus dominant JH $p = 0.026$, subordinate JH versus no treatment control $p = 0.016$). There were no aggression differences between trials where dominants were treated with JH and no treatment control trials ($p = 0.82$). Year was also linked with dominant aggression, as wasps were more aggressive in 2015 than 2013 ($F_{1,45} = 2.4$, $p = 0.001$). There was no significant interaction between year and treatment ($F_{1,44} = 0.13$, $p = 0.72$).

The increase in dominant aggression was not caused by a change in subordinate aggressive behaviour after methoprene treatment. Subordinate aggression did not differ
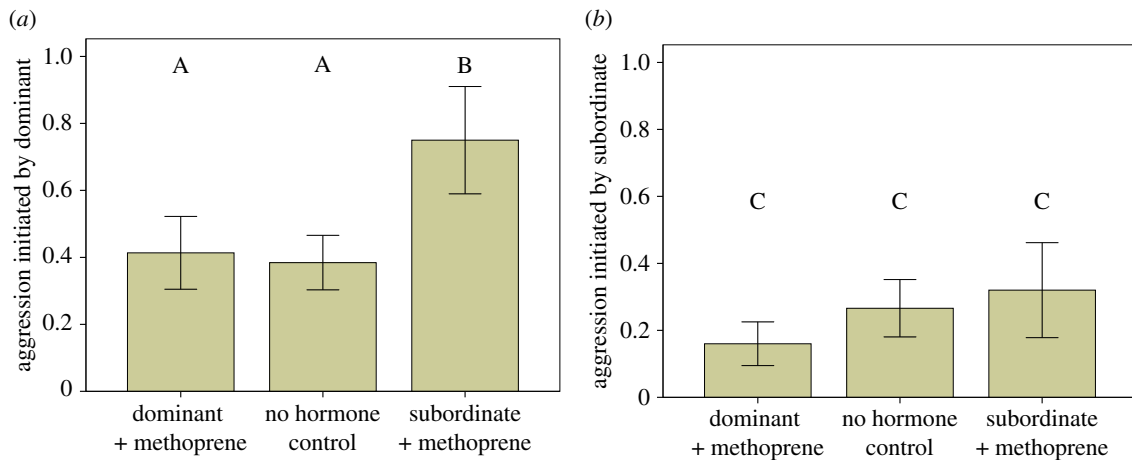
Figure 4. Mean ± s.e. number of aggressive acts initiated (bites, mounts and grapples) by (*a*) dominant and (*b*) subordinate when the dominant or subordinate are treated with methoprene, a JH analogue, or both remain untreated. (Letters denote significant differences). (Online version in colour.)

across treatment groups ($F_{1,45} = 1.4$, $p = 0.25$). Subordinate aggression was higher in 2015 than 2013 ($F_{1,45} = 45.0$, $p = 0.030$), but there was no significant interaction between year and treatment ($F_{1,44} = 0.14$, $p = 0.84$).

4. Discussion

In many taxa, subordinate group members have lower levels of reproductive hormones than dominants [1,4]. However, the factors that cause such physiological suppression are rarely tested experimentally [4]. Here, we measure reproductive hormones in wasp foundress three hours after experimental group formation and find that attaining the lowest rank causes rapid physiological suppression. Wasps who attained rank 3 had lower JH titres than other group members or solitary controls. However, social dynamics during group formation did not influence JH titres of wasps that attain rank 1 or 2 (figures 1 and 3).

There are two non-exclusive explanations for why lowest ranking subordinates have lower JH than dominants: losing a contest may decrease JH and/or low JH may cause wasps to lose contests. Our results illustrate that obtaining the lowest subordinate rank in a group causes wasps to downregulate their JH titres, as there are significant differences between the JH of solitary wasps and wasps randomly assigned to social groups. First, the relationship between JH titres and ovarian development differed in social and solitary wasps. JH mediates ovarian development [36] and *P. dominula* foundress JH titres are linked with ovarian development [31]. In this study, JH was correlated with ovarian development in solitary wasps, but not in social wasps. JH influences ovarian development over days, not hours, so this result is consistent with social interactions rapidly altering JH and disrupting the link between social wasp JH and fertility. Second, solitary wasps had lower variance in JH titres than wasps in social groups, consistent with social interactions rapidly altering JH. Finally, subordinate wasps in social groups had lower JH titres than the randomly selected solitary wasps with the lowest JH titres. If JH was similar between solitary wasps with low JH and wasps that attain subordinate rank after being assigned to social groups, it would suggest that low JH causes wasps to attain subordinate rank. Instead, we found wasps that attained subordinate rank had less JH than solitary wasps

with low JH. Therefore, social interactions cause subordinates to downregulate their JH titres.

In a second experiment, we show dominant behaviour favours subordinate physiological suppression, as dominants are more aggressive to subordinates who are not suppressed. Group membership provides fitness benefits for subordinate paper wasps [29,30] and physiological suppression reduces within-group conflict (this study). Therefore, our results suggest subordinates benefit by reducing their reproductive capacity.

Aggressive punishment by dominants was not a consequence of JH treatment directly altering aggression. JH treatment had no effect on a wasp's own aggression. Instead, dominant aggression was altered by subordinate JH treatment. In fact, dominants were significantly more aggressive when subordinates were treated with JH than when dominants were treated with JH. These results are initially surprising because JH is positively linked with aggression in many taxa [35,49]. However, they are consistent with extensive vertebrate research which has shown that the effects of androgens on aggression vary with the social and ecological context [37]. Therefore, it makes sense that the effects of JH on aggression vary with social context like rank.

This study provides direct experimental evidence that dominants punish subordinates who maintain high reproductive capacity. In many species, dominants appear to 'punish' subordinates, as they direct apparently costly behaviour towards subordinates, including eviction, aggression, social stress and infanticide [4,15]. Subordinate behaviour can induce such 'punishment': dominants are more aggressive to subordinates who do not work [50] and dominants may evict subordinates who grow large enough to be a threat [17]. However, previous work has not explicitly tested whether subordinate reproductive state influences dominant punishment. Here, we follow the suggestions of Young and Cant [4,18] by measuring how dominants respond to subordinates who are experimentally forced to maintain high reproductive capacity. Our results show subordinate wasps that maintain high levels of reproductive hormones are aggressively punished by dominants, perhaps because subordinates with high JH are perceived as a greater threat than subordinates with low JH. Therefore, the threat of aggressive punishment by dominants favours subordinate physiological suppression.

Increased aggression towards subordinates with high reproductive capacity requires that dominants can assess

subordinate reproductive physiology. How do dominants detect subordinates who are not physiologically suppressed? Dominant punishment may be a response to differences in subordinate behaviour. While there is no evidence that subordinate members of stable groups with high JH are more aggressive than subordinate members of stable groups with low JH (this study), JH may cause more subtle behavioural changes. Alternatively, dominants may assess JH via cuticular hydrocarbon profiles (CHCs). CHCs are important signals of fertility across the social insects that are not volatile and are sensed via antennal contact [51]. In paper wasps, CHCs are correlated with JH titres and fertility [52]. Cuticular hydrocarbons are often thought to change slowly, though dominant crickets change their CHC profile to more closely resemble the CHC profile of a subordinate within one day of losing a fight [53]. Future research will be useful to establish how rapidly CHCs change in response to changes in JH titres and whether CHCs provide information about rapid, short-term changes in a wasp's physiological state.

Studying the mechanisms underlying reproductive suppression can provide insight into the basis of reproductive skew in social groups [54]. This study supports transactional rather than tug-of-war models of reproductive sharing. Tug-of-war models hypothesize that reproductive sharing occurs because the dominant is unable to monopolize reproduction and group members constantly struggle over reproductive shares [55]. It is unlikely that *Polistes* compete over reproduction throughout the nesting cycle, as subordinates downregulate their reproductive hormones within hours of social group formation. Instead, rapid subordinate physiological suppression is consistent with transactional skew models which posit that reproductive shares are offered as a reward for cooperative behaviour [55]. There are many reproductive skew models and it can be difficult to distinguish between the models based on patterns of reproductive sharing alone [56]. Considering the proximate mechanisms underlying reproductive sharing in social groups may provide a useful new perspective for understanding reproductive skew.

Rapid JH downregulation in subordinates has parallels to the loser effect seen in some solitary taxa. In both social and solitary species, individuals who obtain a subordinate rank are less aggressive and less likely to win future contests than individuals who obtain a dominant rank [35,57]. These behavioural changes are often linked with a rapid decrease in hormone titres (steroid hormones in vertebrates, JH in insects) [37,58]. Although there are parallels between the loser effect in solitary taxa and physiological suppression in social taxa, there are also key differences. Many solitary species do not exhibit a loser effect. Other solitary species only show a loser effect in certain social or ecological contexts [57,59]. By contrast, physiological suppression in social taxa is common and produces substantial, long-term hormonal changes in subordinates [4]. Explicitly considering loser effects and subordinate physiological suppression in the same framework may provide a useful perspective on social modulation of hormone titres.

Previous work on physiological suppression in social insects has primarily focused on worker reproductive suppression. Suppression of eusocial workers is sometimes treated as distinct from suppression of subordinate cooperative breeders because workers have fewer reproductive options than subordinates. In some social insects, workers are sterile. In other social insects, the timing of worker production means workers are typically unable to mate or start their own successful nests. Despite these differences, there are also striking parallels between social insect workers and subordinate cooperative breeders [60]. For example, *Polistes* workers can upregulate their reproductive capacity if the queen disappears or is unable to reproduce [49,61,62]. In some social insects, workers that upregulate their reproductive capacity experience social punishment, including egg-eating and aggression [63,64].

Overall, attaining the lowest rank in cooperative groups causes nest-founding queen wasps to rapidly downregulate their JH titres. JH downregulation has social benefits, as dominants are aggressive to subordinates who maintain high JH titres after group formation. These results suggest that subordinates may restrain their own reproductive capacity to facilitate the formation of stable cooperative groups. Many social species, including paper wasps, experience rapid transitions between conflict and cooperation. Social modulation of hormone titres allows animals to match their behaviour to their changing social environment and facilitates the evolution of stable cooperative groups.

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