Honesty of Larval Begging Signals Covaries With Colony Kin Structure in *Formica* Ants

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Social insects live in highly complex societies with efficient communication systems. Begging is one display commonly used by offspring to signal their nutritional state, however begging behavior has received very little attention in social insects. Theory predicts that begging can be either an honest (i.e., honest-signaling strategy) or a dishonest (i.e., scrambling competition) signal of need, with dishonest signals expected to be more likely when relatedness within the group is low. To investigate the presence and honesty of begging, as well as the nature of the involved signals, we used a comparative approach with four species of the ant genus *Formica* known to differ in the degree of intra-colony relatedness. We investigated the behavior of starved and non-starved larvae of *F. aquilonia*, *F. pressilabris* (both low intra-colony relatedness), *F. exsecta* (intermediate relatedness), and *F. fusca* (high relatedness). In addition, we assessed the attraction of conspecific workers toward odors extracted from these two classes of larvae and analyzed the larval cuticular hydrocarbon profiles. We found that in *F. fusca* and *F. exsecta*, larvae signaled significantly more when starved. In contrast, larvae of *F. aquilonia* signaled significantly more when they were non-starved, while there was no significant difference in the behavior of starved vs. non-starved larvae in *F. pressilabris*. Our results show that workers were not preferentially attracted to the odor of starved larvae, and we also did not detect any differences between the cuticular hydrocarbon profiles of starved and non-starved larvae. Overall, this study demonstrates among species variation in larval hunger signaling in *Formica* ants, and encourages further studies to confirm the link between kin structure variation and the honesty of begging signals.

Keywords: social insects, kin conflict, parent-offspring conflict, honest signal, scramble competition, relatedness, cuticular hydrocarbons

INTRODUCTION

Communication systems allow individuals to base their adaptive decisions on the behavior, morphology, or physiology of others (Endler, 1993). Signals have evolved to convey information to receivers, so that the information extracted from the signal elicits a response and results, on average, in positive fitness consequences for both the sender and the receiver (Laidre and Johnstone, 2013).

To be efficient these interactions must depend on a repertoire of signs and rules shared between the participants of the interaction (Maynard Smith and Harper, 2003; Witzany, 2010). Communication and signaling systems thus allow individuals to affect the plastic decisions of others, possibly generating coevolution (Font and Carazo, 2010).

Despite the social nature of communicative signals, the adaptive function of signals from the receiver's perspective may be fundamentally different from that of the sender (Marler, 1961). Traditionally, communication has been seen as a voluntary transfer of information from the sender to the receiver, when the inclusive fitness interests of the sender and the receiver converge. However, when the inclusive fitness interests of the sender and the receiver diverge, the ultimate aim of the sender is not the sharing of mutual beneficial information, and conflicts over information use arise (Owren and Rendall, 1997, 2001; Seyfarth and Cheney, 2003). In such cases, selection should favor senders, whose signals affect the behavior of the receiver at their own advantage, and receivers, who are able to extract information from the signals (Endler, 1993; Font and Carazo, 2010). Kinship and the inclusive fitness benefits gained through helping relatives (Hamilton, 1964) should influence the type of communication. Closely related individuals should be more likely to communicate honestly in order to cooperate, whereas the opposite would be expected for non-relatives.

Begging is commonly used by offspring to influence the quantity and quality of received food, with consequences for survival (Hodar et al., 2002) and other fitness-related traits (Breteler and Gonzalez, 1988; Chapman, 1998; Shafiei et al., 2001; Engels and Sauer, 2007). Godfray (1991, 1995) developed the idea that begging should be an honest signal of need. Food provisioning by parents is obviously a cooperative behavior but contains an element of conflict as well. This is because the existence of relatedness asymmetries leads offspring to value themselves more than they value their siblings, and thus, to selfishly beg for a larger part of parental investment. However, for a parent, providing one offspring with a larger investment influences the trade-off between the expected fitness returns from this offspring vs. the others (Trivers, 1972, 1974). Therefore, parents would benefit from accurately assessing the level of need expressed through the begging signal. Other models, called scramble competition models, predict that this conflict between parent and offspring over the distribution of resources is increased by competition among siblings (Parker and Macnair, 1979). In such a case, each offspring is assumed to beg more than other offspring even when already fed, leading to an exaggeration of need (Godfray, 1995; Royle et al., 2002; Johnstone, 2004; Akçay, 2012). Scramble competition, and consequently dishonest signaling, should thus be more prevalent when competition occurs among a greater number of siblings or when the relatedness among brood is low (Parker et al., 2002; Johnstone, 2004). This is because the inclusive fitness costs of dishonestly taking food from other brood members are lower under low relatedness. In contrast, signals should be honest indicators of need under high relatedness.

Both honest and dishonest begging has been demonstrated previously. Food-deprived pigeon squabs (*Columba livia*) spend more time begging than recently fed ones (Mondloch, 1995), giving support to honest signaling models. Conversely, begging calls of yellow-headed blackbird chicks varied with the intensity of sibling competition, independent of need (Price et al., 1996). Empirical studies on begging behavior are taxonomically highly biased toward birds and consequently also heavily focused on vocal and visual signals (Kedar et al., 2000; Leonard and Horn, 2005; Marques et al., 2008; Klenova, 2015; Caro et al., 2016). However, offspring of insects with maternal care also display various begging behaviors, mainly in order to solicit protection and food (reviewed in Mas and Kölliker, 2008). For instance, burying beetle larvae (*Nicrophorus*) beg by approaching their parents' mouthparts, pushing their heads against them and touching them with their legs (Milne and Milne, 1976; Rauter and Mooref, 1999).

Colonies of social insects—ants, bees, wasps, and termites—are especially interesting to study from a signaling perspective because they form large, complex societies, which function based on chemical, visual, acoustic, vibrational, and tactile signals (Leonhardt et al., 2016). In ants, kinship among colony members varies widely due to variation in queen number and queen mating frequency. This makes them particularly suitable for testing predictions concerning signaling honesty. Recently, selfish brood behaviors were shown to be correlated with low relatedness, raising the possibility that brood might indeed respond to kin structure variation in adaptive ways (Schultner et al., 2013, 2014). However, offspring begging as a classic conflict-related trait has been largely overlooked in social insects. The few empirical studies that have characterized begging in social Hymenopteran larvae show that various behavioral hunger signals exist, from scraping mandibles against cell walls (vespine wasp larvae: Ishay and Schwartz, 1973) to flexing the head or whole body to attract workers (ant larvae: Brian, 1977; Creemers et al., 2003; Kaptein et al., 2005). For example, *Gnamptogenys striatula* ant larvae perform two types of movement to signal their hunger toward workers after a 24 h starvation period: "swaying," which corresponds to a repositioning of the larval body to reach food or workers and "stretching," which corresponds to a rhythmic stretching of the body (Kaptein et al., 2005). Hungry larvae perform these movements more often, indicating that begging signals honestly reflect need in this species. Similar behavioral patterns have been detected in *Myrmica* ant larvae (Creemers et al., 2003). Additionally, larvae may solicit food from workers using chemical hunger signals in bees (den Boer and Duchateau, 2006; He et al., 2016), and fire ants (Cassill and Tschinkel, 1995). While these studies provide first evidence for the ability of social insect larvae to influence their food intake via begging, comparative studies are needed to test how begging in social insects is influenced by colony kin structure.

We used a comparative approach to investigate larval hunger signaling in ants. Our aim was to assess the existence of hunger signals and the effect of kinship on the honesty of these signals. We focused on *Formica* ants because their colony kin structures range from single queen colonies with high intra-colony relatedness to multiple queen colonies with extremely low relatedness among nestmates (Rosengren et al., 1993; Sundström et al., 2005). For four species of *Formica* ants, representing a

range of intra-colonial relatedness, we assessed the behavior of starved and non-starved larvae. We furthermore analyzed the cuticular odors of starved and non-starved larvae and experimentally tested for potential chemical hunger signals by presenting workers with larval cuticular extracts. In line with inclusive fitness predictions, we find that starved larvae signaled significantly more than non-starved larvae in *Formica fusca* and *F. exsecta*, i.e., species with high and intermediate levels of intra-colony relatedness. In contrast in *F. aquilonia*, a low relatedness species, larvae signaled significantly more when they were not starved, while there was no significant difference in the behavior of starved vs. non-starved larvae in the other low relatedness species. Workers were not preferentially attracted to the odor of starved larvae, and we also did not detect any differences between the cuticular hydrocarbon profiles of starved and non-starved larvae. Our study demonstrates that larval hunger signaling varies among species in ants and suggests links between kinship and the honesty of hunger signaling in social insects.

MATERIALS AND METHODS

Study Species and Experimental Setup

Formica ants are characterized by remarkable intra- and interspecific variability in within-nest relatedness, largely due to variation in queen numbers (Rosengren et al., 1993; Sundström et al., 2005). We studied four *Formica* species covering the full range of relatedness values: *F. aquilonia* forms large networks of interconnected nests (so-called polydomy), where each nest contains hundreds of queens (Rosengren et al., 1993; personal observation), and relatedness among nestmates is very low ($r = 0.06 \pm 0.09$ CI, Schultner et al., 2014, 2016). *F. pressilabris* is also typically polydomous and nests contain multiple queens, usually some dozens ($r = 0.21 \pm 0.09$ CI, Schultner et al., 2014). These species are therefore classified as "low-relatedness" species. In contrast, *F. fusca* nests typically contain ~1–10 queens (Hannonen and Sundström, 2003; Helanterä et al., 2011), and each colony comprises one nest only (so-called monodomy). This species is thus considered a "high-relatedness" species ($r = 0.46 \pm 0.14$ CI, Schultner et al., 2014). *F. exsecta* can form both monodomous colonies containing single queens and polydomous colonies in which each nest contains dozens of queens (Rosengren et al., 1993). The *F. exsecta* colonies used in this study were collected from a polydomous population ($r = 0.09 \pm 0.08$ CI, Schultner et al., 2014). However, in our study area most nests of this species form monodomous populations, in which colonies have only one queen and very high relatedness ($r = 0.71 \pm 0.03$ SE; Sundström et al., 2003). Compared to *F. aquilonia* and *F. pressilabris*, which predominantly live in polydomous, low relatedness populations, and *F. fusca*, which always live in monodomous, high relatedness populations, *F. exsecta* is therefore considered an "intermediate relatedness" species. Note that of the four species, only *F. fusca* founds colonies independently, while the other three species are so-called temporary social parasites, which found colonies in the nests of other ants, including *F. fusca* (Buschinger, 2009), or dependently with the help of conspecific workers. All colonies used in thi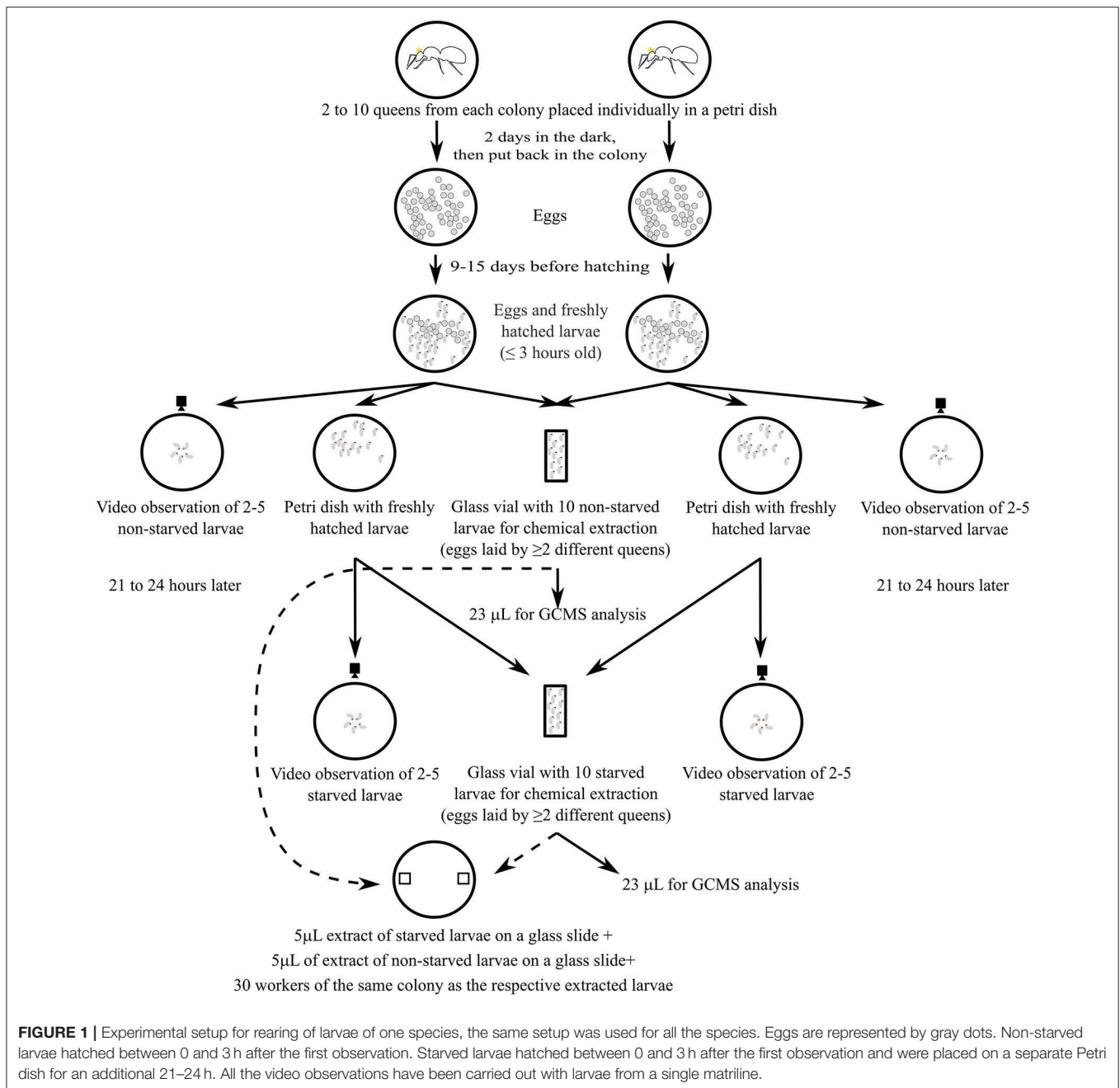s study were collected from established populations in which the host species have already been eradicated, and none contained any host workers.

From each species, colony fragments including queens, workers, and nest material were collected in the field and brought back to the laboratory. Colony fragments were then placed in containers lined with Fluon, together with a ceramic plate, some compost as nest material and *Sphagnum* moss to maintain humidity. The colonies were fed with Bhatkar-Whitcomb diet (Bhatkar and Whitcomb, 1970) and watered daily. Colony fragments were checked daily for the presence of eggs. When the first eggs were observed, up to 10 queens were taken from each fragment, placed individually in Petri dishes with two pieces of $1 \, cm \times 1 \, cm$ humid sponge and kept in the dark at room temperature. After 2 days, queens were removed and put back into their colony fragments. Eggs deposited on the Petri dishes were then counted and checked every 2 days to remove fungal spores, re-humidify sponges and remove dead eggs. Eggs from all species hatched within 9–15 days.

We created two groups of larvae differing in regard to their state of hunger for behavioral observations and chemical assays. Freshly hatched larvae (0–3 h old) were considered not to be hungry ("non-starved" hereafter), and were either moved to a new Petri dish, in which their behavior was observed immediately (see "Quantification of larval behavior"), or directly collected in glass vials for chemical analyses (**Figure 1**). To obtain hungry ("starved" hereafter) larvae, freshly hatched larvae were moved to a new Petri dish containing a piece of $1 \, cm \times 1 \, cm$ sponge, where they remained for 21–24 h. Larvae were thereafter subjected to behavioral or chemical analyses as described for non-starved larvae (**Figure 1**). Larvae did not have the opportunity to cannibalize one another on the Petri dish because they were physically separated, and the total number of larvae on each Petri dish was documented. No larvae disappeared from any of the Petri dishes during the starvation period, indicating that cannibalism did not occur. The number of replicates conducted for behavioral assays and the number of samples used in chemical analyses are summarized in **Table 1**.

Quantification of Larval Behavior

To quantify begging behavior, groups of 2–5 starved or non-starved larvae were placed on their backs in a Petri dish (**Figure 1**). The Petri dish was put under a stereomicroscope onto which a camera (DigiMicroScale) was installed and behaviors were recorded for 10 min. Each group consisted only of larvae produced by the same queen, thus groups were genetically homogenous. To avoid any matriline bias, several groups of larvae produced by different queens from each colony were used (at least two different queens). To avoid observer bias, videos were renamed by someone unfamiliar with the experimental setup prior to analysis. We observed two different kind of behaviors performed by the larvae. The first one, termed "swaying" in reference to Kaptein et al. (2005), consisted of the larva moving its body upwards, remaining in this position for some time and/or rhythmically repeating the movement. The second recorded behavior was a rhythmic opening and closing of the mandibles. This behavior has been implicated in larval begging in attine ants (Wheeler and Bailey, 1920),

FIGURE 1 | Experimental setup for rearing of larvae of one species, the same setup was used for all the species. Eggs are represented by gray dots. Non-starved larvae hatched between 0 and 3 h after the first observation. Starved larvae hatched between 0 and 3 h after the first observation and were placed on a separate Petri dish for an additional 21–24 h. All the video observations have been carried out with larvae from a single matriline.

and preliminary observations suggest it may correlate with nutritional status in Formica ants. For each larva, the number and duration (in seconds) of swaying movements and the number and duration of mandible movements were recorded with the software Boris (version 6.3.1, Friard and Gamba, 2016).

Intracolony Relatedness and Larval Sex Ratios

Levels of selfishness–in the form of egg cannibalism–exhibited by *Formica* ant larvae depend on intra-colony relatedness and larval sex (Schultner et al., 2013, 2014). While female larvae behave less selfishly when within-colony relatedness is high, male larvae

do not adjust their levels of selfishness to variation in colony kin structure. In order to estimate the effect of intra-colony relatedness and larval sex on begging behavior, we determined relatedness and sex of larvae from genotype data using six microsatellite loci previously tested in our focal species (FE 19, FE 42, FE 51, FY 4, FY7, FL20; Schultner et al., 2013, 2014, 2016). To this end, we collected 2–7 larvae from 10 to 15 colonies of each species in 70% EtOH (**Table 1**). For DNA extraction, each larva was placed in an individual well with a 2.5 μl: 100 μl Proteinase K–Chelex solution (6%), and incubated overnight at 56°C. After inhibition of Proteinase K activity at 100°C for 10 min, 1 μl of DNA was used in simplex PCR reactions, together

TABLE 1 | Number of larvae, workers, and colonies used in behavioral assays and for chemical and genetic analyses.

Experiment species		Starved treatment		Non-starved treatment	
Behavioral assays		**Replicates**	**Colonies**	**Replicates**	**Colonies**
Larval begging behavior	F. fusca	131	14	41	8
	F. exsecta	86	10	43	7
	F. pressilabris	92	10	80	9
	F. aquilonia	100	10	68	8
Worker response to larval extracts	F. fusca	135	15	135	15
	F. exsecta	72	8	72	8
	F. pressilabris	108	12	108	12
	F. aquilonia	108	12	108	12
Chemical analysis		**Colonies**		**Colonies**	
Larval cuticular hydrocarbon profiles	F. fusca	7		8	
	F. exsecta	3		2	
	F. pressilabris	7		8	
	F. aquilonia	11		10	
Genetic analysis		**Replicates**		**Colonies**	
Larval genotypes	F. fusca	66		15	
	F. exsecta	41		10	
	F. pressilabris	48		10	
	F. aquilonia	56		9	

For larval begging behavior, the replicates represent the number of individual larvae whose behavior was recorded (when we did not exclude the 5 colonies with species-level values of relatedness and sex ratio, see Statistical Analyses). For worker response to larval extracts, the replicates represent the number of data points collected for each species (e.g., the number of colonies used in a species multiplied by 9, the number of hours during which we extracted the maximum number of workers on each glass slide). For chemical analysis, each colony represents one replicate of a pool of 10 larvae. For genetic analyses, larvae were collected randomly from starved and non-starved treatments; each replicate corresponds to one larva.

with 5 µl of TAQ polymerase, 3 µl of H_2O, and 1 µl primer (0.5 µl forward primer, 0.5 µl reverse primer). PCRs were run with protocols optimized for each primer and PCR products analyzed in a 310 ABI PRISM sequencer (Applied Biosystems). Each microsatellite peak was scored manually with GeneScan 3.1 software (Applied Biosystems).

Intra-colony relatedness was estimated with Relatedness 5.0.8 software (Queller and Goodnight, 1989) using background allele frequencies calculated from the genotypes generated for this study. For all relatedness estimates, standard errors were calculated by jackknifing over colonies. To determine larval sex, individuals were scored as females if they were heterozygous at one or more loci and as males if they were homozygous at all six loci. In some cases, not all loci amplified successfully; for putative males, which were homozygous at <6 loci (15 out of 29 homozygous individuals), we calculated the probability of wrongly assigning a larva as a male using previously established methods (Schultner et al., 2014), and scored larvae with a misclassification probability below 10% as males (0 putative male

larvae excluded). For each colony, the average larval sex ratio was calculated from sexed individuals, with 0 = all females and 1 = all males.

Worker Response to Larval Odors

From several (at least two) queens from each colony fragment, we collected and froze pools of 10 starved and 10 non-starved larvae in individual glass vials (Sigma Aldrich) (**Table 1**). Cuticular compounds were extracted from each group of 10 larvae for 1 min with 30 µL of hexane. Thereafter, 28 µl of the solvent were transferred to a new, clean vial. From each pooled sample (e.g., each group of 10 larvae), 5 µl were used to investigate chemical hunger signaling. The test setup consisted of two 0.5 cm × 0.5 cm glass slides that were placed in a round container (7 cm diameter). The containers' sides were covered with black paper and the containers themselves were covered with red transparent paper and placed in a cardboard box to minimize luminosity and disturbance. In each container, one glass slide was covered with 5 µL of cuticular extract from non-starved larvae (hereafter, "non-starved odor") while the other one was covered with 5 µL of cuticular extract from starved larvae (hereafter, "starved odor") (**Figure 1**). After the solvent had evaporated (30 min), 30 workers were added from the same colony fragment as the respective extracted larvae. After 30 min of adaptation, 10 pictures were taken at 5 s intervals at every full hour for 9 h, for a total of 90 pictures per container. Each camera (two in total, camera model: SONY HDR-SR8E) was able to capture 12 containers at the same time. The number of workers with at least one body part (legs, antennae) on the glass slide was recorded in each picture for each glass slide. To avoid any side bias, the position of the cuticular extracts was randomized among replicates for each species. Prior to analyses, the pictures were renamed by someone unfamiliar with the experimental setup to avoid any observer bias. We predict that if a chemical signal is produced by larvae to signal their state, workers should be more attracted toward the odors of starved larvae than to the odors of non-starved larvae, irrespective of the species.

Characterization of Larval Chemical Profiles

Chemical analyses were conducted on the remaining 23 µl of each sample. Samples were fractionated on silica columns (Chromabond SiOH, 1 mL/100 mg) to remove fatty acids (likely originating internally, as the larval cuticle is thin and not all larvae remained intact throughout the extraction). The non-polar hexane fraction was carefully evaporated under a stream of nitrogen, and then re-dissolved in 23 µl of hexane. Two µl per sample were then subjected to gas chromatography with coupled mass spectrometry (GCMS, Shimadzu GCMS-QP2010 Plus) fitted with a SGE BPX-5 column (31 m × 0.25 µm × 0.25 mm). Helium gas (1 mL/min) served as carrier gas, and injection was splitless. The temperature program started at 70°C isothermal for 1 min, after which the temperature was raised to 200° C at 30°C/min and then from 200 to 320°C at 5°C/min, where it was held for 5 min. As larval samples showed a number of unusual peaks, we also ran control samples of Petri dishes, which were considered a likely cause of contaminations. For

that purpose, four clean Petri dishes were separately rinsed with 30 µL of hexane for 1 min each and the resulting samples were analyzed following the protocol above. The chromatograms of each sample were analyzed using ChemStation software (E.02.01.1177). Compounds detected in samples of the clean petri dishes were also found in larval samples and were thus excluded from further analyses. Tentative identification of the remaining peaks was based on their retention indices and mass spectra. We used letters to indicate different but unidentifiable peaks, and "x" and "y" to indicate different positions of double-bonds or methyl groups (**Supplement Table 1**). Relative amounts for each of the remaining peaks were calculated following Aitchison (1982):

$$\ln \frac{\text{area of compound A of individual } L_x}{\text{geometric mean of all compound areas of individual } L_x}$$

Statistical Analyses

We conducted all statistical analyses with R software 3.5.1 (R Core Team, 2018). To investigate larval begging behavior, we ran generalized linear mixed models that account for zero-inflated data following a Poisson distribution (function glmmTMB in R package glmmTMB, Brooks et al., 2017). Each of the four measures of begging intensity (number of swaying movements, swaying duration, number of mandible movements, mandible movement duration) were treated as dependent variables and investigated separately. To test for overall differences in begging intensity between species, we constructed two global models. The first model included the effects of species and the interaction between mean intra-colony relatedness and larval state as fixed terms, and the group of larvae observed at the same time as a random term:

$$\text{Global model 1}: \quad \textit{Measure of begging intensity} \\ \sim \textit{relatedness} * \textit{state} + \textit{species} + (1|\textit{group})$$

The second global model tested the effects of species and the interaction between mean intra-colony sex ratio and larval state as fixed terms, and the group of larvae observed at the same time as a random term:

$$\text{Global model 2}: \quad \textit{Measure of begging intensity} \\ \sim \textit{sex ratio} * \textit{state} + \textit{species} + (1|\textit{group})$$

For global models, overall p-values for each term were obtained using Type II Wald X^2 tests (R function anova). For each species, we furthermore constructed two species-specific models. The first species-specific model tested the effect of larval state and its interaction with mean intra-colony relatedness (fixed terms), while the group of larvae observed at the same time was used as a random term:

$$\text{Species} - \text{specific model 1}: \quad \textit{Measure of begging intensity} \\ \sim \textit{relatedness} * \textit{state} + (1|\textit{group})$$

The second species-specific model tested the effect of larval state and its interaction with mean intra-colony sex ratio (fixed terms),

while the group of larvae observed at the same time was used as a random term.

$$\text{Species} - \text{specific model 2}: \quad \textit{Measure of begging intensity} \\ \sim \textit{sex ratio} * \textit{state} + (1|\textit{group})$$

The effects of mean intra-colony relatedness and mean intra-colony sex ratios were tested separately because these factors covary for each individual colony as they are based on one set of genotypes per colony. For five colonies, larval genotypes were not available (*F. aquilonia* = 1 colony, *F. exsecta* = 1, *F. fusca* = 2, *F. pressilabris* = 1); here we substituted species-level mean relatedness and sex ratio values. We ran the two global models and the eight species-specific models without these five colonies to verify whether the results stayed the same. As there were no major differences in the statistical results between the two data sets (compare **Tables 2, 3**, **Supplement Tables 2–4** and **Supplement Tables 5–9**), we retained the entire data set for all analyses given in the main text.

To compare intra-colony relatedness and sex ratio levels across species, we used Kruskal-Wallis rank sum tests for non-parametric data (R function kruskal.test).

To investigate worker responses to larval odors, we performed generalized linear mixed effects models following a Poisson distribution (function glmer in R package lme4, Bates et al., 2014). We determined the maximum number of workers on a glass slide in each treatment (i.e., starved or non-starved larvae odor) in the 10 pictures from each hour and used it as a response variable. For all species combined, we constructed a model where treatment and species were included as fixed terms while the hour when the picture was taken and the colony from which larvae and workers were sampled were used as random terms:

$$\text{Global model 3}: \quad \textit{Maximum number of workers} \sim \textit{treatment} \\ + \textit{species} + (1|\textit{hour}) + (1|\textit{colony})$$

We also constructed a separate model for each species, where treatment was used as a fixed term and the hour when the picture was taken and the colony from which larvae and workers were sampled were used as random terms:

$$\text{Species} - \text{specific model 3}: \quad \textit{Maximum number of workers} \\ \sim \textit{treatment} + (1|\textit{hour}) + (1|\textit{colony})$$

To characterize the chemical profiles of starved and non-starved larvae we performed principal component analyses (PCA) on transformed proportions of compounds (function fviz_pca in R package factoextra). We performed four separate PCAs using the proportions of all compounds, only alkanes, only methylated alkanes or only alkenes. Each of these PCAs was performed on the combined data from all the species, and for each species separately. The 95% confidence ellipses for each species and treatment were calculated by bootstrapping based on the function coord.ellipse in R package FactoMineR (Lê et al., 2008).

TABLE 2 | Results of Generalized Linear Mixed Models using Template Model Builder linking the number of swaying movements, swaying duration, number of mandible movements, or mandible movement duration to the state and species of the larvae, intra-colony relatedness, and intra-colony sex ratio.

	~Relatedness*state + species + (1|group)				~Sex ratio*state + species + (1|group)			
	Effect	**Chisq**	**df**	***p*-value**	**Effect**	**Chisq**	**df**	***p*-value**
Number of swaying movements (n = 641)	Relatedness	2.009	1	0.156	Sex ratio	0.946	1	0.331
	State	0.912	1	0.340	State	1.091	1	0.296
	Species	0.776	3	0.855	Species	0.55	3	0.908
	Relatedness:state	2.246	1	0.134	Sex ratio:state	9.091	1	**0.003**
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.76 ± 0.87			(1|group)	0.43 ± 0.66		
Swaying duration (n = 641)	Relatedness	2.928	1	0.087	Sex ratio	1.616	1	0.204
	State	17.901	1	**<0.001**	State	18.383	1	**<0.001**
	Species	11.400	3	**0.0097**	Species	11.175	3	**0.011**
	Relatedness:state	0.568	1	0.451	Sex ratio:state	2.164	1	0.141
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	36.54 ± 6.05			(1|group)	36.86 ± 6.07		
Number of mandible movements (n = 641)	Relatedness	2.816	1	0.093	Sex ratio	1.118	1	0.290
	State	0.130	1	0.718	State	0.184	1	0.668
	Species	12.379	3	**0.006**	Species	11.088	3	**0.011**
	Relatedness:state	5.360	1	**0.021**	Sex ratio:state	1.503	1	0.220
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.35 ± 0.59			(1|group)	0.37 ± 0.61		
Mandible movement duration (n = 641)	Relatedness	0.405	1	0.524	Sex ratio	0.186	1	0.666
	State	2.096	1	0.148	State	7.359	1	**0.007**
	Species	11.202	3	**0.011**	Species	7.81	3	**0.050**
	Relatedness:state	6.471	1	**0.011**	Sex ratio:state	0.0001	1	0.992
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	1.16 ± 1.08			(1|group)	19.26 ± 4.39		

Significant p-values are written in bold. n represents the number of starved and non-starved larvae used in each model.

RESULTS

Quantification of Larval Behavior

Overall, larvae swayed slightly less often when they were starved, with the number of swaying events averaging 0.47 ± 0.05 SE in starved larvae and 0.58 ± 0.14 SE in non-starved larvae. Swaying duration was also shorter in starved larvae (12.05 s $\pm$ 14.55 SE) than in non-starved larvae (12.85 ± 32.29 SE). Similarly, starved larvae moved their mandibles less often (starved: 1.01 ± 0.08 SE; non-starved: 1.06 ± 0.11 SE) and for shorter periods of time (starved: 24.93 s $\pm$ 29.67 SE; non-starved: 25.94 ± 46.70 SE). However, only swaying duration differed significantly between starved and non-starved larvae (**Table 2**, fixed term state in global model "Swaying duration," $\chi^2 = 17.90$, $p < 0.001$). Swaying duration, the number of mandible movements and mandible movement duration also differed significantly between species, with *F. aquilonia* larvae signaling significantly more than larvae of the other three species (**Table 2**, Global models "Swaying duration," "Number of mandible movements," "Mandible movement duration").

In contrast, there were no significant effects of intra-colony relatedness or sex ratio on the number of swaying events, swaying duration, number of mandible movements or mandible movement duration (**Table 2**). However, we did
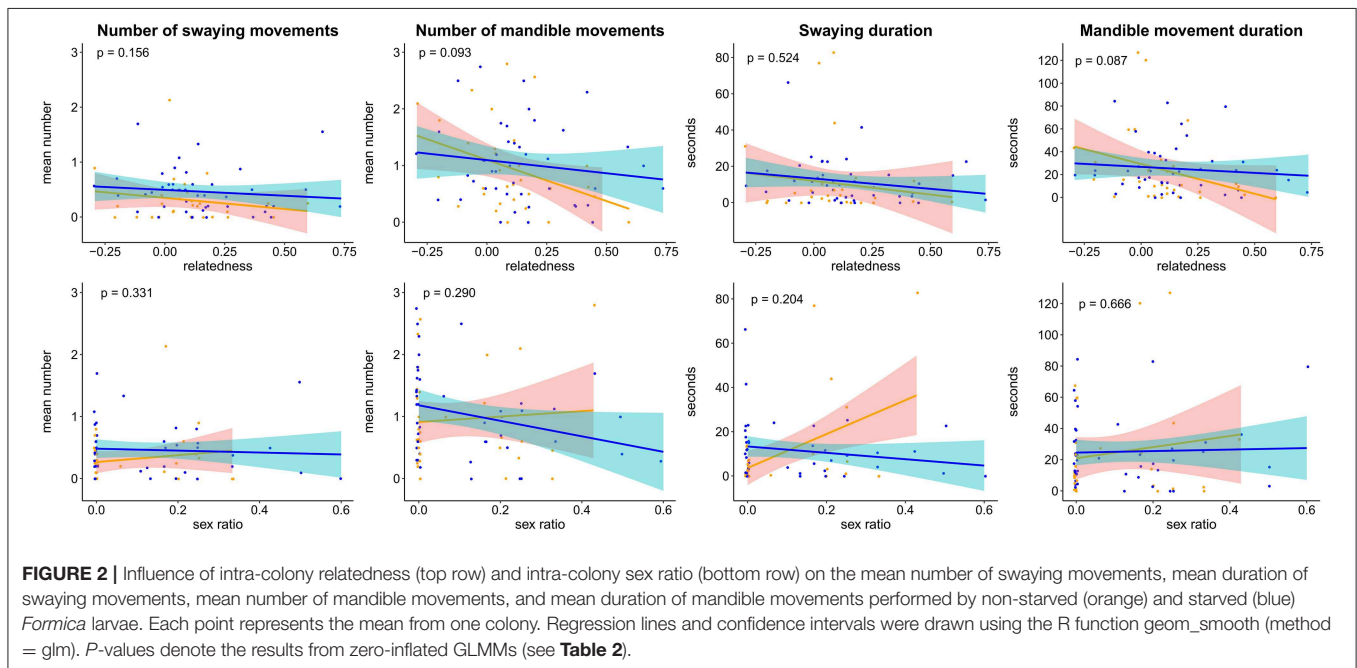
find a significant negative effect of the interaction between intra-colony relatedness and larval state on the number and duration of mandible movements (**Figure 2**, **Table 2**, fixed term relatedness*state in global models "Number of mandible movements": $\chi^2 = 5.36$, $p = 0.021$, "Mandible movement duration"; $\chi^2 = 6.47$, $p = 0.011$), indicating that the number and duration of mandible movements decreased significantly with increasing intra-colony relatedness in non-starved but not starved larvae. Additionally, the interaction between intra-colony sex ratio and state had a significant positive effect on the number of swaying events (**Figure 2**, **Table 2**, fixed term state*sex ratio in global model "Number of swaying movements," $\chi^2 = 9.09$, $p = 0.003$). This suggests that the number of swaying movements increased significantly faster with increasing male bias in non-starved compared to starved larvae.

Within-species analyses showed that *F. fusca* and *F. exsecta* larvae swayed more often when starved (**Figure 3**, **Table 3**, fixed term state, *F. fusca*: $z = 2.69$, $p = 0.007$; *F. exsecta*: $z = 2.39$, $p = 0.017$), while *F. aquilonia* larvae swayed more when not starved (**Figure 3**, **Table 3**, fixed term state, *F. aquilonia*: $z = -2.84$, $p = 0.005$). The number of swaying movements performed by *F. pressilabris* larvae did not vary depending on nutritional state (**Figure 3**, **Table 3**, fixed term state, *F. pressilabris*: $z = -0.20$, $p =$

TABLE 3 | Results of Generalized Linear Mixed Models using Template Model Builder linking the number of swaying movements to the state of the larvae, intra-colony relatedness, and intra-colony sex ratio for each species separately.
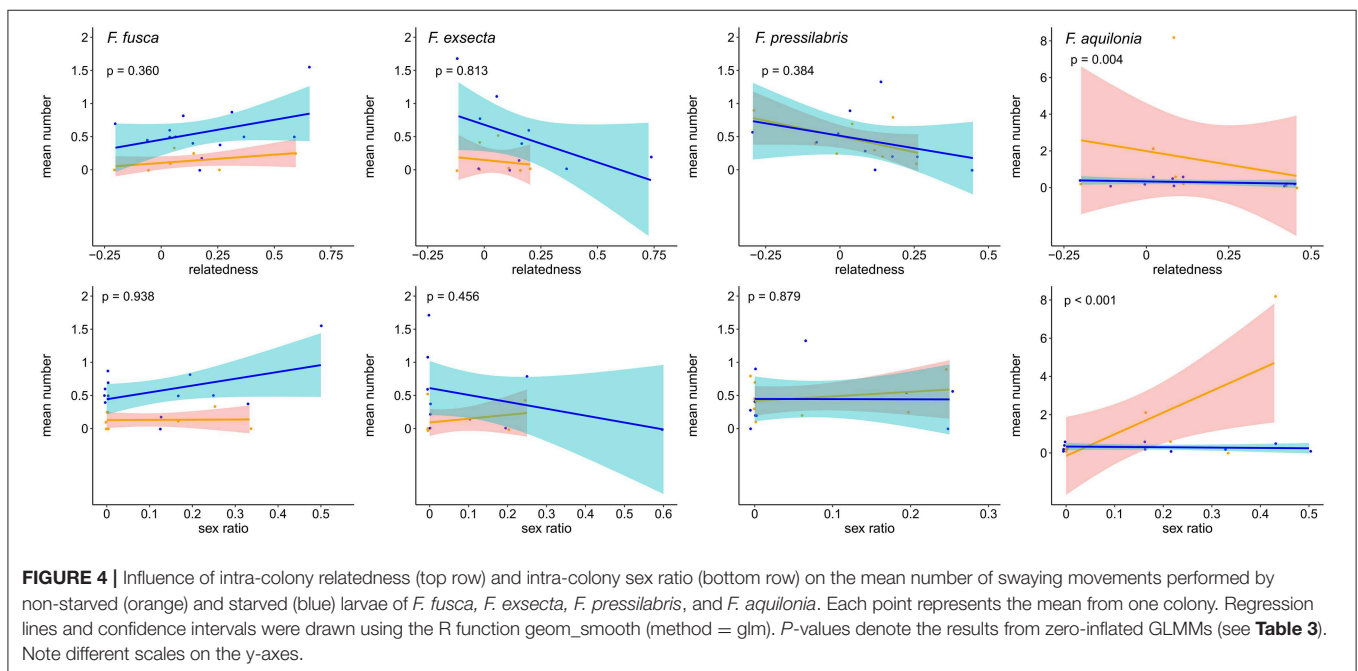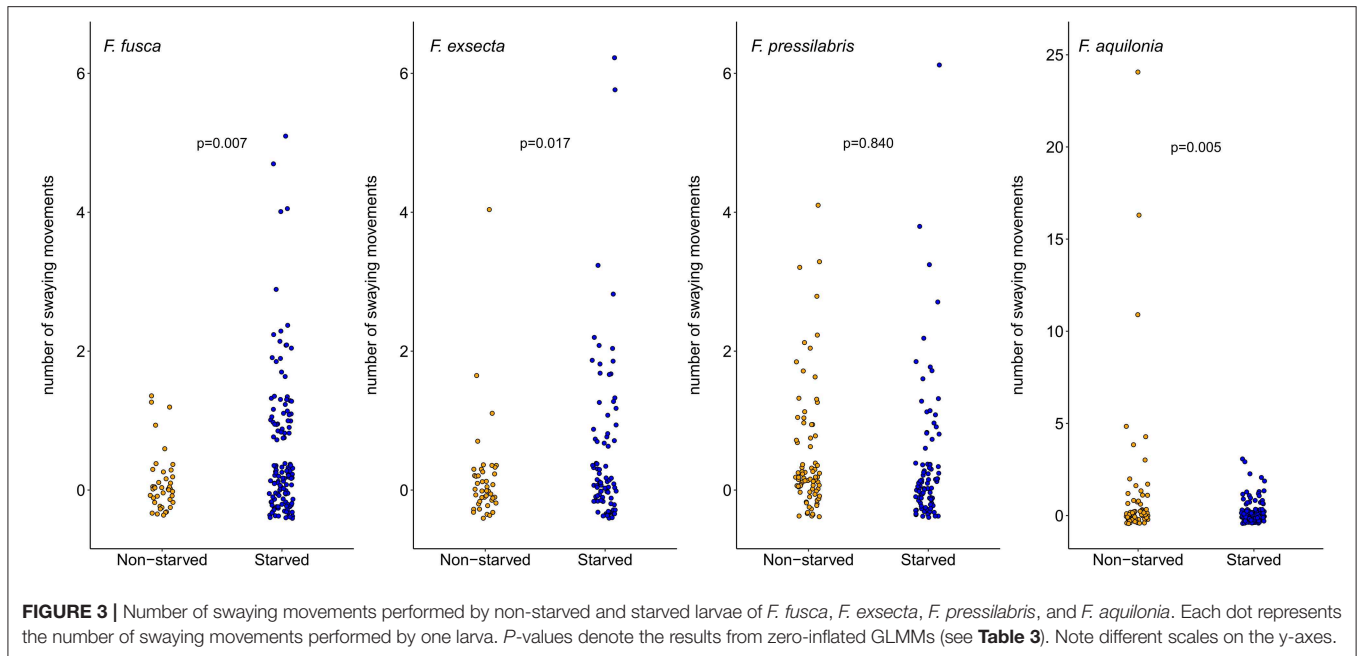
Number of swaying movements	~Relatedness*state + (1|group)				~Sex ratio*state + (1|group)			
	Effect	β ± SE	z	p-value	Effect	β ± SE	z	p-value
F. fusca (n = 172)	Relatedness	1.78 ± 1.95	0.92	0.360	Sex ratio	0.32 ± 4.11	0.08	0.938
	State	1.60±0.60	2.69	**0.007**	State	1.38 ± 0.60	2.29	**0.022**
	Relatedness:state	−0.73 ± 2.02	−0.36	0.716	Sex ratio:state	1.21 ± 4.18	0.29	0.772
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.02 ± 0.14			(1|group)	$4.31*10^{-8} \pm 2.08*10^{-4}$		
F. exsecta (n = 129)	Relatedness	−1.29 ± 5.44	−0.24	0.813	Sex ratio	3.89 ± 5.21	0.75	0.456
	State	1.60 ± 0.67	2.39	**0.017**	State	2.00 ± 0.78	2.55	**0.011**
	Relatedness:state	−2.37 ± 5.73	−0.41	0.679	Sex ratio:state	−8.40 ± 5.81	−1.45	0.148
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	1.08 ± 1.04			(1|group)	1.28 ± 1.13		
F. pressilabris (n = 172)	Relatedness	−1.07 ± 1.22	−0.87	0.384	Sex ratio	0.29 ± 1.88	0.15	0.879
	State	−0.06 ± 0.30	−0.20	0.840	State	−0.10 ± 0.37	−0.28	0.779
	Relatedness:state	−0.69 ± 1.64	−0.42	0.674	Sex ratio:state	0.87 ± 2.64	0.33	0.741
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.12 ± 0.34			(1|group)	0.06 ± 0.24		
F. aquilonia (n = 168)	Relatedness	−5.27 ± 1.82	−2.89	**0.004**	Sex ratio	7.46 ± 1.44	5.19	**<0.001**
	State	−1.51 ± 0.53	−2.84	**0.005**	State	0.09 ± 0.47	0.20	0.845
	Relatedness:state	4.21 ± 2.31	1.83	0.068	Sex ratio:state	−8.07 ± 1.95	−4.13	**<0.001**
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.82 ± 0.91			(1|group)	0.11 ± 0.33		

Significant p-values are written in bold. n represents the number of starved and non-starved larvae used in each model.

FIGURE 2 | Influence of intra-colony relatedness (top row) and intra-colony sex ratio (bottom row) on the mean number of swaying movements, mean duration of swaying movements, mean number of mandible movements, and mean duration of mandible movements performed by non-starved (orange) and starved (blue) *Formica* larvae. Each point represents the mean from one colony. Regression lines and confidence intervals were drawn using the R function geom_smooth (method = glm). *P*-values denote the results from zero-inflated GLMMs (see **Table 2**).

0.840). There was no difference between starved and non-starved larvae in the other three measures of begging intensity, with the exception of swaying duration in *F. exsecta* (**Supplement Table 2**, fixed term state, *F. exsecta*: z = 4.72, p < 0.001) and number of mandible movements in *F. aquilonia* (**Supplement Table 3**, fixed term state, *F. aquilonia*: z = −2.90, p = 0.004). The effect of

FIGURE 3 | Number of swaying movements performed by non-starved and starved larvae of *F. fusca*, *F. exsecta*, *F. pressilabris*, and *F. aquilonia*. Each dot represents the number of swaying movements performed by one larva. *P*-values denote the results from zero-inflated GLMMs (see **Table 3**). Note different scales on the y-axes.

FIGURE 4 | Influence of intra-colony relatedness (top row) and intra-colony sex ratio (bottom row) on the mean number of swaying movements performed by non-starved (orange) and starved (blue) larvae of *F. fusca, F. exsecta, F. pressilabris*, and *F. aquilonia*. Each point represents the mean from one colony. Regression lines and confidence intervals were drawn using the R function geom_smooth (method = glm). *P*-values denote the results from zero-inflated GLMMs (see **Table 3**). Note different scales on the y-axes.

larval state on these behaviors was the same as for the number of swaying movements, with *F. exsecta* larvae swaying longer when starved and *F. aquilonia* larvae exhibiting more mandible movements when non-starved.

Analyses of begging behavior within species furthermore revealed that variation in intra-colony relatedness and sex ratio do not influence begging signals to the same degree in all species (**Figure 4**, **Table 3**, **Supplement Tables 2–4**). For example, increasing relatedness had significant negative effects while increasing male-bias in sex ratio had significant positive

effects on the number of swaying movements in *F. aquilonia* (**Figure 4**, **Table 3**, fixed term relatedness, *F. aquilonia*: $z = -2.89$, $p = 0.004$; fixed term sex ratio, *F. aquilonia*: $z = 5.19$, $p < 0.001$) but not the other three species. Conversely, increasing relatedness had significant negative effects on the number of mandible movements performed by larvae in *F. fusca*, *F. pressilabris*, and *F. aquilonia*, but not *F. exsecta* (**Supplement Table 3**, fixed term relatedness, *F. fusca*: $z = -2.14$, $p = 0.033$; *F. pressilabris*: $z = -2.31$, $p = 0.021$; *F. aquilonia*: $z = -2.42$, $p = 0.007$; *F. exsecta*: $z = 4.02$, $p = 0.091$).
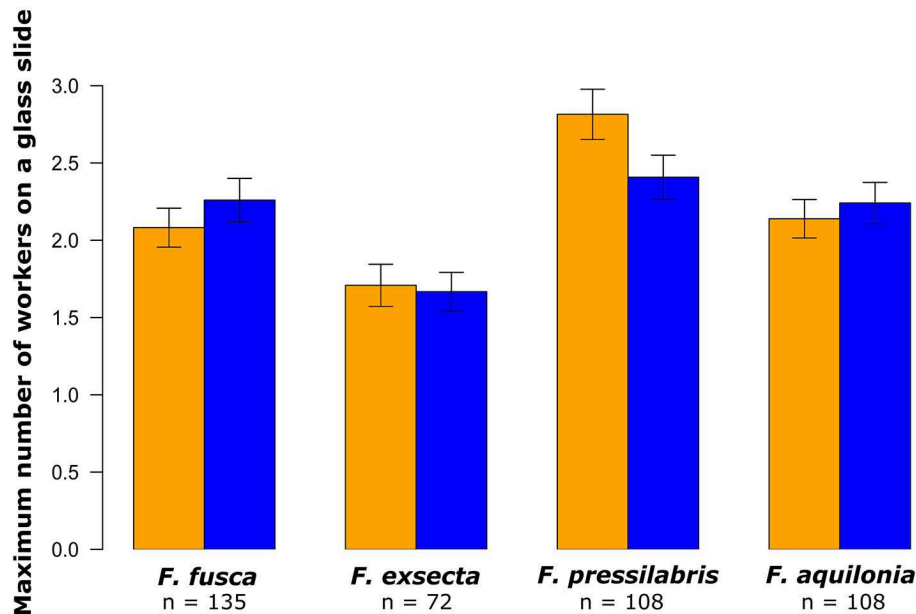
FIGURE 5 | Maximum hourly number of *F. fusca*, *F. exsecta*, *F. pressilabris*, and *F. aquilonia* workers on glass slides covered with odors from non-starved (orange) and starved (blue) larvae. The number of replicates is noted below each species name. The error bars refer to the 95% confidence interval of the mean.

Intra-colony Relatedness and Larval Sex Ratios

F. fusca had a mean intra-colony relatedness of 0.18 ± 0.05 SE and an intra-colony sex ratio (proportion of males) of 0.13 ± 0.19 SD. *F. exsecta* had a mean intra-colony relatedness of 0.16 ± 0.06 SE and an intra-colony sex ratio of 0.11 ± 0.20 SD. *F. pressilabris* had a mean intra-colony relatedness of 0.14 ± 0.07 SE and an intra-colony sex ratio of 0.06 ± 0.11 SD. *F. aquilonia* had a mean intra-colony relatedness of 0.09 ± 0.05 SE and intra-colony sex ratio of 0.21 ± 0.22 SD. There were no differences in intra-colony sex ratios between species (Kruskal Wallis rank sum test, $X^2 = 3.357$, $p = 0.339$). Similarly, intra-colony relatedness estimates showed large overlap and there were no differences in relatedness estimates between species (Kruskal Wallis rank sum test, $X^2 = 0.033$, $p = 0.998$). Intra-colony relatedness was nevertheless included as an explanatory variable in the statistical analyses of begging intensity in order to test whether begging behavior is plastic, i.e., larvae adjust begging intensity to intra-colony relatedness. When interpreting results obtained from comparisons between species, we additionally relied on more robust relatedness estimates obtained from larger data sets in previous studies (Sundström et al., 2003, Schultner et al., 2014, 2016).

Worker Response to Larval Odors

On average, 1.3 (± 1.3 SD) *F. fusca* workers, 0.8 (± 0.9 SD) *F. exsecta* workers, 1.3 (± 1.4 SD) *F. pressilabris* workers, and 1.2 (± 1.3 SD) *F. aquilonia* workers approached larval odors during the observation period. Workers did not move significantly more toward the odor of starved larvae than toward the odor of non-starved larvae, neither when combining data from all species nor when analyzing each species separately (**Figure 5**, **Table 4**, all species: $p = 0.61$, *F. fusca*: $p = 0.32$, *F. exsecta*: $p = 0.85$, *F. pressilabris*: $p = 0.06$, *F. aquilonia*: $p = 0.61$).

Characterization of the Larval Chemical Profiles

Overall, we found 46 peaks after exclusion of Petri dish contaminations (**Supplement Table 1**). When analyzing all species together, visual inspection of PCAs carried out with all compounds showed no difference in the cuticular hydrocarbons of starved and non-starved larvae (**Figure 6A**). Similarly, there was no difference in the cuticular hydrocarbons of starved and non-starved larvae across all species when compound groups where analyzed separately: alkanes (**Figure 6B**), methylated alkanes (**Figure 6C**), alkenes (**Figure 6D**). We also did not find strong differences between the cuticular hydrocarbon profiles of starved and non-starved larvae when analyzing each species separately (**Supplement Figures 1–4**), with the exception of *F. exsecta*. However, the apparent difference in the chemical compounds of starved and non-starved *F. exsecta* larvae may be due to the smaller number of colony replicates for this species. While we did not find any treatment effects on larval odors, the analysis did reveal differences between species (**Figure 7**). Species differences in larval odor profiles were most apparent when analyses included all compounds (**Figure 7A**) and only alkenes (**Figure 7D**). Additionally, the hydrocarbon profiles of *F. fusca* larvae differed from those of all other species in the composition of methyl-branched alkanes (**Figure 7C**).

TABLE 4 | Results of Generalized Linear Mixed effects Models linking the maximum number of workers on a glass slide to the odor of starved or non-starved larvae on this slide.

Maximum number of workers on a glass slide	~State*species + (1|group)		
All species (n = 423)	**Effect**	**Chisq**	**df**	**p-value**
	State	0.08	1	0.781
	Species	33.94	3	**<0.001**
	State:species	4.63	3	0.201

Maximum number of workers on a glass slide	~State + (1|group)		
	Effect	**β ± SE**	**z**	**p-value**
F. fusca (n = 135)	State	0.08 ± 0.08	0.99	0.322
F. exsecta (n = 72)	State	−0.02 ± 0.13	−0.19	0.847
F. pressilabris (n = 108)	State	−0.16 ± 0.08	−1.85	0.064
F. aquilonia (n = 108)	State	0.05 ± 0.09	0.51	0.613

The first model is a global model for all the species combined whereas the following models are species-specific. Significant p-values are written in bold. n represents the number of data points used in each model.

DISCUSSION

So far, there have been few studies on the influence of kinship on signaling honesty in insects. Caro et al. (2016) found that offspring from around 60 species of birds are generally less honest when facing competition from distantly related nestmates. Begging honesty has mostly been studied in birds and mammals, even though the large natural range of relatedness variation makes social insects great models for understanding the evolution of offspring signals. The results we obtained by comparing larval begging in ant species with varying kin structure are generally consistent with the idea that dishonesty is more prevalent under low relatedness, when inclusive fitness costs suffered through depriving nestmates of food are low. Larvae from high and intermediate relatedness species (*F. fusca, F. exsecta*) begged when hungry, and thus honestly signaled their need. In contrast, larvae from low-relatedness species (*F. aquilonia*), showed behaviors more resembling scramble competition or dishonest signaling. However, given the small number of species studied, and potential confounding factors that could explain the species differences discussed below, further studies are necessary for strong conclusions.

Begging intensity varied with species-specific traits in complex ways. Most importantly, we did not find an overall significant effect of intra-colony relatedness on begging levels. However, this does not mean that species-specific kin structures do not influence begging. Instead, in the global analyses we may not have been able to detect species-level changes in begging behavior caused by intra-colony relatedness variation due to the large variation in our relatedness estimates, which do not perfectly reflect estimates obtained in previous studies (e.g., Sundström

et al., 2003; Schultner et al., 2014, 2016). There are two main reasons that could explain why our estimates deviate from those of previous studies. First, in order to obtain enough larvae for experiments, we preferentially used multiple queen colonies for *F. fusca,* even though these have lower relatedness levels than single queen colonies (multiple queen colonies: $r = 0.2 \pm 0.18$ SD, single queen colonies: $r = 0.68 \pm 0.12$, Helanterä et al., 2011). This likely biased intra-colony relatedness toward lower values in this species. Second, we were limited by small sample sizes, which generally results in imprecise allele frequency estimates both in group level allele frequencies and the population wide background allele frequencies, thus increasing the uncertainty of relatedness estimates (Queller and Goodnight, 1989). Nevertheless, the difference in kin structure is one of the most prominent traits that characterizes our focal species, as other traits such as nesting behavior show large overlap between the species. For example, *F. exsecta*, *F. pressilabris*, and *F. aquilonia* can found their colonies dependently in nests of other ant species, while *F. fusca* often acts as a host (Seifert, 2018). In terms of colony sizes, *F. fusca* has the smallest colonies, with *F. exsecta* and *F. pressilabris* having intermediate and *F. aquilonia* by far the largest colonies. Finally, adult size is largest in *F. aquilonia* compared to the other three species, but large intraspecific variation both within and between colonies occurs in all species.

Two measures of begging intensity were significantly affected by the interaction between state and intra-colony relatedness in the global analyses, indicating that kin structure may modulate state-dependent begging behaviors. In addition, in the species-specific analyses, increasing relatedness generally had a negative influence on begging intensity, as expected if begging were generally correlated with kin competition. Although these effects were not always statistically significant, and suffer from the uncertainty of our relatedness estimates, this suggests that kin structure may shape begging behavior both over evolutionary time (i.e., on a species level) and over shorter time spans (i.e., on a colony level). This is surprising, as begging behavior was quantified in sibling groups, and larvae presumably had no access to information about average colony kin structure. This suggests that the relatedness effect is either mediated through a maternal effect—as the mother and the workers who tend to her do have access to information on kin structure (see e.g., Helanterä and d'Ettorre, 2015)—or factors that covary with both kin structure and brood provisioning levels. Further studies that include more species with varying kin structures and, ideally, studies that experimentally manipulate relatedness and its potential covariates such as colony size, are now needed to better comprehend the impact of kin structure on larval behavior within and between species.

Within-colony sex ratios also explained some of the variation in begging intensity (but note that the same proviso about confounding variables applies here as well). Specifically, the number and duration of swaying events was higher when larval sex ratios were male-biased in *F. aquilonia*. Similar differences in begging intensity between males and females have been recorded in zebra finches (von Engelhardt et al., 2006). In addition, in *F. aquilonia* as well as over all species combined, only non-starved
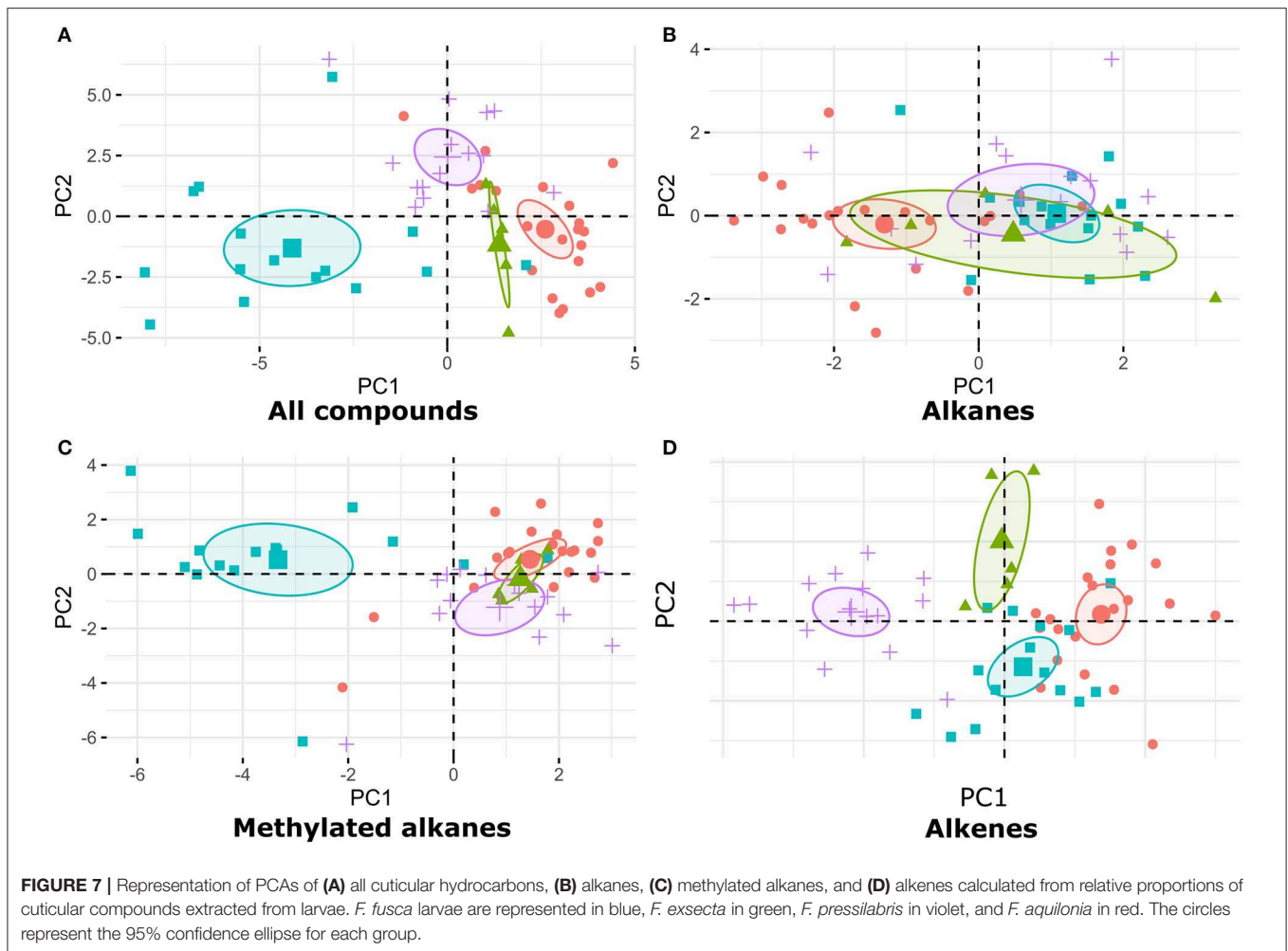
FIGURE 6 | Representation of PCAs of **(A)** all cuticular hydrocarbons, **(B)** alkanes, **(C)** methylated alkanes, and **(D)** alkenes calculated from relative proportions of cuticular compounds extracted from larvae of four *Formica* species. Non-starved larvae are represented in orange and starved larvae are represented in blue. The circles represent the 95% confidence ellipse for each group.

larvae swayed more often in response to male-biased sex ratios. Selfishness has been shown to be more common in male *Formica* larvae in the context of cannibalism (Schultner et al., 2013, 2014), and males may be under selection to compete more fiercely for resources, in particular if nutritional gains increase fitness-related traits such as sperm quantity, quality (Delisle and Hardy, 1997) or adult body size (Wiernasz et al., 1995; Osawa, 2002). In addition, if competition for mating opportunities among males is stronger than among females, male-biased sex ratios may result in high levels of male-male competition. Such competition may be especially intense when larvae can afford to expend energy for begging, i.e., when they are in a non-starved state. In ants, selfish larval behavior in the form of cannibalism is predicted to depend on an interaction between kinship and sex ratio (Schultner et al., 2014), and the complex patterns observed here suggest that similar models, which take into account haplodiploidy and kin structure variation, are needed for a better understanding of begging in social insects.

One factor that may potentially confound the results is the age of larvae. Indeed, it is possible that older, starved, larvae are simply more experienced and better at begging than young, non-starved larvae. Conversely, it is possible that young, non-starved, larvae had more resources to invest in begging, resulting in more intense begging. Finally, contrasting patterns of begging intensity in the focal species may stem from differences in larval size,

developmental rates or metabolism, rather than from species-specific responses to nutritional status. While these are plausible alternative explanations, there are several reasons why we think begging intensity is likely more dependent on nutritional status than larval age. First, the behavior of starved and non-starved larvae in *F. pressilabris* was very similar, indicating that larvae know how to beg innately and can do so regardless of their age or nutritional status. Second, in *F. exsecta* and *F. fusca* older, starved larvae begged more than young, non-starved larvae, indicating that starved larvae were not too deficient in energy to prevent them from begging. Finally, like in other insects, temperature is an important factor influencing development rates in ants (e.g., Porter, 1988). As larval rearing and behavioral assays were conducted under controlled temperature conditions, it is unlikely that larvae from different species underwent development at fundamentally different rates.

While we found clear patterns when looking at the number of swaying events, the patterns of swaying duration, and the number and duration of mandible movement, were not as clear; nevertheless, the data revealed similar trends. In *Gnamptogenys striatula* ants, Kaptein et al. (2005) found that starved larvae swayed significantly longer than non-starved larvae in the presence of workers. It is thus possible that swaying duration depends on the presence of workers, which reflects the difficulty of interpreting behaviors of isolated larvae. It is also possible

FIGURE 7 | Representation of PCAs of **(A)** all cuticular hydrocarbons, **(B)** alkanes, **(C)** methylated alkanes, and **(D)** alkenes calculated from relative proportions of cuticular compounds extracted from larvae. *F. fusca* larvae are represented in blue, *F. exsecta* in green, *F. pressilabris* in violet, and *F. aquilonia* in red. The circles represent the 95% confidence ellipse for each group.

that mandible movements are not related to begging, or that the quality of the videos did not allow us to correctly count all incidents of mandible movement.

As signaling through cuticular hydrocarbons in social insects is particularly well-developed (e.g., Blomquist and Bagnères, 2010), we hypothesized that larvae emit chemical hunger signals, and that workers should preferentially respond to odors of starved larvae. This hypothesis was based on previous studies showing that *Bombus terrestris* workers reacted to extracts of starved larvae with increased feeding (den Boer and Duchateau, 2006), and that female burrower bugs provisioned more when exposed to extracts from nymphs reared in low food conditions (Kölliker et al., 2005). In contrast to these results, *Formica* ant workers did not preferentially approach the odors of starved larvae compared to the odors of non-starved larvae. In line with this, chemical analysis of larval cuticular hydrocarbon profiles did not reveal any differences between starved and non-starved larvae. Nevertheless, there are several reasons why we cannot rule out that chemical hunger signaling plays a role in *Formica* ants. First, we may have overlooked potential chemical signals, for instance because relevant compounds are more polar, their concentrations lay below the detection limit, or because the

compounds had lower or higher molecular weights than those included in our analysis. Second, our study was not designed to test for volatile compounds such as those known to play a role in larval chemical hunger signaling in honey bees (He et al., 2016). Third, chemical signaling by larvae may be context-dependent, and, if it is costly, may only occur in the presence of workers. Finally, workers may respond to larval chemical signals only when they are accompanied by other visual, tactile or behavioral cues. In the ant *Formica cunicularia* for example, workers accept heterospecific brood only when it resembles conspecific brood in both size and odor (Mori and Le Moli, 1988). Further studies are thus needed to rule out the existence of chemical hunger signals in *Formica* larvae.

Although we did not find any hunger-related differences in larval cuticular chemistry, chemical analyses confirmed the presence of species-typical chemical profiles (Martin and Drijfhout, 2009) in *Formica* ant larvae. However, some inconsistencies remain. For instance, not all *F. fusca* samples clustered together. This suggests that larval odors are less strong, or less species-specific, than adult odors (Carlin, 1988), or that individual colonies have very clear and distinct profiles. The last hypothesis is particularly likely for high-relatedness species,

where each colony is genetically distinct. Indeed, the chemical profiles of *F. fusca* eggs are known to be more colony-specific than those of species with lower relatedness such as *F. aquilonia* (Helanterä and d'Ettorre, 2015). Chemical analyses of larval odors also highlighted an interesting phenomenon. Previous studies on cuticular hydrocarbons of ants have typically focused on eggs and adults (van Zweden and d'Ettorre, 2010). In some of these studies, individuals were placed on Petri dishes before being moved to glass vials for extraction. Our samples were handled in a similar manner, and we consistently observed contamination of larval profiles, most likely originating from Petri dishes. This may be due to the fact that, unlike eggs, which are protected by a chorion, freshly hatched larvae like those used in our experiments lack thick cuticles. In natural colonies, this may allow young larvae to pick up colony odors easily. As the only available studies of larval cuticular chemistry in ants have focused on larvae in later stages of development (Brian, 1975; Hare, 1996; Akino et al., 1999; Viana et al., 2001; Villalta et al., 2016; Penick and Liebig, 2017), further studies are required to assess how development stage-dependent differences in cuticle composition may affect odor acquisition.

Begging as a classic conflict-related trait has been largely overlooked in social insects. In particular, the importance of chemical signals as indicators of nutritional state, and the impact of relatedness on the honesty of begging signals have not yet been studied. Our study revealed that, in *F. fusca* and *F. exsecta*, starved larvae begged more frequently than non-starved larvae. In these species with predominantly high intra-colony relatedness, this can be interpreted as an honest signal of need. This is in line with what has been found in *Myrmica rubra* (Creemers et al., 2003) and *Gnamptogenys striatula* (Kaptein et al., 2005). Accordingly, *G. striatula* nests exhibit relatively high levels of relatedness ($r = 0.65 \pm 0.25$ SE, Giraud et al., 2001). In *M. rubra*, intra-colony relatedness varies strongly depending on the study population ($r = 0–0.82$, Seppä and Walin, 1996). As relatedness among *M. rubra* larvae was not estimated in the previous study, it is difficult to draw conclusions about the influence of kin structure on begging honesty in this species. In contrast, larvae in low-relatedness species were more inclined to convey dishonest signals about their state. Thus, as predicted by inclusive fitness theory, begging in social insects may be influenced by colony kin structure. However, it remains unclear whether dishonestly signaling larvae from low-relatedness nests actually receive more food from workers as a result of increased begging, i.e., whether the dishonest signal pays off. Furthermore, our study does not allow us to infer how individual traits such as future reproductive caste may influence a larva's propensity to beg. For example, individual begging intensities may vary

with species-level traits such as the degree of queen-worker dimorphism, with more begging expected to occur when the dimorphism is more pronounced. As caste of developing females has previously been shown to affect worker behavior (e.g., Brian, 1973; Passera et al., 1995; Penick and Liebig, 2012, 2017), it is furthermore possible that larval caste affects worker responses to begging.

Overall, this study provides the first test of inclusive fitness predictions in the context of hunger signaling in social insects. By demonstrating that the honesty of larval hunger signaling varies among species with different colony kin structures, our study highlights yet another social conflict in which kinship covaries with social insect behavior, thus providing further evidence that larvae are active players in colony life (Schultner et al., 2017).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

MP, HH, and ES contributed conception and design of the study, and carried out field collections. MP, RL, TP, and ES collected the data. MP, TP, ES, and HH analyzed the data. MP wrote the first draft of the manuscript. MP, TP, JH, HH, and ES contributed to manuscript revision and all authors read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00398/full#supplementary-material

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