Received: 19 January 2021 | Accepted: 16 July 2021

DOI: 10.1111/1365-2435.13895

RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

Resource limitation, intra-group aggression and brain neuropeptide expression in a social wasp

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Funding information
NSF-IOS Doctoral Dissertation Improvement Grant, Grant/Award Number: 1701887

Handling Editor: Sheena Cotter

Abstract

1. Nourishment can have profound effects on social behaviour, including aggressive interactions between individuals. The prevailing theoretical and empirical understanding is that when nutritional resources are limited, inter-individual competition and aggression will increase. Alternatively, studies from some group-living species suggest limited nutrition can lead to increased cooperation, including by a reduction in inter-individual aggression. Thus, a general model for understanding how and why nutritional resource limitation affects aggressive behaviour remains elusive.
2. We suggest that the link between nourishment and future reproductive potential may be a key missing element of models that predict how nutritional resource availability affects inter-individual aggression in social animals.
3. We investigated how nourishment influenced intra-colony aggression and its molecular correlates in colonies of the social paper wasp *Polistes fuscatus*, which contain workers that maintain flexible reproductive potential as adults. We subjected colonies to either a high or low feeding treatment, and examined subsequent effects on behaviour, nutritional/reproductive physiology and brain gene expression.
4. We found that nutritional restriction reduced aggressive interactions. Thus, resource limitation was linked to reduced intra-group conflict. Thus, individual worker paper wasps appear to have the capacity to adjust their behaviour (e.g. reduced aggression) in response to nutritional stress; this suggests they may invest nutritional resources in the colony when resources are limiting, and in the self (and possible future reproduction) when resources are abundant.
5. Differential brain gene expression results implicate two well-known neuropeptides associated with aggression and/or nutrient signalling across taxa, *Tachykinin* and *Neuropeptide F*, as possible mediators of nutritionally dependent intra-colony aggression. This adds to a growing understanding that deeply conserved genes associated with core, conserved behaviours such as feeding and aggression in solitary insects can play a role in the regulation of social plasticity in more highly social species.

1 | INTRODUCTION

Classical competition theory predicts that when resources are limited, aggression between conspecifics will increase as individuals are spurred into conflict over diminishing resources (Titman, 1976; Volterra, 1926). This is supported by empirical studies on food resource limitation that have demonstrated, in various animal systems, that caloric restriction can lead to increased aggression among conspecifics (Collie et al., 2020; Fattorini et al., 2018; Fiocca et al., 2020; Vitousek et al., 2004). However, this classical model may not accurately predict intraspecific aggression across all types of social groups. For example, there are instances across taxa demonstrating that individuals may instead tend towards enhanced cooperation when resources are limited, from blood meal sharing in vampire bats (Wilkinson, 1984) to the formation of multicellular fruiting bodies in aggregations of free-living *Dictyostelium discoideum* amoebae (Bonner, 1982; Kuzdzal-Fick et al., 2007). In particular, classical competition theory may fail to predict the patterns of aggression within kin groups, where individuals have the option to increase inclusive fitness through cooperating to secure resources, rather than competing for them. Therefore, in strongly kin-structured groups, conditions where food resources are consistently scarce may be more likely to promote selection for increased cooperation and decreased aggression towards group members.

One excellent place to examine the impacts of nutritional restriction on intra-group aggression is in insect societies, some of which possess extremely high intra-group relatedness (Boomsma, 2009; Strassmann, 2001). Social insects (ants, termites and the social bees and wasps) live in highly cooperative and socially integrated societies, where many individuals share the duties of maintaining and defending the colony (Wilson, 1971). Different social insect species show a continuum of degrees of cooperation, with some more 'primitively' social species showing marked conflict within the society (Pardi, 1948; Ratnieks & Reeve, 1992; Strassmann, 1981; West-Eberhard, 1967). For example, in many highly social species such as honeybees, intra-colony aggression is very low, and reproductive competition is rarely seen between colony members. In contrast, species such as *Polistes* have constant overt aggression between colony members, and the degree of behavioural dominance is directly related to reproductive capacity (Röseler et al., 1984, 1985). Queens are singly mated, and intra-colony relatedness among her daughters is generally very high, approaching the theoretical maximum of $r = 0.75$ in some species (Strassmann, 2001). These traits make *Polistes* an excellent system to examine how nutritional resource limitation affects intra-colony aggression (Hunt, 1991; Rossi & Hunt, 1988; Wcislo & West-Eberhard, 1995).

Additionally, studies of nutrition and social behaviour can provide a bridge to understanding connections between environmental and molecular determinants of social plasticity. If nutritional resource limitation is an important regulator of intra-colony aggression, deeply conserved molecular pathways related to nutrient signalling may contribute to the regulation of this behaviour. Recent studies have suggested that nutrient signalling pathways well-known from solitary insects may be important in the evolution and regulation of insect sociality (Okada et al., 2017; Toth, 2017). Neuropeptides, in particular, are known to be important mediators of food-related behaviours across animals (Nässel & Winther, 2010; Nässel & Zandawala, 2019). Previous studies on both honeybees and paper wasps suggests genes related to nutrient signalling, for example some members of the deeply conserved insulin pathway, are related to the regulation of worker foraging behaviour (Ament et al., 2008; Daugherty et al., 2011). The neuropeptide hormone Neuropeptide F (NPF) is another example of a canonical nutrient signalling gene, widely distributed through both the central nervous and digestive systems, and is involved in the regulation of feeding across a wide variety of taxa. There is evidence from *Drosophila* that NPF modulates aggression as well as food-seeking behaviour (Bubak et al., 2019; Dierick & Greenspan, 2007). NPF may serve to signal to an individual its own nutritional status, which can in turn affect behavioural outcomes, including their degree of cooperation or aggression. Similarly, the neuropeptide Tachykinin has been implicated in regulating aggression in many insect groups (Asahina et al., 2014; Bubak et al., 2019; Howe et al., 2016; Pavlou et al., 2014), as well as foraging behaviour in honeybees (Brockmann et al., 2009). Thus, neuropeptides such as insulin-like peptides, NPF and Tachykinin are excellent candidates as molecular intermediaries between nutrient signalling and aggressive behaviour in social insects.

Previous research supports the supposition that many pathways related to reproductive physiology are also associated with social traits in wasps, as well as bees. Ovarian development and activation is linked to pollen foraging behaviour in worker honeybees (Amdam et al., 2006; Kocher et al., 2008; Wang et al., 2010) and reproductive dominance in worker paper wasps (Fletcher & Ross, 1985). This link may be in part regulated by the yolk precursor protein vitellogenin and the gonadotropin juvenile hormone (Amdam & Omholt, 2003; Hartfelder, 2000; Röseler et al., 1985). Based on prior work on honeybees, Walton et al. (2018) proposed that the way in which nutritional stress affects cooperative behaviour in social insects is related to the level of reproductive plasticity individuals possess. In honeybees, reproductively plastic larvae develop into more cooperative adults when they experience nutritional stress, whereas reproductively fixed adults exhibit lower cooperative behaviour. These findings suggest that, when workers are reproductively plastic, they may selfishly invest excess nutritional resources in their own reproduction. But, if nutritional resources are low, or if they cannot invest resources in their own reproduction, workers invest energy and resources in the colony's fitness (Walton et al., 2018). To test the generality of these results in social insects and understand whether deeply conserved genes related to both nutrient signalling and reproduction may play a role, we explored the role of nutritional restriction on cooperation and aggression in reproductively totipotent adult paper wasps.

In *Polistes* sp., which have evolved sociality independently from honeybees, adult paper wasp workers have plastic reproductive potential, and can mate, lay eggs and take over as queen of the colony if the resident queen dies (Reeve, 1991). Thus, adult paper wasps

more closely resemble larval honeybees in their flexibility in reproductive potential than adult honeybees. In a typical *Polistes* colony, members work together to build and defend the nest, collectively forage, allo-groom each other and share food. However, cooperation can sometimes be low (e.g. when a dominant wasp dies, leading to elevated colony-wide aggression among nestmates), and workers or subordinate queens may even abandon the nest to reproduce and found colonies of their own (Hunt, 2007; Reeve, 1991). Thus, in a more primitively social system such as *Polistes* wasps, environmental conditions may have an even stronger effect on the cooperativeness and aggression of individuals than in the more derived and likely more canalized society of the honeybee.

Here, we describe findings that investigate connections between nutritional restriction, behaviour, reproductive and nutritional physiology, and brain reproductive and nutrient signalling pathways in the paper wasp *Polistes fuscatus*. Our results provide an assessment of the connection between nutrient limitation and intra-group aggression, in relation to an individual's reproductive potential, along with new data on conserved molecular correlates of nutritionally mediated changes in social behaviour. These findings contribute to our understanding of the environmental and molecular forces that underlie the evolution of extreme forms of cooperation such as in social insects.

2 | MATERIALS AND METHODS

2.1 | Paper wasp colonies

We collected *Polistes fuscatus* from field sites in central Iowa. To attract wasps to construct nests, we set out wooden boxes (14 cm × 14 cm × 14 cm) in April 2017 at two sites: The Iowa 4-H Center (Madrid, IA; 41°55′40.0″N 93°51′45.6″W) and Chichaqua Bottoms Greenbelt (Maxwell, IA; 41°47′37.1″N 93°25′46.6″W). We affixed boxes to the tops of metal posts, and wasps were able to build nests by entering boxes through the bottoms, which were open but for a coarse wire screen. We regularly monitored boxes, and marked foundresses with paint pens to distinguish them from workers later during experimentation.

We collected 17 wasp nests (three from the 4-H Center and 14 from Chichaqua Bottoms Greenbelt) from the field and moved them into the laboratory in June 2017, just before adult workers began emerging. Wasps built their nests on the roofs of each box, which were detachable. To collect a nest, we removed the wood lid containing nest and foundress and placed them onto the top of a laboratory nest box (see below). We collected nests at night, between 21:00 and 5:00 hr, to ensure that colony members were present.

Additionally, we collected nine nests from parking canopies at Brighton Park Apartments (3815 Tripp St., Ames, IA 50014, N 42°1′14.95″, W 93°40′11.22″). These nests were also in the founding phase of the colony lifecycle (before workers emerge). To collect these nests, we placed Ziploc bags over the nest and foundress and severed the nest's pedicel from the parking enclosure ceiling with forceps. We marked foundresses with paint pens and affixed nests to cardboard squares with a hot glue gun, and then placed them onto the tops of laboratory nest boxes.

2.2 | Laboratory conditions

Moving colonies to the laboratory in late June 2017 ensured that all workers in the experiment would be from the foundress' first batch of brood. Thus, we excluded any pre-overwintering queens, or 'gynes', which appear later in the season (Hunt, 2007), so that experimental colonies consisted of only a foundress and workers. Although we did not explicitly measure the relatedness of workers, *Polistes* foundresses are almost exclusively monogamous (Strassmann, 2001), so nestmate workers were most likely full sisters (with $r = 0.75$). Subsequently, we maintained colonies in an indoor rearing room at Iowa State University in Ames, IA. We placed wasp nests in 30 cm × 30 cm × 30 cm clear Plexiglas laboratory nest boxes, with a 9 cm × 9 cm opening at the top, with nests affixed to either wooden field box roofs or glued to cardboard. Full-spectrum lights were set to a day–night cycle, with lights turning on at 6:00 and turning off at 20:00. Temperature was maintained at 27°C. We provided colonies with construction paper to build and maintain their nests, water and sugar rock candy ad libitum and prey according to feeding treatment (see below). Every day, nest box positions were rotated, so that each nest experienced any potential rearing room microclimate equally, and so that colonies were not observed in the same order during behavioural observations.

2.3 | Feeding treatments

We provided colonies with prey (*Galleria mellonella* waxworms purchased from local bait vendors, or *Trichoplusia ni* cabbage loopers from Frontier Scientific Services) according to their adult and larval population (0.5 prey per adult and 0.083 prey per larvae on the nest; quantities based on a similar study with the species *Polistes metricus* by Daugherty et al., 2011). Insect larvae are the primary diet of developing wasp larvae, as well as a food resource for adult workers (Hunt, 1984). We censused colonies by counting adults and larvae every other day. We assigned colonies to either a high or low feeding treatment ($n = 13$ nests per treatment), being careful to evenly distribute different colony sizes across feeding treatments. We fed colonies in the high treatment group daily, and fed the low treatment colonies prey every fourth day. Prior to treatment assignment, we fed all colonies a high-diet treatment for the 2 days following their move to the laboratory to allow them to recuperate from the move and adjust to laboratory conditions. Upon treatment assignment, we fed colonies their respective diets for 4 days before behavioural observations commenced, and continued throughout the experiment until all wasps were sampled at the same time at the end of the experiment (after 13 days of treatment), and kept at −80°C until they were processed for physiological and gene expression analyses.

2.4 | Behavioural observations

Starting 4 days after the initiation of feeding treatments, we recorded two observational periods a day (an observation period each morning between 08:00 and 11:00, and each afternoon between 13:00 and 17:00) for 8 days. An observation period consisted of observing all colonies sequentially for 5 min and then immediately repeating (thus each observation period consisted of two 5-min observations of each colony, for a total of four 5-min observations of each colony each day). We tallied instances of trophallaxis (food sharing between adults), foraging (on caterpillars and sugar) and aggression (lunging, biting and grappling) for each colony per observation period. Separate statistical analyses were performed for all behavioural observation periods (averaged) to investigate long-term effects of the treatments, as well as a subset of observation periods, that is those that occurred 6 hr before (AM observation) or directly after (PM observation) all colonies had been fed (so that behaviours related to prey capture and processing were not biased to one treatment).

2.5 | Ovaries, mass and lipids

At the conclusion of experimentation, we dissected the abdomens of 24 workers from each of the diet treatments to remove organs, leaving the fat body adhered to the cuticle. We removed ovaries and scored ovarian development in a manner similar to the protocol used for honeybees (Velthuis, 1970) and other polistine wasp studies (Daugherty et al., 2011; Desuó et al., 2011; Gobbi et al., 2006; Walton et al., 2020; see Table S1). Next, we weighed the abdomens using a balance (Mettler AE 100) to the nearest 0.01 mg, and extracted lipids in 2:1 chloroform: methanol. We quantified lipid content using a well-established sulfophospho-vanillin spectrophotometry assay previously used on both honeybees and *Polistes* wasps, using a SpectraMax 190 multi-well spectrophotometer (Daugherty et al., 2011; Jandt et al., 2015; Toth & Robinson, 2005) with a standard curve of known amounts of cholesterol to estimate total abdominal lipid content. Using these measurements, we acquired measurements of abdominal mass, total abdominal lipid content and calculated percent lipid content (lipid content per mass) for each individual wasp.

2.6 | Gene expression

Two hours after all colonies received their final prey feeding, we freeze-dried wasp worker heads ($n = 12$ individuals per treatment, one wasp randomly selected from each colony) at 300 mTorr and −85°C for 60 min, and dissected brains over dry ice. We carefully removed cuticle, fat and glands with a scalpel to isolate the brain from surrounding tissue. We sampled wasps at the conclusion of the experiment to be sure to capture gene expression differences associated with the long-term effects of treatment (as opposed to short-term fluctuations in hunger states).

To identify candidate genes for social cohesion and nutrition in *Polistes fuscatus*, we selected genes that have previously shown associations with nutrient signalling, reproduction and social behaviour in wasps and honeybees (Table 1). We selected 12 genes as candidates for differential gene expression across diet treatments: the neuropeptides *tachykinin* and *NPF*, the NPF receptor *NPF-R*, the octopamine receptor *Octβ2R*, the RNA-binding protein *Rasputin*, the insulin-like peptide *Ilp1*, the insulin-like receptor *InR1*, the insulin-like receptor *InR2*, the nutrient signalling kinase gene *TOR* (target-of-rapamycin), the ecdysone-inducible nuclear hormone receptor gene *HR46*, the egg-yolk precursor vitellogenin gene *Vg* and the vitellogenin receptor *VgR*. We identified gene sequences by BLASTing previously published *Apis mellifera* sequences for each gene (Honeybee Genome Sequencing Consortium, 2006) against a *Polistes fuscatus* Transcriptome Shotgun Assembly (Berens et al., 2015). We designed primers with the Primer Quest tool from Integrated DNA Technologies. Primer sequences of focal genes are in the supplementary materials (Table S2).

We extracted brain RNA using a Qiagen RNeasy Mini Kit and protocol (Qiagen), and treated it with DNaseI (Ambion). To control for technical errors that may occur during cDNA synthesis or pipetting error, we spiked in an external reference gene, *mCherry* (RNA isolated from a cnidarian of the genus *Discosoma*, Carrillo-Tripp et al., 2014). Two hundred nanograms of isolated RNA was used as a template for cDNA synthesis with SuperScript III First-Strand Synthesis System (Invitrogen).

For RT-qPCR, we used 2 μl of cDNA in 10 μl volume reactions (ran in triplicate as technical replicates) of the 2X SYBR® Green PCR Master Mix (Applied Biosystems) with the CFX384 TouchTM Real-Time PCR Detection System. We used an internal reference gene *rp49* to normalize gene expression data. The internal reference gene and the external reference gene cycle thresholds did not differ across treatments (*rp49*: linear model: $F = 0.25$, $df = 1$, $p = 0.13$; *mCherry*: linear model: $F = 0.05$; $df = 1$; $p = 0.82$, $n = 7$ and 12 wasp brains for high-diet and low-diet treatments, respectively, Figure S1). We used the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) to calculate relative gene expression, with expression normalized to the internal control gene *rp49*, and shown relative to the high-diet treatment as the 'reference' group.

2.7 | Statistics

We performed statistical analyses using R version 3.4.3 (R Team Core, 2017). To analyse the effect of diet on colony behaviour, we calculated the 'average behaviour rate' of a particular behaviour for each colony: we summed the occurrence of the behaviour during an observation period, divided by the number of wasps present in the colony to obtain a 'behaviour proportion' for the observation. This proportion helps control for differences in colony size while still capturing colony-level behaviour (London & Jeanne, 2003). Then, we averaged the 'behaviour proportions' across observation periods to obtain the 'average behaviour rate' of each behaviour for each

TABLE 1 Candidate genes for social cohesion and nutrition in *Polistes fuscatus* examined in this study. '*p*' indicates *p*-value (from ANOVAs of relative gene expression; significant differences in expression are in bold) and sample sizes in high and low-diet treatment groups represented by n_{High} and n_{Low} respectively

		Association with social insect behaviour (previous studies)			Pattern of expression (this study)
Gene name	**Gene product putative function**	**Trait**	**Species**	**Study**	**Expression trend higher in (Treatment group, *p*, n_{High}, n_{Low})**
Tachykinin	Neuropeptide	Aggression Foraging Starvation	*Acromymex echinatior* *Apis mellifera* *Polistes metricus*	Howe et al. (2016) Brockmann et al. (2009) Daugherty et al. (2011)	High, **$p = 0.03$**, $n = 7, 11$
NPF	Neuropeptide	Foraging	*Apis mellifera*	Ament et al. (2011)	High, **$p = 0.006$**, $n = 7, 10$
Octβ2R	Octopamine receptor	Foraging	*Solenopsis invicta*	Qi et al. (2018)	High, **$p = 0.06$**, $n = 7, 12$
NPF-R	Neuropeptide receptor	Foraging/starvation	*Polistes metricus*	Daugherty et al. (2011)	Low, $p = 0.64$, $n = 6, 12$
Rasputin	RNA-binding protein	Dominance	*Polistes dominula*	Manfredini et al. (2018)	High, $p = 0.18$, $n = 7, 12$
Ilp1	Insulin-like peptide	Foraging	*Apis mellifera*	Ament et al. (2008)	High, $p = 0.29$, $n = 10, 10$
InR1	Insulin-like receptor	Foraging	*Apis mellifera*	Ament et al. (2008)	High, $p = 0.17$, $n = 7, 11$
InR2	Insulin-like receptor	Foraging	*Apis mellifera*	Ament et al. (2008)	High, $p = 0.26$, $n = 10, 10$
TOR	Nutrient signalling kinase	Foraging	*Apis mellifera*	Ament et al. (2008)	High, $p = 0.21$, $n = 7, 11$
HR46	Ecdysone-inducible nuclear hormone receptor	Foraging/ reproductive traits	*Apis mellifera*	Wang et al. (2009)	High, $p = 0.38$, $n = 7, 11$
Vg	Egg-yolk precursor	Dominance Foraging	*Polistes dominula* *Apis mellifera*	Manfredini et al. (2018) Nelson et al. (2007)	High, $p = 0.45$, $n = 10, 10$
VgR	Vitellogenin receptor	Reproductive status Worker ovary activation	*Bombus lantschouensis* *Apis mellifera*	Du et al. (2019) Guidugli-Lazzarini et al. (2008)	High, $p = 0.46$, $n = 10, 10$

colony. We tested the effect of diet on behaviour across all observation periods (see *Results: All observations*), as well as only the observation periods that occurred the same day as experiment-wide feedings, both before and after the introduction of prey (see *Results: Observations at specific time points*). For the analysis of behaviour across all observations, we calculated average behaviour rates across all 16 observation periods (8 observation days, 2 observation periods per day). For the analysis of behaviour before or just after feeding, we calculated average behaviour rates across two morning observation periods or two afternoon observation periods (because low-diet treatment colonies were only fed every 4 days, there were only 2 days when *all* colonies were fed). The impetus for focusing on behaviours at these specific time points was to capture behaviour when the colonies from different treatments had the same, or most similar, prey availability—prior to feedings all colonies had no, or little, prey; and post-feeding, all colonies had prey available. At all other observation points, the high-diet colonies had prey available, and the low-diet colonies did not. This caused a bias in which behaviours a colony could invest: low-diet colonies could not perform prey foraging, and high-diet treatments could perform prey foraging at the expense of other behaviours. By focusing on the time points before and (even more accurately) after all colonies were fed, we removed this bias. We analysed behavioural data with Wilcoxon rank-sum tests using the 'wilcox.test' function. We compared relative gene expression for each gene of interest across treatments with one-way ANOVAs using the 'aov' function. We excluded samples with low RNA quality/quantity or poor PCR amplification, resulting in some differences in sample sizes between genes. We made linear mixed models for abdominal mass, lipids and ovaries with colony as a random factor using the 'lmer' function in the LME4 package (Bates

et al., 2015). We performed post hoc using the 'lsmeans' functions in the package LSMEANS (Lenth, 2016).

3 | RESULTS

3.1 | Behavioural observations

3.1.1 | All observations

When behaviours were averaged across all observations, wasp colonies from the low-diet treatment foraged on sugar more than colonies from the high-diet treatment (Wilcoxon rank-sum test: $W = 20$, $p = 0.001$; Figure 1a). Conversely, colonies from the high-diet treatment foraged on caterpillars more than colonies from the low-diet treatment (Wilcoxon rank-sum test: $W = 142$, $p = 0.002$; Figure 1a). All other behaviours were not significantly different across treatments (Table S3).

3.1.2 | Observations at specific time points

Because wasps often responded immediately to the introduction of prey items (A. Walton, personal observation), behaviour was also analysed at specific time points, that is observation periods that occurred 6 hr before or directly after all colonies in both treatments had been fed caterpillars. For the subset of observation times directly after prey was introduced, wasp colonies in the high-diet treatment exhibited higher aggression than those from the low-diet treatment (Wilcoxon rank-sum test: $W = 120$, $p = 0.022$, $n = 13$

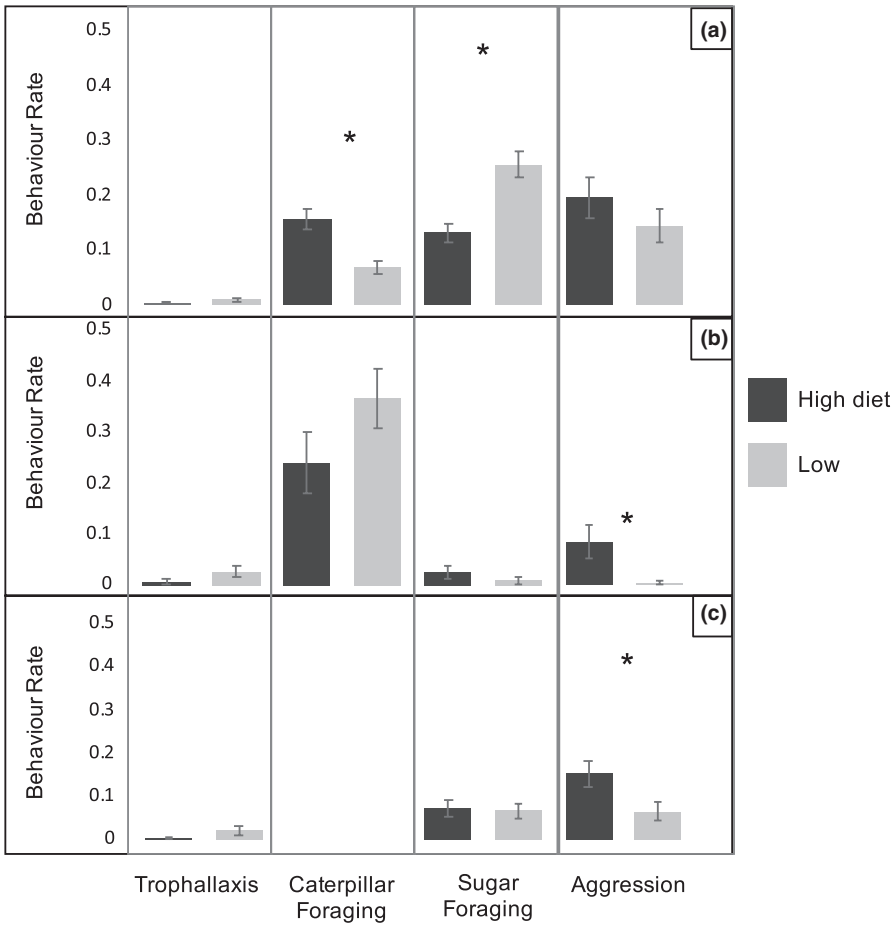

FIGURE 1 Behaviour rates (a colony's average behaviour proportion, where a behaviour proportion is the proportion of wasps performing a behaviour during an observation period) for trophallaxis between adults, caterpillar foraging, sugar foraging and aggressive interactions in high and low-diet-treated colonies. Observation periods were comprised of two sequential rotations of 5-minute observation bouts of each colony, with a running sum of all behaviours observed. Observation periods occurred twice daily (morning and afternoon) for 8 days. (a) Average behaviour rates across all observation periods. Wasp colonies from the low-diet treatment foraged on sugar more than colonies from the high-diet treatment (Wilcoxon rank-sum test: $W = 20$, $p = 0.001$, $n = 13$ colonies per treatment) and colonies from the high-diet treatment foraged on caterpillars more than colonies from the low-diet treatment (Wilcoxon rank-sum test: $W = 142$, $p = 0.002$, $n = 13$ colonies per treatment). (b) Average behaviour rates during observation periods immediately following experiment-wide prey feeding. Only aggression was significantly different between treatments (Wilcoxon rank-sum test: $W = 120$, $p = 0.022$, $n = 13$ colonies per treatment). (c) Average behaviour rates during observation periods prior to experiment-wide prey feeding. Only aggression was significantly different between treatments (Wilcoxon rank-sum test: $W = 124$, $p = 0.042$, $n = 13$ colonies per treatment)

colonies per treatment; Figure 1b). Wasp colonies did not differ in trophallaxis rates across treatments (Wilcoxon rank-sum test: $W = 53.5$, $p = 0.11$, $n = 13$ colonies per treatment; Figure 1b), nor in foraging behaviours (Table S4).

Additionally, behavioural observations were compared from the morning before experiment-wide prey feeding occurred to confirm that the pattern observed above was consistent when colonies had no prey available. Again, colonies in the high-diet treatment exhibited higher aggression than those from the low-diet treatment (Wilcoxon rank-sum test: $W = 124$, $p = 0.042$; Figure 1c), and this was the only behaviour that differed significantly between treatments (Table S5).

3.2 | Mass and physiological measurements

Workers from the high-diet treatment had a higher abdominal mass than workers from the low-diet treatment (mean abdominal mass: high = 36.97 mg, low = 25.92 mg; linear mixed effects model: *t*-value = 3.01, $p = 0.004$, $n = 29$ high and 24 low; Figure 2a). Total lipid content did not differ between treatment groups (mean lipid content: high = 3.51 mg, low = 2.89 mg; linear mixed effects model: *t*-value = 1.13, $p = 0.27$, $n = 29$ high and 24 low; Figure 2b). Relative lipid (lipid content divided by mass) did not differ between treatment groups (mean percent lipid: high = 0.11, low = 0.13; linear mixed effects model: *t*-value = −0.88, $p = 0.38$, $n = 29$ high and 24 low). Ovary scores did not differ between diet treatment groups (mean ovary score: high = 1.60, low = 1.56; linear mixed effects model: *t*-value = −0.23, $df = 46$, $p = 0.82$, 24 wasps per feeding treatment; Figure 2d).

Treatment affected the larval population of the nest. Although the preponderance of nests in both diet treatments had declines in larval population over the course of the experiment (which is common for laboratory-reared *Polistes* nests, Jandt et al., 2015), nests in the low-diet treatment had a higher net loss of larvae (mean larvae lost: high = 1.08, low = 2.6; linear model: $F = 4.29$, $p = 0.049$; $n = 13$ nests per treatment; Figure 2c). Net larvae lost was calculated as the number of larvae present on the nest at the start of the experiment minus the number of larvae present at the conclusion of the experiment.

3.3 | Gene expression

Both *Tachykinin* and *NPF* had higher gene expression in workers from the high-diet treatment than the low-diet treatment (*Tachykinin*: ANOVA; $F = 5.6$, $df = 1$, $p = 0.03$, $n = 7$ high-diet and 11 low-diet

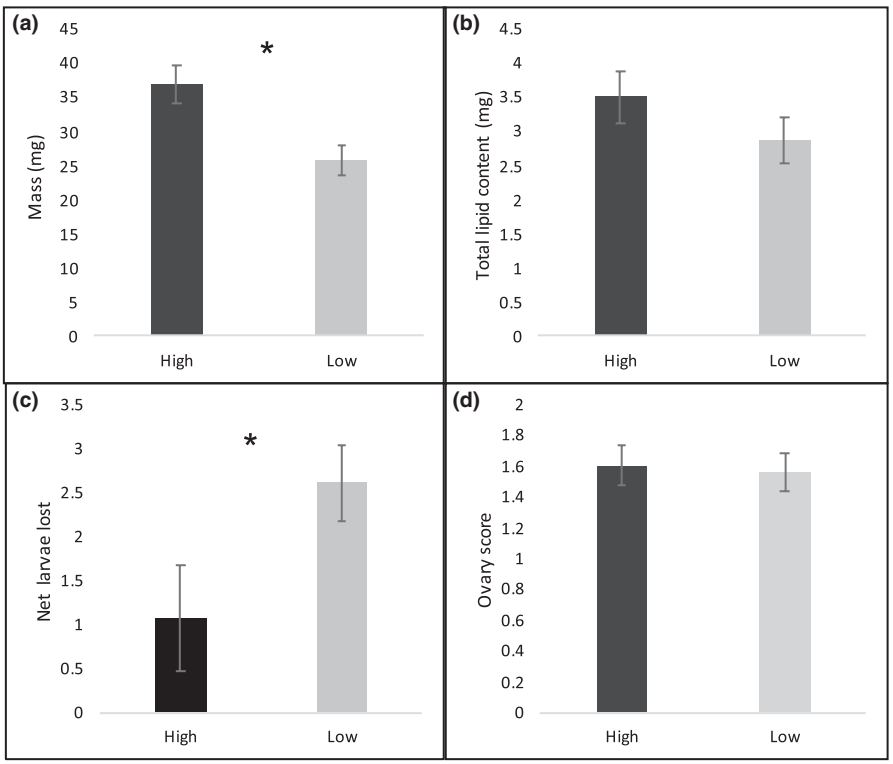

FIGURE 2 Mass and physiological measurements. (a) Workers from colonies in the high-diet treatment had a higher abdominal mass than workers from the low-diet treatment (linear mixed model: *t* ratio = 3.20, $p = 0.003$, $n = 24$ wasps per treatment). (b) Total lipid content did not differ between treatment groups (linear mixed model: *t* ratio = 0.68, $p = 0.49$, $n = 24$ wasps per treatment). (c) Net larval loss. Nests in the low-diet treatment lost more larvae over the course of the experiment than nests in the high-diet treatment (linear model: $F = 4.29$, $p = 0.049$, $n = 13$ nests per treatment). Net larvae lost was calculated as the number of larvae present on the nest at the start of the experiment minus the number of larvae present at the conclusion of the experiment. (d) Ovary development. There was no difference in average ovary score between diet treatments (*T* test: $t = 0.23$, $df = 45.89$, $p = 0.82$, $n = 24$ wasps per treatment)

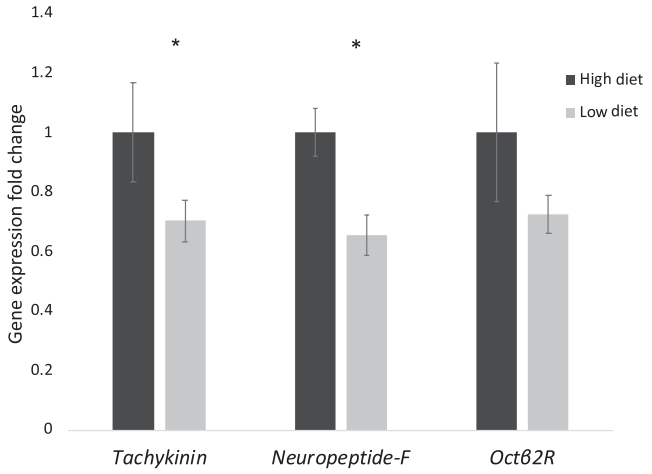

FIGURE 3 Brain gene expression for *Tachykinin*, *Neuropeptide F* (*NPF*) and *Octβ2R* as determined by real-time quantitative RT-PCR. Both *Tachykinin* and *NPF* had higher relative expression in wasps from colonies in the high-diet treatment versus the low-diet treatment (*Tachykinin*: ANOVA; $F = 5.6$, $df = 1$, $p = 0.03$, $n = 7$ high-diet and 11 low-diet wasp brains. *NPF*: ANOVA; $F = 10.5$, $df = 1$, $p = 0.006$, $n = 7$ high-diet and 10 low-diet wasp brains). *Octβ2R* had a marginally significant higher expression in the high-diet treatment relative to the low-diet treatment (ANOVA: $F = 3.99$, $df = 1$, $p = 0.06$). We confirmed that the internal reference gene (*rp49*) and the external reference gene (*mCherry*) cycle thresholds did not differ across treatments (see Figure S1)

wasp brains; *NPF*: ANOVA; $F = 10.5$, $df = 1$, $p = 0.006$, $n = 7$ high-diet and 10 low-diet wasp brains; Figure 3). Also, there was a marginally significant difference, with a trend showing workers in the high-diet treatment have higher expression of *Octβ2R* relative to the low-diet treatment (ANOVA: $F = 3.99$, $df = 1$, $p = 0.06$; Figure 3). Workers from the two diet treatments did not differ in brain gene expression for any of the other genes we measured (Table 1; Figure S2): *NFP-R* (ANOVA: $F = 0.22$; $df = 1,16$; $p = 0.64$), *Rasputin* (ANOVA: $F = 2.00$, $df = 1$, $p = 0.18$), *Ilp1* (ANOVA: $F = 1.18$; $df = 1,18$; $p = 0.29$), *InR1* (ANOVA: $F = 2.02$; $df = 1,16$; $p = 0.17$), *InR2* (ANOVA: $F = 1.34$; $df = 1,18$; $p = 0.26$), *TOR* (ANOVA: $F = 1.74$; $df = 1,16$; $p = 0.21$), *HR46* (ANOVA: $F = 0.81$; $df = 1,16$; $p = 0.38$), *Vg* (ANOVA: $F = 0.59$; $df = 1,18$; $p = 0.45$) or *VgR* (ANOVA: $F = 0.57$; $df = 1,18$; $p = 0.46$).

4 | DISCUSSION

Here, we present evidence that nutritional environment has the potential to affect intra-colony aggression in the social paper wasp *Polistes fuscatus*. Specifically, we found that low prey availability is associated with less aggression towards nestmates. As predicted by Walton et al. (2018) and proposed previously (Hunt, 1991, 2007), these data provide further support for the idea that when nutritional resources are scarce, individuals of highly social species may be selected to behave cooperatively to promote family group-level reproduction. Alternatively, when resources are abundant, individuals can more readily invest in their own fitness, and group cohesion may begin to degrade. We observed higher nutrition associated with more aggressive interactions. This is in contrast to classical competition theories, which predict that a decrease in resources will lead to increased aggression between conspecifics (Maynard Smith & Harper, 1988) and siblings (Hodge et al., 2009; Mock et al., 1987). Our results highlight a potential alternative strategy of resource allocation and competition within kin groups (Hunt, 1991; Rossi & Hunt, 1988; Wheeler, 1986), where nutritional availability influences investment in individual versus inclusive fitness.

Our results demonstrate that nourishment is an important regulator of social behaviour in paper wasp workers. To investigate how nutritional state influences cooperative behaviours, we focused on aggression and trophallaxis. Specifically, we recorded behaviours following prey feeding for all experimental colonies (every 4 days). We focused on these times because these were the only observation periods in which the high and low-diet treatments were on a 'level playing field' with respect to prey availability. During these observation periods, aggression was higher in the high-diet treatment. However, it could be possible that the low-diet treatment spent more effort foraging for prey during these observation periods because prey is rarer and thus of higher priority to wasps in this treatment. If that were so, we would predict higher prey foraging post-feeding in low-diet treatments than high-diet treatments, as observed when behaviour rates were averaged across the course of the experiment (Figure 1a). Yet, there was no difference in prey foraging post-feeding between treatments following prey feeding (Figure 1b; Table S4). Further, we recorded behaviours on the mornings prior to experiment-wide prey feeding, when no colonies had prey to forage. Here, we observed the same pattern as post-feeding: higher aggression in the high-diet treatment (Figure 1c; Table S5). Thus, lower aggression in the low-diet treatment was not a result of wasps in this treatment focusing on prey foraging during observation. When nutritionally restricted, wasps were less aggressive towards nestmates, even during time periods in which they are not intensively focused on feeding.

Importantly, we were able to verify that our nutritional restriction treatment was successful—diet restriction led to decreased abdominal mass in workers. Furthermore, treatment affected colony demography—diet restriction led to a higher net loss of larvae present on the nest. However, diet restriction did not affect worker lipid content or relative lipid content. Thus, although diet restriction led to decreased mass, workers from this treatment were able to maintain normal fat body lipid stores. This was likely accomplished by increased sugar consumption, which is corroborated by the increased sugar foraging observed in prey diet-restricted colonies. Together, the decrease in abdominal mass and the increase in sugar foraging confirm the efficacy of the diet restriction method used in the study. Future work could more explicitly investigate how prey limitation affects the protein/carbohydrate balance of the colony's diet and how shifting the ratio of protein and carbohydrate intake might affect social behaviour.

We predicted that nutritional restriction would result in decreased ovary size in wasp workers, as is true of honeybees that

experience nutritional stress (albeit, as larvae, not as adults; Hoover et al., 2006; Walton et al., 2018; Wang, Campbell, et al., 2016; Wang, Kaftanoglu, et al., 2016; Wang et al., 2014). However, we did not observe a difference in ovarian size between diet treatments. Although wasps examined in this study had very low variation in ovary size, the ovary sizes observed are typical of *Polistes* workers (Toth et al., 2009). Thus, differences may have been non-existent or too small to detect. Alternatively, it is possible that the level of nutritional stress imposed by our treatment was not severe enough to result in a change in ovary size. In addition, it is possible that if diet treatments had continued for longer, differences in ovarian development may have emerged.

Our data implicate two deeply conserved neuropeptide genes associated with nutrient signalling and aggression in insects. Both *NPF* and *Tachykinin* were upregulated in the brains of workers from high-diet-treated colonies. These two peptides have been associated with regulating feeding-related processes in insects (Ament et al., 2011; Huang et al., 2011; Kwok et al., 2005; Nässel, 2002; Pabla & Lange, 1999; Van Wielendaele et al., 2013). Additional work has linked these peptide genes to aggression behaviour in expansive animal taxa (Asahina et al., 2014; Bubak et al., 2019; Howe et al., 2016; Pavlou et al., 2014). Here, we saw an increase in expression of these genes paired with both an increase in nutritional status and elevated aggression. Thus, NPF and Tachykinin may act as internal signallers of nutritional status, and influence behavioural response to increased or decreased nourishment. *Tachykinin* was also upregulated under high-diet conditions. In contrast with our findings in *P. fuscatus*, Daugherty et al. (2011) recorded an upregulation of *Tachykinin* in starved workers of *P. metricus*. It is not clear why there would be opposite expression patterns in these congenerics, as starvation in some other insects has been associated with a decrease in *Tachykinin* expression (Lange, 2001; Nagai-Okatani et al., 2016), but the fact that both studies found a neurogenomic response by *Tachykinin* to similar, but distinct nutritional manipulations in paper wasps suggest this gene may be highly sensitive to changes in the nutritional environment. In addition to an upregulation of *NPF* and *Tachykinin*, we also found a nearly significant upregulation in expression of the octopamine receptor gene *Octβ2R* in the brains of workers from the high-diet treatment. In insects, the neurohormone octopamine plays a large role in the regulation of food-seeking behaviour (Roeder, 1994), including the transition to foraging behaviour in honeybees (Schulz & Robinson, 2001). The octopamine receptor *Octβ2R* has higher expression in the heads of foragers than nurses in the imported red fire ant *Solenopsis invicta* (Qi et al., 2018). Here, we see an increase in expression in the brains when more prey is available.

These findings contribute to a growing body of evidence that conserved nutrient sensing genes are part of a 'genetic toolkit' that regulate cooperative behaviour in social insects (Toth, 2017; Toth & Robinson, 2007). The evolution of social traits need not require major genomic change, but instead may have been aided by novel functions of conserved genes brought about by changes in genetic regulation. Thus, genes that signal to an individual their own nutritional status (e.g. *NPF*, *Tachykinin* and *Octβ2R*) may also help regulate how an individual allocates those nutritional resources—selfishly or to benefit the group. In the case *P. fuscatus*, the behavioural response to nutritional status includes the level of aggression towards nestmates, which affects the social cohesion of the whole kin group.

Although this study examined an extant species and the immediate effect of nutritional restriction on the reduction of intra-colony aggression, the results have the potential to be reflective of the conditions that promoted social insect evolution historically (Hunt & Nalepa, 1994). The lack of nutrition can make personal reproduction difficult or impossible, and so investing in the fitness of the group (via cooperative behaviour) may be the best or only option under these circumstances (Hunt, 1991). This approach would be especially true of kin groups with maternal care, where individuals are closely related and the inclusive fitness pay-off of cooperation is highest. Thus, we suggest these results add to a growing understanding that nutrition regulates aggression and cooperation in social groups, but that this depends on the level of sociality and group members' potential to reproduce (Walton et al., 2018). On one side of the spectrum, in groups where all individuals have full reproductive potential, aggression should be high when nutritional resources are low, because individuals will compete to hoard resources to invest in their own potential reproduction. This situation may characterize most non-social species, and explain observations of resource limitation and enhanced aggression in some vertebrate groups (Vitousek et al., 2004). On the other side of the spectrum, in groups where individuals have no reproductive potential (e.g. the fixed adult worker caste of highly social honeybees), an abundance of nutritional resources should promote cooperation and reduce intra-colony aggression because individuals are constrained reproductively, and would thus be selected to reinvest resources to benefit the colony. In this case, a dearth of nutritional resources may not affect cooperation at all, or even allow individuals to invest more energy in cooperative activities, such as care of the queen (as shown in Walton et al., 2018). In intermediately social groups, such as in the small societies of *Polistes fuscatus* where all individuals, including workers, have reproductive plasticity (Reeve, 1991), increased nutritional resources lead to higher aggression because all individuals have the option to reproduce, and will try to do so when resources are available. In such systems, a scarcity of nutritional resources will lead to reduced aggression because individuals no longer have a viable option to reproduce, instead opting to invest in cooperative behaviour and the fitness of the group. This reduction of individual conflict by a decrease in intra-colony aggression enhances the social cohesion of the colony (the degree to which individuals perform behavioural acts that promote the interests of the group over the interests of the individual). A previous study on this species showed evidence that high food availability is associated with a breakdown of a colony's social cohesion, via a reduction in time spent on the nest engaging in cooperative behaviour (Jandt et al., 2015). When coupled with our findings that low prey availability reduces intra-colony aggression, these findings suggest that paper wasps adjust their cooperative strategies in relation to the nutritional environment they are currently experiencing. Furthermore,

the social cohesiveness of a colony may be particularly sensitive to the nutritional environment.

Our results add to a growing understanding of the interrelationships between nutrition, nutrient signalling pathways and animal social organization. This study supports the idea that physiological and molecular pathways related to fundamental forms of solitary insect feeding and reproductive behaviour reinforce cooperative behaviour in highly social insect systems (Toth & Robinson, 2007). Thus, in social Hymenoptera colonies, decreased individual conflict and increased group cohesion may be achieved by maintaining a workforce of nutritionally restricted daughter-helpers (Rossi & Hunt, 1988; Wheeler, 1986). We suggest nutritional restriction could be an important internal regulator of cooperation among the individuals that make up social insect colonies, in turn promoting the emergent group trait of cohesion and maintenance of the superorganism.

ACKNOWLEDGEMENTS

The authors thank Cameron Fay for assistance with wasp nest collection; Amy Geffre for assisting with qPCR and wasp nest collection; David Stein for lipid extraction and quantification; and Fred Janzen, Russell Jurenka, John Nason, Jeanne Serb and the members of the Toth Lab for comments on the manuscript. This research was funded by an NSF-IOS Doctoral Dissertation Improvement Grant awarded to A. Walton (award no. 1701887).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

A.W. and A.L.T. conceived the ideas and designed the methodology; A.W. collected and analysed the data; A.W. and A.L.T. wrote the manuscript. Both authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.gqnk98sng (Walton & Toth, 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Walton, A., & Toth, A. L. (2021). Resource limitation, intra-group aggression and brain neuropeptide expression in a social wasp. *Functional Ecology*, 00, 1–13. https://doi.org/10.1111/1365-2435.13895