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Ant Larvae as Players in Social Conflict: Relatedness and Individual Identity Mediate Cannibalism Intensity

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ABSTRACT: Conflict arises among social organisms when individuals differ in their inclusive-fitness interests. Ant societies are excellent models for understanding how genetic relatedness mediates conflict intensity. However, although conflicts within colonies typically arise over offspring production, the role of larvae as actors in social conflict has received little attention. We develop and empirically test kin-selection theory of larval egg cannibalism in ant societies. Specifically, we investigate how selection for cannibalism is mediated by nestmate relatedness and larval sex in a mathematical model and then test the model's predictions by measuring cannibalism levels in eight ant species with varying nestmate relatedness. In line with our theoretical predictions, cannibalism levels in larvae were significantly influenced by relatedness and sex. Increased relatedness was associated with reduced levels of cannibalism, indicating that larval behavior is mediated by inclusive-fitness considerations. Levels of cannibalism were significantly higher in male larvae, and our model suggests that this is due to sex differences in the benefits of cannibalism. By examining the selfish interests of larvae and the constraints they face in a social environment, our study presents a novel perspective on conflict in ants and on the evolution of selfish elements in social systems in general.

Keywords: kin selection, behavior, power, *Formica*, social insects.

Introduction

In social organisms, the environment of developing individuals is largely shaped by social interactions between group members (Alexander 1974). Offspring access to resources can be influenced both by the provisioning behavior of parents and competition among siblings and by the behavior of focal offspring themselves (Trivers 1974; Mock and Parker 1997). Individuals typically disagree about optimal resource allocation because each is under

selection to maximize its own inclusive fitness. Thus, while parents prefer investing equal amounts of resources in each developing individual (because they are equally genetically related to all offspring), every individual offspring prefers more investment in itself, because it is genetically more closely related to itself than to its family members (Trivers 1974; Queller 1994). Levels of offspring selfishness and the overall potential for conflict between individuals within social groups are predicted to vary with genetic relatedness among group members (following inclusive-fitness theory; Hamilton 1964; see also Taylor 1996; Mock and Parker 1997; Ratnieks et al. 2001, 2006; Bourke 2011a, 2011b), with lower levels of selfish behavior expected when offspring compete against close relatives (Mock and Parker 1998).

Across taxa, conflict over parental investment has led to an evolutionary tug-of-war where both offspring and parents can display complex behavioral traits to gain control over resource allocation (e.g., Harper 1986; Godfray 1995; Mock and Parker 1998; Godfray and Johnstone 2000). Developing individuals follow their own interests when they compete for resources with siblings or nestmates. They may display power through begging (Kilner and Johnstone 1997; Godfray and Johnstone 2000), establishment of dominance hierarchies (Cotton et al. 1999; Smiseth and Moore 2007; Forbes 2011), or, more drastically, fatal aggression (reviewed in Mock and Parker 1997). Rivalry reaches extreme levels when developing individuals resort to cannibalizing their siblings or nestmates in order to increase survival, development, and growth rate or future adult fitness (e.g., insects: Ohba et al. 2006; spiders: Bilde and Lubin 2001; snails: Baur 1988; sharks: Gilmore et al. 2005; birds: Mock et al. 1990). Parents, in turn, can attempt to control the outcome of within-family conflict, for instance, by using signals to assess the hunger status of offspring chicks and adjusting feeding frequency ac-

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cordingly (such as mouth color in birds; Götmark and Ahlström 1997; Kilner 1997) or by producing similar-sized offspring to reduce competitive asymmetries between individuals (in spiders; Iida 2003). In line with evolutionary theory, levels of offspring selfishness and the potential for competition within broods vary with intrabrood relatedness (e.g., tadpoles: Pfennig et al. 1993; bird chicks: Briskie et al. 1994; earwig nymphs: Dobler and Kölliker 2009).

Social control of offspring environment is especially complex in the colonies of eusocial insects such as termites, wasps, bees, and ants, where fertile queens lay eggs while their worker offspring—composed of only females in the social Hymenoptera (wasps, bees, and ants) and of male and female offspring in the social Blattodea (termites)—specialize on cooperative brood care (Wilson 1971). Here, resource allocation to brood is mainly controlled by workers (e.g., through differential provisioning of larvae; Brian 1973; Wheeler 1986; Le Conte et al. 1995; Ribeiro et al. 1999; Boot et al. 2006; Linksvayer et al. 2011) and by queens (e.g., through differential provisioning of immature eggs; Bier 1952, 1954*a*, 1954*b*). In the social Hymenoptera, larval nutrition plays a particularly important role during the processes of female caste determination (i.e., whether a female larva develops into a queen or a worker; Wheeler 1986) and male lifetime spermatogenesis (Hölldobler and Bartz 1985), both of which occur during larval development and are crucial determinants of adult fitness. Similar to those of offspring in other taxa, behavioral traits that allow individuals to selfishly regulate nutrient intake in order to influence fitness should therefore be positively selected in social Hymenoptera larvae.

Indeed, social Hymenoptera larvae have been shown to influence worker provisioning by using behavioral (Creemers et al. 2003; Kaptein et al. 2005) or chemical (Le Conte et al. 1995) signals and to selfishly regulate nutrient intake by engaging in cannibalism (Baroni Urbani 1991; Rüger et al. 2007; Schultner et al. 2013). In some taxa, female larvae seem capable of selfishly developing into queens instead of workers, especially when queens and workers are reared in similar-sized cells (e.g., in *Melipona* stingless bees; Ratnieks 2001; Wenseleers et al. 2003, 2004*a*; Wenseleers and Ratnieks 2004). Female larvae can also develop into miniature queens (e.g., in *Plebeia* stingless bees [reviewed in Ribeiro et al. 2006] and many ants [reviewed in Rüppell and Heinze 1999]), which suggests that female offspring can play an active role in the conflict over female caste fate (Bourke and Ratnieks 1999; Beekman et al. 2003). Male offspring, on the other hand, may avoid being killed in the course of queen-worker conflict over sex allocation (Bourke and Franks 1995; Sundström et al. 1996; Hammond et al. 2002; Rosset and Chapuisat 2006; Helanterä and Ratnieks 2009) by mimicking the smell of females (Nonacs 1993). Finally, larvae can play an im-

portant cooperative role within colonies, for instance, as a specialized nutritional processing caste in ants (Wheeler 1918; Petralia et al. 1980; Cassill et al. 2005; Dussutour and Simpson 2009) and wasps (Hunt et al. 1982; Hunt 1984; Hunt and Dove 2002). Overall, these studies indicate that social Hymenoptera offspring possess the power to act according to their inclusive fitness interests (Beekman and Ratnieks 2003), in spite of their low mobility and apparent dependence on workers (Beekman et al. 2003).

Past work has revealed that much of a colony's social life revolves around brood care and the attempts of adult queens and workers to follow their inclusive-fitness interests by influencing offspring production and development (e.g., in conflict over sex allocation [Bourke and Franks 1995; Sundström et al. 1996; Hammond et al. 2002; Rosset and Chapuisat 2006; Helanterä and Ratnieks 2009], male parentage [Wenseleers and Ratnieks 2006*a*; Helanterä and Sundström 2007], or queen rearing [Bourke and Ratnieks 1999]). The potential for such conflict typically varies with relatedness among nestmates (Ratnieks et al. 2006). However, even though all of the well-studied social conflicts arise because adults disagree over reproduction, few studies have investigated how social environment shapes selfish traits in social Hymenoptera offspring themselves (but see Wenseleers and Ratnieks 2004 for a rare exception).

Here, we investigate selfish behavior in ant larvae in the form of egg cannibalism, a common means of selfish provisioning and one of the most extreme and taxonomically widespread examples of selfish behavior found in nature (Elgar and Crespi 1992). Cannibalism is known to increase growth rate and adult body size in insects (Osawa 1992, 2002; Michaud and Grant 2004; Roy et al. 2007) and may be important for ant larvae as a way of regulating nutrient intake (Baroni Urbani 1991; Rüger et al. 2007). Cannibalism has a negative effect on the direct fitness of cannibalized victims, and the high inclusive-fitness costs associated with eating close relatives makes cannibalism behavior particularly likely to be mediated by relatedness between cannibals and their victims (Polis 1981; Pfennig 1997). A recent study of larval egg cannibalism in the ant *Formica aquilonia*, furthermore, suggests that cannibalism propensity can be affected by individual traits such as larval sex (Schultner et al. 2013), demonstrating that larval behavior is variable and thus subject to selection.

We explore the role of larvae in social conflict over egg cannibalism by taking a twofold approach. First, we develop a mathematical model in order to formulate predictions as to how the inclusive-fitness effects of cannibalism are mediated by nestmate relatedness and larval sex. Second, we test these predictions by measuring levels of egg cannibalism in eight species of *Formica* ants that show variation in average nestmate relatedness (Rosengren

et al. 1993; Sundström et al. 2005; Seppä et al. 2009; this study) and brood sex ratios (Pamilo and Rosengren 1983; Pamilo and Seppä 1994; Sundström 1995; this study). With this study, we investigate how larval behavior is modulated by individual traits and social environment, with the aim of providing insight into the role of developing individuals as players in social conflict in animal societies.

Methods

Mathematical Analysis

We develop a mathematical model in which larvae cannibalize nestmate eggs in order to increase their future competitiveness against same-sex nestmates. We employ a neighbor-modulated-fitness approach to analyze the action of kin selection in this model (Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). This approach focuses attention on the eggs that are threatened with cannibalism and considers how their survival to adulthood and their fitness as adults are modulated by the cannibalism behavior exhibited by the larvae in their nest (including themselves, when they reach that stage of development). The neighbor-modulated-fitness approach to kin selection yields exactly the same results as the traditional inclusive-fitness approach (Hamilton 1964; Gardner et al. 2011), which focuses attention on the enactors of the social behavior (in this case, the cannibalistic larvae).

We assume monogamy, outbreeding, and haplodiploid inheritance. We also assume that each nest contains n equally fecund queens and that these have sorority a (i.e., the probability that two given queens in the same nest are sisters as opposed to nonrelatives). We consider that a focal egg's survival to adulthood decreases as the average level of cannibalism in their nest increases. Mathematically, we denote the probability of survival as $S(zy_m + (1 - z)y_f)$, where z is the sex ratio (proportion male) among larvae, y_m is average investment of male larvae into larval cannibalism in the nest, y_f is the average investment of female larvae into larval cannibalism in the nest, and S is a monotonically decreasing function of its argument (i.e., $dS(\alpha)/d\alpha < 0$). We assume that same-sex larvae compete within nests for access to resources and that increasing resource share through cannibalism carries a benefit. This can occur either during competition among brood (e.g., cannibal larvae are more likely to be reared until pupation) or later in life (e.g., because cannibalism increases reproductive success by heightening competitive abilities of males and gynes or by decreasing numbers of same-sex competitors for mating and/or founding opportunities). We consider that a surviving adult has expected reproductive success $R_m(x_m/y_m)$ for males and $R_f(x_f/y_f)$ for females, where x_m is the amount that the focal individual will invest into larval cannibalism, conditional on surviving to become a larva and developing as a male; x_f is the amount that the focal individual will invest into larval cannibalism, conditional on surviving to become a larva and developing as a female; and R_m and R_f are both monotonically increasing functions of their arguments (i.e., $dR_m(\beta)/d\beta > 0$ and $dR_f(\beta)/d\beta > 0$). The expected reproductive success R may differ between males and females, for instance, if the sexes differ in their average investment in cannibalism (y_m and y_f).

To calculate fitness, we multiply the probability of survival to adulthood by the expected adult reproductive success, conditional on surviving to adulthood: this yields $w_m = S \times R_m$ for males and $w_f = S \times R_f$ for females. Thus, the average fitness among males is $\bar{w}_m = w_m|_{x_m = y_m = \bar{y}_m, x_f = y_f = \bar{y}_f}$, and the average fitness among females is $\bar{w}_f = w_f|_{x_m = y_m = \bar{y}_m, x_f = y_f = \bar{y}_f}$, where $\bar{y}_m$ is the population average level of larval cannibalism by males, $\bar{y}_f$ is the population average level of larval cannibalism by females, and hence the relative fitness for a focal male is $W_m = w_m/\bar{w}_m$ and the relative fitness for a focal female is $W_f = w_f/\bar{w}_f$.

Genetical traits may be associated with different fitnesses in different classes of individuals (i.e., males vs. females). Accordingly, in deciding whether there is an overall positive association between trait and fitness, an average must be taken over all the classes in the population. In computing this average, each class is weighted by its reproductive value, that is, its asymptotic contribution of genes to distant future generations, as the overall effect of selection acting in this class on the total genetic change of the population is proportional to this quantity (Fisher 1930, p. 291; Price and Smith 1972; Taylor 1990). Thus, when a gene is picked at random from the population from a locus that controls female investment into larval cannibalism and its genic value is denoted g, the condition for natural selection to favor an increase in female investment into larval cannibalism is $dW/dg > 0$, where $W = c_f W_f + c_m W_m$ is a class reproductive value–weighted average of relative fitness over the two classes (Taylor 1996). This yields the condition for increase $c_f dW_f/dg_f + c_m dW_m/dg_m > 0$, where

$$\frac{dW_f}{dg_f} = \frac{\partial W_f}{\partial x_f} \times \frac{dx_f}{dG_f} \times \frac{dG_f}{dg_f} + \frac{\partial W_f}{\partial y_f} \times \frac{dy_f}{dG_f'} \times \frac{dG_f'}{dg_f}$$

and

$$\frac{dW_m}{dg_m} = \frac{\partial W_m}{\partial y_f} \times \frac{dy_f}{dG_f'} \times \frac{dG_f'}{dg_m}$$

(Taylor and Frank 1996). Here, G_f represents a female's genetic "breeding" value for the cannibalism trait of interest, that is, a weighted sum of the frequencies of the alleles that she carries at the focal locus, the weights being

determined by least squares linear regression analysis and defining the genic values of those alleles (Price 1970). Accordingly, the quantity dx_f/dG_f describes the mapping between genotype and phenotype. Similarly, G'_f represents the average genetic value for the cannibalism trait among all the female larvae in the focal female's nest, and the quantity dy_f/dG'_f represents exactly the same mapping (i.e., $dy_f/dG'_f = dx_f/dG_f$). The quantity dG_f/dg_f is the probability that two genes, drawn at random (with replacement) from the focal locus in the focal female, are identical by descent. This defines the consanguinity of a female to herself, or p_f (Bulmer 1994). Similarly, $dG'_f/dg_f = p_{ff}$ is the consanguinity of two female larvae in the same nest, and $dG'_f/dg_m = p_{fm}$ is the consanguinity of a female larva to a male larva in the same nest.

This yields the condition for increase

$$\bar{y}_f < \frac{R'_f}{S'} \times \frac{1}{1-z} \times \frac{c_f(p_{ff} - p_f)}{c_f p_{ff} + c_m p_{fm}},$$

where $S' = dS(\alpha)/d\alpha|_{\alpha = z\bar{y}_m + (1-z)\bar{y}_f}$ and $R'_f = dR_f(\beta)/d\beta|_{\beta = (\bar{y}_f/\bar{y}_f) = 1}$. Consequently, under the action of natural selection, the average female investment into larval cannibalism converges on the value

$$y^*_f = \frac{R'_f}{S'} \times \frac{1}{1-z} \times \frac{c_f(p_{ff} - p_f)}{c_f p_{ff} + c_m p_{fm}}.$$

Under the same approach, the average male investment into larval cannibalism is found to converge on the value

$$y^*_m = \frac{R'_m}{S'} \times \frac{1}{z} \times \frac{c_m(p_{mm} - p_m)}{c_m p_{mm} + c_f p_{fm}},$$

where p_m is the consanguinity of a male to himself and p_{mm} is the consanguinity of two male larvae in the same nest.

The ratio of female to male cannibalism is thus $F = y^*_f/y^*_m$, or

$$F = \frac{R'_f}{R'_m} \times \frac{z}{1-z} \times \frac{c_f(p_{ff} - p_f)(c_f p_{fm} + c_m p_{mm})}{c_m(p_{mm} - p_m)(c_f p_{ff} + c_m p_{fm})}. \quad (1)$$

This is a product of three terms: (1) a ratio of sex-specific benefits of cannibalism (R'_f/R'_m), (2) the number of males per female ($z/(1-z)$), and (3) a ratio of sex-specific consanguinities and reproductive values ($[c_f(p_{ff} - p_f)(c_f p_{fm} + c_m p_{mm})]/[c_m(p_{mm} - p_m)(c_f p_{ff} + c_m p_{fm})]$). Assuming no sex differences in costs and benefits and an unbiased sex ratio, the first two terms are both unity. The term describing sex-specific consanguinities and reproductive values is solely responsible for any sex differences in larval cannibalism and can be expressed in terms of queen number and sorority. Specifically, we have $c_f = 2/3$ and $c_m = 1/3$ (Price 1970; Bulmer 1994; Taylor 1996); $p_f = 1/2$ and $p_m = 1$ (Bulmer 1994); $p_{ff} = (1/n)(3/8) + [(n-1)/n]a(3/$

32), because two female juveniles share the same mother with probability $1/n$ and hence are full sisters with consanguinity 3/8, or otherwise they share different mothers with probability $(n-1)/n$ but their mothers are sisters with probability a and hence the juveniles are cousins with consanguinity 3/32; $p_{fm} = (1/n)(1/4) + [(n-1)/n]a(3/16)$, because a female and a male juvenile share the same mother with probability $1/n$ and hence are full siblings with consanguinity 1/4, or otherwise they share different mothers with probability $(n-1)/n$ but their mothers are sisters with probability a and hence the juveniles are cousins with consanguinity 3/16; and $p_{mm} = (1/n)(1/2) + [(n-1)/n]a(3/8)$, because two male juveniles share the same mother with probability $1/n$ and hence are brothers with consanguinity 1/2, or otherwise they share different mothers with probability $(n-1)/n$ but their mothers are sisters with probability a and hence the juveniles are cousins with consanguinity 3/8. Making these substitutions yields

$$F = \frac{[4 + 3a(n-1)][12 + 3a(n-1) - 16n]}{[8 + 3a(n-1)][4 + 3a(n-1) - 8n]}. \quad (2)$$

Assessing Cannibalism Levels across Species

Study Colonies and Collections. The genus *Formica* is extremely diverse and currently contains 175 described species (Dlussky 1967; Bolton 1995; Goropashnaya et al. 2012). *Formica* ants are especially abundant in southern Finland, where they dominate a variety of ecosystems, including forests, sandy beaches, peat bogs, clear-cut areas, and rocky coastline. In addition to their diverse ecology, *Formica* ants exhibit both inter- and intraspecific variation in colony kin structure, mainly because of varying queen numbers (Rosengren et al. 1993; Pamilo et al. 2005; Sundström et al. 2005; Pamilo 2008). Furthermore, unlike many other ant species (Le Masne 1953; Franks and Sendova-Franks 1992), *Formica* ants do not separate brood by developmental stage, and larvae therefore have easy access to eggs. Finally, *Formica* ants exhibit a clear seasonal pattern in the production of sexuals, with eggs laid in spring comprising both sexes (Gösswald 1951; Helanterä and Sundström 2007) and typically giving rise to males and new queens, while summer eggs are usually female and develop into workers (Bier 1952, 1954b; Gösswald and Bier 1953, 1954; Gösswald 1989). This makes it possible to test conflict predictions in sexual spring brood, where the majority of larvae are potential future reproductives.

We chose three species that form nests with few queens (high-relatedness species, with ~1–10 queens per nest) and five species with many queens (low-relatedness species, with >20 queens per nest; Rosengren et al. 1993; Sundström et al. 2005; Seppä et al. 2009; H. Helanterä, personal

observation), to test whether nestmate relatedness affects the intensity of larval egg cannibalism. We collected whole nests (high-relatedness species *F. fusca* and *F. lemani*) or nest fragments (high-relatedness species *F. sanguinea* and low-relatedness species *F. aquilonia, F. cinerea, F. fennica, F. pressilabris*, and *F. truncorum*) containing sexual spring brood in 2010–2012 around Tvärminne Zoological Station near Hanko in southwestern Finland. Nests were collected either within a single population (*F. fusca, F. sanguinea, F. lemani, F. cinerea, F. fennica*, and *F. pressilabris*) or from two distinct populations separated by 15 km or more (*F. aquilonia* and *F. truncorum*). Ants were transferred to plastic nest boxes (40 cm × 30 cm × 20 cm) coated with Fluon on the inner walls to prevent them from escaping. Some of the original nest material, peat, and *Sphagnum* moss were added so that ants could build a small nest in the box. A ceramic plate (15 cm × 15 cm) was placed in each box as a nest site. Boxes were kept in the lab under a natural light regime and a controlled temperature of 25°C and were moistened daily with a spray can. Ants were fed once daily with Bhatkar-Whitcomb diet (Bhatkar and Whitcomb 1970).

Cannibalism Experiments. To assess how relatedness affects cannibalism intensities, we measured the level of egg cannibalism in larvae from several nests of each species. Since the theoretical analysis points to intrabrood relatedness as the most relevant mediator of the inclusive-fitness effects of larval cannibalism, relatedness was estimated by genotyping the larvae used in bioassays. From genotypes we also determined individual larval sex and average sex ratios, which allowed testing for differences in cannibalism behavior between males and females.

Bioassays. From each lab nest, brood was removed within 5 days of collection and classed visually into (1) eggs, (2) small larvae (1–3 days old), (3) medium-sized larvae (4–7 days old), or (4) large larvae (8–10 days old). Size classifications were based on personal observations of larva development during previous experiments (Schultner et al. 2013). Eggs were placed in piles of five on fresh petri dishes, and a single larva was placed ventrally on top of each pile. The number of eggs a larva consumed was recorded once a day for two consecutive days. When larvae cannibalize eggs, they pierce the outer shell and consume the egg contents; thus, eggs were recorded as cannibalized when the outer shell remained or a larva was observed actively feeding on an egg.

Genetic Analyses: Within-Brood Relatedness. A subset of larvae used in bioassays ($n = 409$ larvae) were collected in individual Eppendorf tubes filled with 90% ethanol and genotyped at polymorphic microsatellite loci designed for *Formica* species and tested for successful cross amplification in our focal species: FE13, FE16, FE19, FE21, FE42,

and FE51 (Gyllenstrand et al. 2002); FL12, FL20, and FL21 (Chapuisat 1996); and FY4, FY7, and FY13 (Hasegawa and Imai 2004). For DNA extraction, whole larvae were placed in individual wells together with a 2.5 : 100 μL proteinase K–Chelex solution and left to incubate overnight at 56°C. Polymerase chain reactions (PCRs) were run in 10-μL reactions using 5 μL Qiagen Type-It microsatellite multiplex buffer, 3 μL deionized water, 1 μL optimized primer mix, and 1 μL DNA. PCR protocols were run according to Qiagen recommendations, products were analyzed in 1 : 200 dilutions in a 3730 ABI sequencer, and microsatellite peaks were scored with Genemapper software, version 4.1. For each species, the eight loci that showed the highest level of polymorphism (table A1, available online) were used to estimate mean nestmate relatedness according to the relatedness index r (Queller and Goodnight 1989), implemented in Relatedness, version 5.0.8. From some nests (17 out of 59 total nests), fewer than three larvae were genotyped; relatedness estimates for these nests were based on worker genotypes ($n = 10$ workers per nest) obtained from one leg per worker with the methods described above. To obtain relevant relatedness estimates, background allele frequencies calculated from whole-population genotypes were used (10 workers per nest; table A1). For species sampled from two populations (*F. aquilonia* and *F. truncorum*), relatedness was calculated separately for each population, since for these so-called supercolonial species this reflects the relevant competitive neighborhood (Helanterä et al. 2009).

Sex Bias in Cannibalism. The sex of *Formica* ant larvae can be easily distinguished because males develop from unfertilized, haploid eggs and females from fertilized, diploid eggs. From larva genotypes, sex was thus determined by classifying larvae that were heterozygous at one or more loci as females and individuals that were homozygous at all successfully amplified loci as males. From whole-population allele frequencies calculated from worker genotypes (table A1), we calculated the probability of wrongly assigning a diploid individual to the male category for all combinations of loci. When all eight loci amplified successfully, this probability was below 1% in all species. When fewer than eight loci amplified (46 of 409 total sexed larvae), only samples with a misclassification probability below 3% were coded as males. Two samples did not fulfill these criteria, so their sex was coded as missing. Because sex was determined in a subset of individuals, missing sexes were imputed by calculating species-specific sex ratios from larva genotypes.

Statistical Analysis. We used the R package lme4 (Bates et al. 2012) to analyze the data because it allows logistic regression with random effects. To test for a global effect of relatedness across species, we fitted the global slope regression model $z \sim$ species + relatedness + sex + size +

(relatedness × sex) + nest, treating nest as a random factor. We modeled cannibalism as a binary variable (z, presence/absence) because only 14% of larvae ate more than one egg. We tested for species-specific effects of relatedness by fitting a second model in which the regression coefficient was nested within species (random-slope regression). The two models were compared via the Akaike Information Criterion (AIC; Akaike 1974) and the Bayesian Information Criterion (BIC; Schwarz 1978). For different factor levels in species and larva-size covariates, joint P values were calculated by fitting an alternative model where either the species covariate or the size covariate was absent and comparing the maximal log likelihood of this alternative model with that of the full model, using the likelihood ratio test with appropriate degrees of freedom. The P values of all other parameters were based on results of the Wald test (lme4's standard approach). We also constructed a Bayesian framework to model cannibalism as a two-stage process, that is, as a binary decision to eat or not to eat and a decision regarding the number of eaten eggs (source code, available online), but this revealed little extra information in comparison to the classical regression analysis. Therefore, we base the following discussion on the results of classical regression analysis.

Phylogenetic Independence.   In order to ensure that species-level values are not affected by phylogenetic constraints, we carried out tests for serial independence (Abouheif 1999), as implemented in the R package adephylo (Jombart et al. 2010), on species means of brood sex ratio, cannibalism incidence, and relatedness. The tests used a phylogeny based on Goropashnaya et al. (2012), where we placed *F. fennica* as sister group to *F. manchu*, following Seifert (2000).

## Results

Mathematical Analysis

Modeling cannibalism in a kin-selection framework predicted an overall increase in absolute levels of cannibalism with rising queen numbers for both male and female larvae (fig. 1*A*, 1*B*). Specifically, a linear increase in cannibalism was predicted for both sexes when queens were unrelated ($a = 0$). An increase in between-queen relatedness affected absolute levels of male and female cannibalism differentially, with females exhibiting a slightly steeper increase in cannibalism levels with rising queen numbers than males. Sex differences in cannibalism propensity arise because males are equally related to male and female nestmates, while females are more related to female than to male nestmates (figure A1, available online). In a nest with a single queen, there is thus more incentive for a male larva to cannibalize sibling eggs than for a female (fig. 1*C*).

When queen numbers increase, relatedness drops overall, but this decrease is, on average, more drastic among female nestmates than between males and females and among males. As a result, relative levels of female cannibalism are predicted to increase. However, assuming no sex differences other than in relatedness coefficients, cannibalism levels in females are predicted to be relatively higher when nests contain at least two queens that are full sisters or when they contain an equal mix of full-sister queens and unrelated queens but not when queens are unrelated (fig. 1*C*).

Assessing Cannibalism Levels across Species

The overall proportion of cannibalistic larvae in the raw data ranged from 1.4% ± 1.4% in *F. fennica* to 19.4% ± 4.7% in *F. truncorum* (mean proportion ± 95% confidence interval). The remaining six species showed intermediate levels of cannibalism (table 1). The proportion of eggs eaten by larvae varied in a similar way, with only 0.3% ± 0.3% of eggs eaten by *F. fennica* larvae and 4.6% ± 1.3% eaten by *F. sanguinea* larvae (table 1). As expected from queen numbers observed in nests of focal species, mean relatedness ranged from −0.06 in *F. cinerea* nests to 0.46 in *F. lemani* nests (table 1). Average sex ratios also differed between species: at the extremes, nests of *F. lemani* showed female-biased sex ratios while ratios in *F. pressilabris* nests were highly male biased (table 1). None of the tested variables showed a signal of being phylogenetically constrained (all tests for serial independence: $P > .22$). Thus, species were treated as independent from each other in statistical analyses.

Statistical analysis revealed significant effects of relatedness, sex ratio, and larva size on cannibalism intensity when the data were fitted to either global- or random- (i.e., species-specific) slope models, but the former was preferred by both the AIC (global-slope model: 991; random-slope model: 993) and the BIC (global-slope model: 1,067; random-slope model: 1,075). We therefore base our discussion on the results of global-slope regression (table 2). Decreasing relatedness led to a significant increase in cannibalism (fig. 2*A*), as did increasingly male-biased sex ratios (fig. 2*B*). The interaction between relatedness and sex was also statistically significant, indicating that male and female cannibalism levels respond differently to changes in relatedness. Specifically, for males high relatedness did not prevent cannibalism, whereas females were likely to be cannibalistic only when relatedness was low (fig. 2*C*). Across species, large larvae cannibalized more than small larvae, suggesting that in addition to sex, individual development stage determines cannibalism propensity. Data underlying tables 1 and 2 and figure 2 are
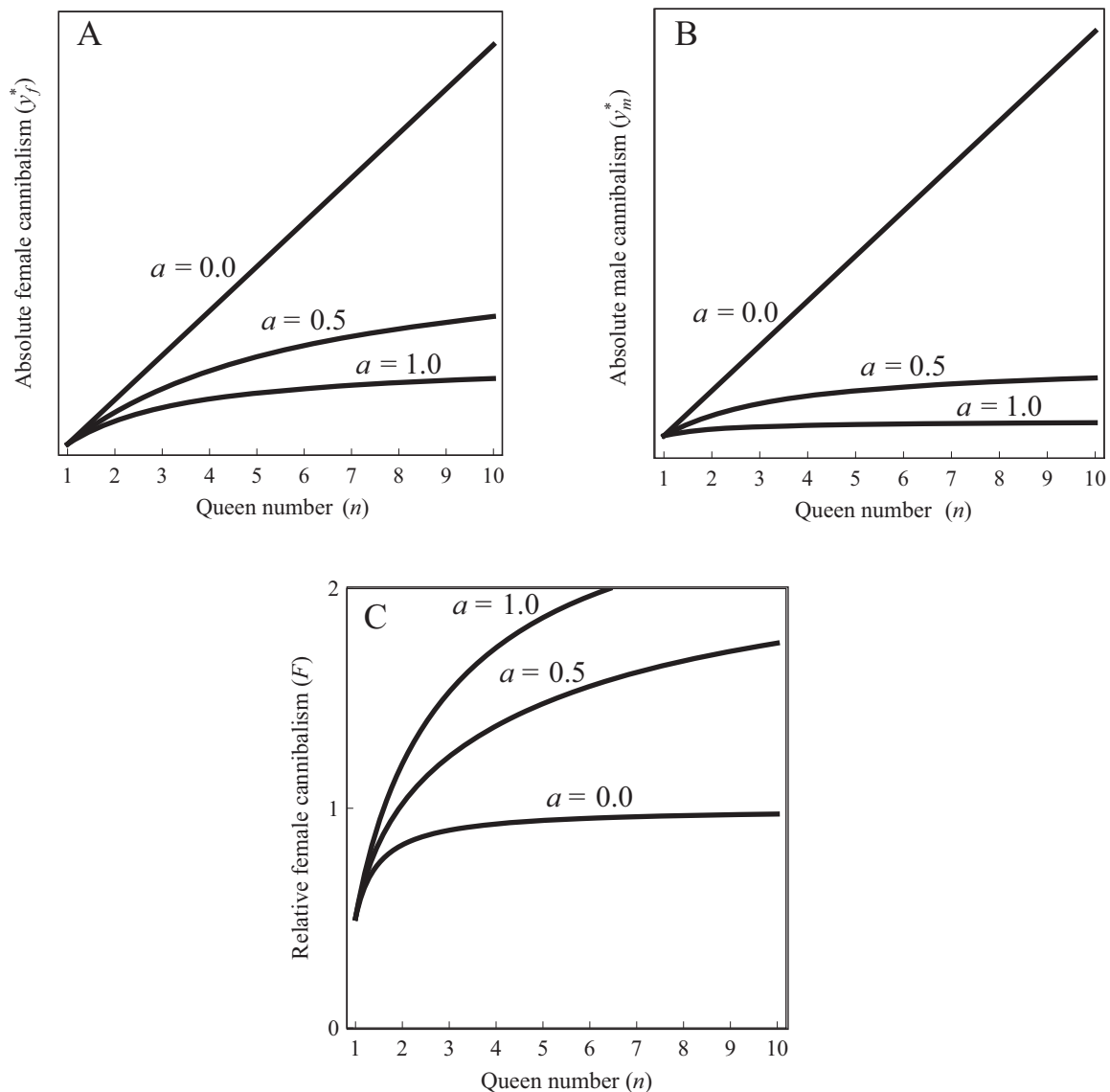
Figure 1: Predicted effects of relatedness and sex on cannibalism. *A*, *B*, Absolute cannibalism levels of female (*A*) and male (*B*) larvae are predicted to increase with decreasing relatedness, simulated by rising queen numbers and/or decreasing levels of consanguinity between queens. Lack of consanguinity strongly amplifies this effect ($a = 0$: queens unrelated; $a = 0.5$: equal mix of unrelated and full-sister queens; $a = 1$: queens are full sisters). *C*, Differences in cannibalism levels between the sexes are predicted to arise because of relatedness asymmetries between males and females (fig. A1, available online).

deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7k5p1 (Schultner et al. 2014).

Discussion

In a diverse range of organisms, social contact between offspring and their parents and siblings shapes the developmental environment, and offspring commonly display selfish behaviors in order to increase their resource share and influence future fitness (Trivers 1974; Godfray 1995;

Forbes 2011). Study of brood conflict has traditionally focused on small, temporary family units where offspring are closely related (e.g., birds; Mock and Parker 1997), which, while allowing assessment of the presence and intensity of selfish offspring behavior, provide only partial resolution of the importance of social structure in determining offspring selfishness. Social insect societies such as ant colonies, on the other hand, provide the perfect framework for testing broad inclusive-fitness predictions of selfish brood behavior because of large inter- and intraspecific

Table 1: Cannibalism levels in *Formica* ants

Species	No. nests	No. replicates	Relatedness	Sex ratio	Eggs eaten (%)	Incidence (%)
F. cinerea	3	70	−.06 ± .02	.73 ± .05	2.3 ± 1.6	10.0 ± 7.0
F. truncorum	5	273	.00 ± .01	.50 ± .04	4.5 ± 1.1	19.4 ± 4.7
F. aquilonia	12	329	.06 ± .02	.37 ± .03	3.0 ± .8	12.5 ± 3.6
F. fennica	10	220	.09 ± .02	.31 ± .02	.3 ± .3	1.4 ± 1.4
F. pressilabris	10	221	.21 ± .02	.98 ± .01	1.9 ± .8	9.5 ± 3.9
F. sanguinea	6	205	.36 ± .02	.63 ± .04	4.6 ± 1.3	18.5 ± 5.3
F. lemani	5	212	.40 ± .01	.18 ± .02	.5 ± .4	2.4 ± 2.0
F. fusca	8	204	.46 ± .03	.41 ± .03	1.4 ± .7	5.9 ± 3.2

Note: Each replicate represents one larva. Sex ratios range from 0 (all females) to 1 (all males). Relatedness, sex ratio, proportion of eggs eaten, and proportion of incidents are reported as means with 95% confidence intervals, calculated from the raw data. Negative relatedness values indicate that nestmates are less related to each other than they are to random individuals from the population.

variation in colony kin structures. However, most studies of evolutionary conflict in ants have focused on the behavior of adult females, who are already constrained in their respective roles, notably leaving workers with limited reproductive options (Wenseleers et al. 2004*b*).

Here, we provide a novel theoretical approach to brood conflict and a comprehensive empirical test of kin-selection theory by focusing explicitly on offspring behavior in the variable social environments of ant colonies. Using a kin-selection framework to model selfish larval egg cannibalism, we found that relatedness and larval sex can affect an individual's decision to cannibalize. In line with our theoretical predictions, *Formica* ant larvae were less

prone to cannibalize nestmate eggs when intrabrood relatedness was high. These results suggest that inclusive-fitness costs curb the evolution of selfishness in ant larvae. However, the effect of relatedness on cannibalism levels was strongly dependent on larval sex: while increasing relatedness led to a strong decline in cannibalism levels of female larvae, male larvae were less sensitive to variation in relatedness. In all species, large larvae engaged in cannibalism significantly more often than small larvae, indicating that developmental stage also plays an important role in determining the magnitude of this novel social conflict in ants.

Our model shows that sex-dependent differences in can-

Table 2: Global slope regression analysis of cannibalism levels in *Formica* ants

Covariate	Regression coefficient	P
Intercept (large *F. aquilonia*)	−2.3 (−3.0 to −1.7)	<.0001***
Species		<.0001***
F. cinerea	−1.3 (−2.3 to −.2)	
F. fennica	−2.2 (−3.5 to −.9)	
F. fusca	−.4 (−1.5 to .6)	
F. lemani	.3 (−1.6 to 1.0)	
F. pressilabris	−1.6 (−2.4 to −.8)	
F. sanguinea	−.05 (−.9 to .8)	
F. truncorum	.16 (−.5 to .8)	
Relatedness	−4.3 (−7.1 to −1.5)	.0029**
Sex	1.9 (1.2 to 2.7)	<.0001***
Larva size		<.0001***
Medium	−.34 (−.71 to .04)	
Small	−.96 (−1.5 to −.46)	
Relatedness × sex interaction	4.5 (1.4 to 7.6)	.0048**

Note: In the global slope model, nests were used as random factors, while species and size were implemented as categorical variables (see text). Consequently, their regression coefficients are contrasts against the baseline rate (intercept), which indicates presence of cannibalism in large *F. aquilonia* larvae. Regression coefficients are reported as maximum likelihood estimates and 95% confidence intervals. For different factor levels in species and larva-size covariates, joint *P* values are presented. These are based on results of likelihood ratio tests with appropriate degrees of freedom. For other parameters, *P* values are based on the Wald test.

** $P < .005$.

*** $P < .001$.
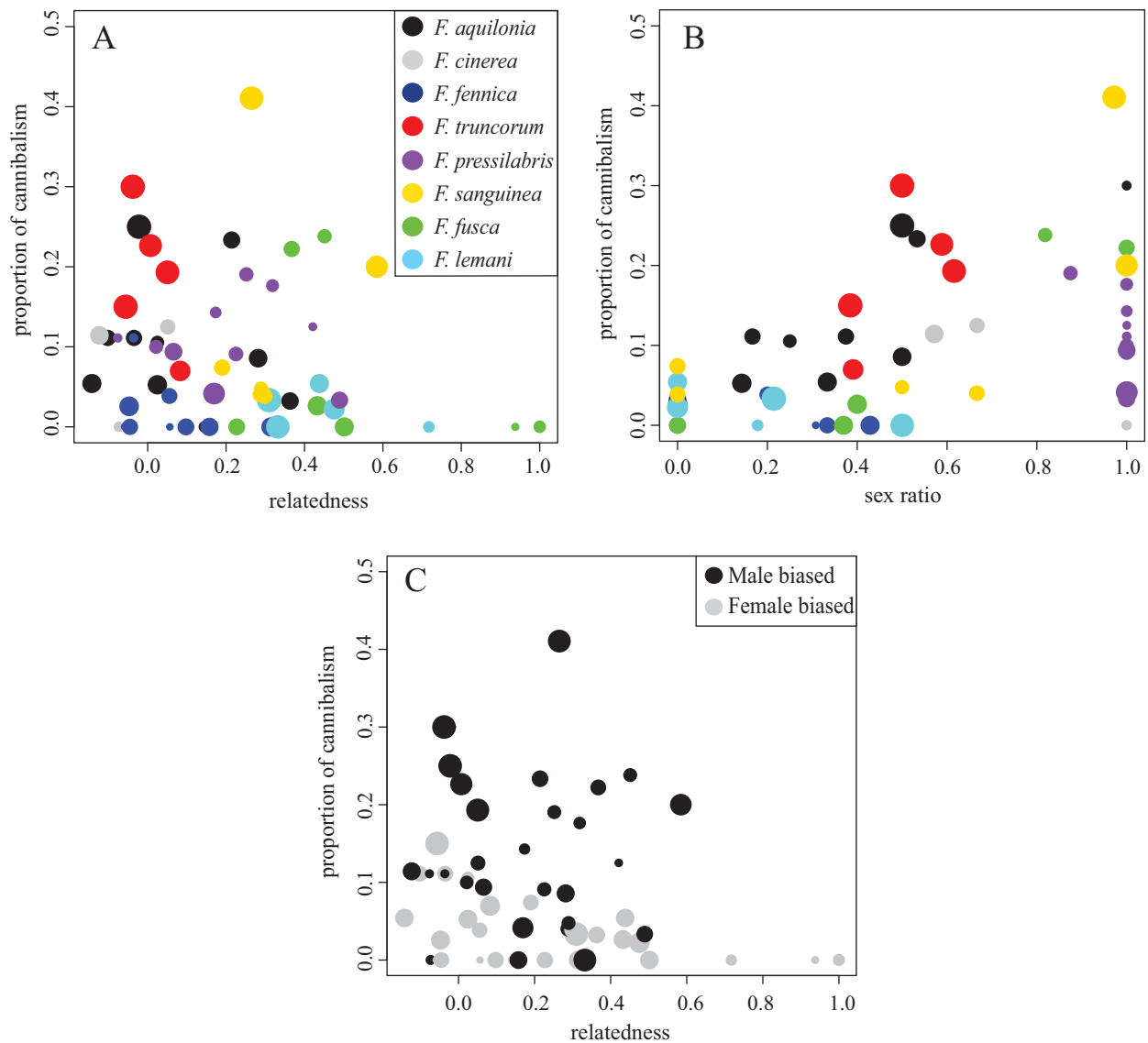
Figure 2: Measured effects of relatedness and sex on cannibalism. *A*, In line with our model's predictions, the proportion of cannibalism incidents in experiments increased significantly with decreasing relatedness. *B*, Cannibalism levels were significantly higher in nests with many male larvae, expressed as colony sex ratio, where 0 = all females and 1 = all males. *C*, We also found a significant positive interaction between relatedness and sex ratio, which indicates that male and female cannibalism levels respond differently to changes in relatedness. Points represent the mean proportion of cannibalism incidents in different nests, color coded by *Formica* species (*A*, *B*) or sex ratio (*C*). In *C*, nests were classified as male or female biased, depending on the sex ratio calculated from larva genotypes (>0.5: male biased; <0.5: female biased). Point size corresponds to the number of replicates for a particular nest (range: 1–60; median: 27).

nibalism propensity can be partly explained by relatedness asymmetries between males and females resulting from haplodiploid sex determination (Hamilton 1964). In particular, higher relatedness among females in single-queen colonies may disincline female larvae from engaging in selfish behavior. In broods composed of male and worker-destined female larvae, differences in cannibalism levels could furthermore arise as a result of lower average ben-

efits to female cannibals (e.g., because cannibalism carries only indirect fitness benefits for worker-destined larvae). In *Formica* ants, however, sexual brood is reared earlier in the season than worker brood (Bier 1954*b*), and the female larvae used in our experiments should thus all be under selection to develop into future queens (Dobata 2012). In addition to the sex of cannibal larvae, the sex of potential victim eggs may influence cannibalism predictions, for in-

stance, if competition among same-sex nestmates is high (e.g., in the case of local mate competition) and larvae thus benefit differentially from cannibalizing eggs of different sexes.

Overall, male cannibalism levels were higher across all relatedness levels in our experiments, indicating that cannibalism is generally more favored in males. Indeed, our model predicted that female : male cannibalism ratios can vary with sex-specific benefits (R'_f/ R'_m) and sex ratio (z, eq. [1]). In particular, if males benefit more from cannibalism than females ($R'_m > R'_f$), this leads to relatively male-biased cannibalism. Such sex-specific benefits have been found in ladybird larvae, where cannibalism has relatively greater effects on male survival and adult body size (Osawa 2002). In ants, males should be under selection to increase their nutrient intake if this leads to higher competitive ability or increased mating success, for example, through improved sperm quality or quantity (Wiernasz et al. 2001) or larger adult body size (Davidson 1982; Wiernasz et al. 1995; Abell et al. 1999). Alternatively, males may cannibalize relatively more to compensate for their higher metabolic rate (Boomsma and Isaaks 1985) or to overcome nutritional restriction through workers, which may arise in the course of queen-worker conflict over sex allocation (e.g., Trivers and Hare 1976; Craig 1980; Bourke and Franks 1995).

Our data suggest that larval egg cannibalism is mediated by kin selection. This raises the question of whether cannibalism levels represent an evolved response to average kin structures encountered by larvae or whether larvae plastically adjust levels of cannibalism to their present colony conditions. A plastic increase in offspring cannibalism and aggression in response to decreased genetic similarity has been found across taxa (Polis 1981; Elgar and Crespi 1992; Pfennig et al. 1993; Pfennig 1997; Giron et al. 2004; Dobler and Kölliker 2009). In spadefoot toads, for instance, cannibal tadpoles exhibit excellent discrimination abilities and consequently associate less often with sibling groups (Pfennig et al. 1993). Our analyses suggest that larvae can also display a plastic response to changes in relatedness and that levels of plasticity are similar across species. We previously showed that larvae of the ant *F. aquilonia* preferentially eat eggs from a foreign population, compared to sibling eggs (Schultner et al. 2013), which suggests that ant larvae possess the relevant discriminatory machinery to adjust behavior to their environment. Behavioral plasticity in response to local social structure is well known from adult ants, for example, when workers adjust colony sex ratio according to the mating frequency of their mother queen (Sundström et al. 1996) or begin producing male eggs in orphaned queenless nests (Miller and Ratnieks 2001; Helanterä and Sundström 2007). Still, the effect of relatedness on cannibalism levels in our study

may also reflect an evolved response to average relatedness levels within species. Understanding how relatedness modulates larval behavior will therefore require careful study of individual behavioral responses, discrimination abilities, and variation in kin structures.

In ants, cannibalism of viable eggs by adults and feeding of eggs to older larvae occur as a result of resource shortages (Wilson 1971; Sorensen et al. 1983; Heinze et al. 1999). Alignment of experimentally measured levels of cannibalism with theoretical predictions in our study suggests that cannibalism of viable eggs by larvae themselves may furthermore be a selfish means of influencing development. Specifically, larvae may engage in cannibalism to increase their survival (Schultner et al. 2013), shorten development time, remove competitors, or influence adult morphology, similar to cannibalistic offspring in other taxa (Osawa 1992, 2002; Wise 2006; Roy et al. 2007; Crossland et al. 2011; Vijendravarma et al. 2013). Cannibalism could be particularly beneficial if its nutritional effects influence future fitness, for example, by increasing a female's chance of developing into a queen (but see Rüger et al. 2007) or increasing an individual's competitive ability by positively influencing body size and morphology (Davidson 1982; Wiernasz et al. 1995, 2001; Bernasconi and Keller 1998; Abell et al. 1999; Wagner and Gordon 1999; Wiernasz and Cole 2003; Fjerdingstad and Keller 2004). Linking cannibalism to developmental processes and ultimately adult fitness is an important next step in understanding this novel conflict in ants.

Assessment of the cost of cannibalism on the colony level is one important aspect that could not be addressed within the scope of this study. In ants, selective forces acting on the colony as a whole can play a major part in shaping individual behavior. One example is when workers destroy eggs laid by other workers even in the absence of kinship benefits because this is less costly to the colony—in terms of productivity—than supporting egg-laying free riders (Ratnieks and Wenseleers 2005; Ratnieks et al. 2006; Wenseleers and Ratnieks 2006b). Cannibalism and other selfish larva behaviors, such as begging (Creemers et al. 2003; Kaptein et al. 2005), could prove similarly costly to the colony if they favor production of excess selfish queen larvae over that of workers (Ratnieks 2001; Amor et al. 2011) or lead to a general decrease in colony output in terms of worker and sexual production. In this case, workers should be under selection to prevent such behavior (Carlin 1988; Baroni Urbani 1991; Dobata 2012). Evidence for selection on worker behavior to prevent costly cannibalism comes from studies of brood-sorting behavior, which reveal that workers of some species put considerable effort into separating larvae from eggs (Le Masne 1953; Franks and Sendova-Franks 1992; Sendova-Franks et al. 2004). In *Hypoponera* ants, where larvae have been shown

to cannibalize other larvae, workers rapidly separate larvae brought into close contact during experiments (Rüger et al. 2007), confirming that worker behavior is adaptively shaped by brood conflict in these species.

In insect societies, larvae embody future generations of both sexuals and workers with individual fitness interests. At the same time, they represent the combined current reproductive investment of all the members in a colony. This makes brood a valuable commodity and a source of conflict within societies. We present a novel perspective on social conflict by investigating the selfish behavior of larvae themselves. We demonstrate how the enormous variation in kin structures in social insects facilitates broad comparative studies of a widespread evolutionary conflict and how exploring offspring behavior in social organisms can give insight into the general evolutionary constraints of competition.

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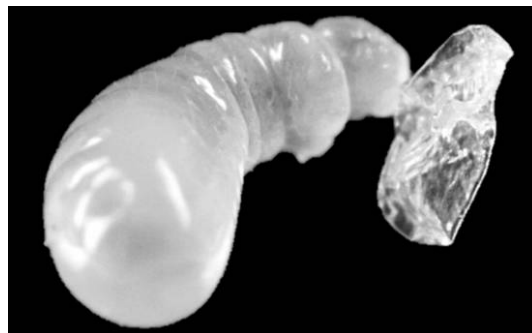
A larva of the ant species *Formica truncorum* cannibalizes an egg by piercing its shell and consuming the contents. Photo credit: Nick Bos.