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Genetic Constraints on Dishonesty and Caste Dimorphism in an Ant

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ABSTRACT: The ultimate causes of honest signaling remain a subject of debate, with questions remaining over the relative importance of costs and constraints. Signal costs may make dishonesty prohibitively expensive, while genetic constraints could make it impossible. We investigated honest signaling using full-sib analysis and parent-offspring regression in the ant *Lasius niger*, in which queens produce a cuticular hydrocarbon-based pheromone that signals fertility and inhibits worker reproduction and aggression. We found multiple lines of evidence that cuticular hydrocarbon production is genetically correlated with oogenesis and that the queen pheromone 3-methylhentriacontane and other 3-methylalkanes have strong genetic links with fertility relative to other cuticular hydrocarbons. These genetic correlations may maintain honesty in the face of directional selection on signaling and explain the putatively widespread use of cuticular hydrocarbons in fertility signaling across the social insects. We also found evidence for a positive genetic correlation for fertility between the castes; that is, the most fertile queens produced especially fertile workers. These results highlight that intercaste genetic correlations could constrain the evolution of queen-worker dimorphism, such that worker reproduction may sometimes reflect a nonadaptive "caste load" rather than positively selected cheating.

Keywords: cuticular hydrocarbons, fertility signal, handicap, index, *Lasius niger*, queen pheromone.

Introduction

Biological signals are actions or structures produced by a sender that elicit a response in one or more receivers (Maynard Smith and Harper 1995). By definition, signals have been shaped by selection to increase the fitness of the sender given the behavior of receivers, although these parties frequently have conflicting evolutionary interests (Andersson 1980; Keller and Nonacs 1993; Brilot and Johnstone 2003; Arnqvist 2006). It is therefore paradoxical that most biological signals appear to convey information reliably (sensu Searcy and Nowicki 2005) because senders often stand to benefit by producing a misleading signal, for example, one that exaggerates their hunger, fighting ability, or quality as a mate. Evolutionary theory has provided a number of potential resolutions to this problem, the best known of which is the handicap principle (Zahavi 1975; Zahavi and Zahavi 1997). This hypothesis states that senders benefit by producing a signal whose strength is proportional to the quality being advertised, because producing a signal of higher intensity would carry fitness costs that outweigh the benefit of the augmented signal. The handicap principle rests on the assumption that senders of differing qualities receive unequal fitness costs and/or benefits per unit of investment in signaling (Grafen 1990; Getty 2006).

Another hypothesis for the maintenance of signal honesty is the index hypothesis, which states that some signals ("indexes") are inherently honest because of evolutionary constraints that preclude dishonesty (Maynard Smith and Harper 1995, 2003). A supposed difference with the handicap principle is that dishonest signaling is impossible rather than simply suboptimal. For example, small-bodied acoustic signalers may be unable to produce calls with the same acoustic properties as larger individuals because of the physics of sound production, such that calls advertising large size cannot be faked (Reby and McComb 2003; Forstmeier et al. 2009; Charlton et al. 2011). Maynard Smith and Harper (1995, 2003) postulated that novel traits improving the relationship between sender quality and signal strength (e.g., traits that make the caller sound larger, such as a descended larynx) should quickly spread to fixation, such that their effect on signal reliability would be transient. Rephrased in evolutionary genetic terms, selection and genetic constraints might collude to exhaust genetic

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variation along the multivariate axis of selection (Blows and Hoffmann 2005). For example, alleles that improve the signal without negatively impacting other traits might all be fixed, such that the only remaining genetic variation in the signal shows antagonistic pleiotropy with nonsignaling traits, constraining any further response to selection and preserving the trait-signal relationship (Clark and Moore 1995; Van Homrigh et al. 2007; Hine et al. 2011).

Recently, Holman (2012) argued in favor of a pluralistic model invoking both costs and constraints in the maintenance of signal honesty. He showed that the cost of signaling optimally depends on the shape of the cost and benefit functions of investing in signaling (see also Lachmann et al. 2001) and the extent to which these functions are affected by the sender's underlying quality (e.g., genetic quality or phenotypic condition). Signals whose cost and benefit functions are constrained strongly by the sender's quality are predicted to evolve to be cheap, while signals whose expression is less dependent on sender quality evolve to be expensive. One can therefore assess a signal's "strategic cost" (the cost over and above the minimum required for adequate signal transmission; Guilford and Dawkins 1991) by testing whether its expression is strongly constrained by the sender's quality. Constraints on signal evolution are rarely studied explicitly, and more data are needed to assess the proximate and ultimate mechanisms responsible for signal honesty and to map and explain variation in strategic signaling costs (which are impossible to measure directly; Számadó 2011).

Here, we employ quantitative genetic methods (full-sib analysis and parent-offspring regression in a cross-fostered breeding design) to identify potential evolutionary constraints on signaling and queen-worker dimorphism, using the queen pheromone of the black garden ant *Lasius niger* as a model system (Holman et al. 2010*a*, 2010*b*; Holman 2012). Queens produce a blend of cuticular hydrocarbons (waxy substances covering the body surface) that differs from that of workers, and one queen-type hydrocarbon (3-methylhentriacontane or 3-MeC$_{31}$) was shown to be a pheromone that inhibits worker reproduction and aggression in experimental bioassays (Holman et al. 2010*b*). Some hydrocarbons, particularly 3-MeC$_{31}$, correlate with queen maturity and fecundity (Holman et al. 2010*a*), suggesting that hydrocarbons signal reproductive output or overall quality. A similar set of queen-type hydrocarbons predict whether a queen will be killed by workers in colonies containing supernumerary queens, and 3-MeC$_{31}$ was again the strongest predictor (Holman et al. 2010*a*). Such pheromone-dependent queen execution may be widespread in ants (West-Eberhard 1983; de Biseau et al. 2004; Wurm et al. 2010).

It is therefore puzzling why queens do not produce high quantities of pheromone at all times. Recent experiments showed that fecundity and 3-MeC$_{31}$ production are both affected by juvenile hormone but in opposite directions, demonstrating an endocrine-mediated trade-off between the two traits and a possible strategic cost of pheromone production (Holman 2012). This result also suggests that fecundity and queen pheromone production may be unable to evolve fully independently, because of their codependence on juvenile hormone. Estimating the genetic correlation between queen pheromones and fecundity can test this hypothesis: if two traits are genetically correlated, one trait cannot evolve without changing the other, potentially constraining both traits from reaching their optimal values. For example, if there were a positive genetic correlation between queen pheromone production and fecundity, queens would not be able to evolve increased pheromone production without also increasing their fecundity. Quantitative genetic parameters therefore provide insight into the strength and nature of evolutionary constraints acting on the evolution of queen pheromones and honest signals in general. Our results also allow us to assess the extent to which queen and worker traits could evolve independently under caste-specific selection (i.e., when different phenotypes are selected for in queens and workers).

Methods

Ant Collection and Cross-Fostering Design

Mated, wingless queen *Lasius niger* were collected during a mating flight in Copenhagen, Denmark, on July 21, 2010, and housed immediately in individual plastic cylinders (26 mm × 38 mm) with a ball of moist cotton wool but no food, mimicking natural colony foundation. Each queen was randomly paired with a group of 10 separately housed "foster workers"; all foster workers were collected from a single large wild colony in Copenhagen and were kept in 8 × 6 × 5-cm plastic boxes with a moist plaster base and ad lib. water, honey, and mealworms. Every 3–4 days, all eggs were transferred from the queen containers to the paired foster worker boxes using a soft paintbrush. Dead foster workers were replaced within 0–2 days, keeping the total number of foster workers constant.

The aims of this rearing procedure were to prevent post-hatching maternal effects from influencing the offspring phenotype and to standardize the biotic and abiotic rearing environment across families as much as possible. Using workers from a single colony as foster parents should greatly diminish the variation in focal worker phenotype caused by variation in the phenotype of the foster parents (indirect genetic and environmental effects; Linksvayer 2006), particularly because mean relatedness among nestmate workers is very high in Scandinavian *L. niger* populations (close to 0.75; Corley and Fjerdingstad 2011). Phenotypic covariation between maternal and offspring traits should therefore be largely attributable to genetic effects and prehatching maternal effects. A limitation of using a standardized environment and foster worker genotype is that we cannot be sure whether estimated quantitative genetic parameters would be the same in other rearing environments. For example, genetic effects could be plastic, depending on factors such as nutrition, colony size, or the genetic composition of rearing workers.

All queens were freeze-killed for later trait measurement 40 days after the mating flight. Their offspring were allowed to mature for an additional 23 days before being frozen. The foster workers were marked at the start of the experiment with a dot of enamel paint on the gaster in order to distinguish them from workers hatched from the lab-reared eggs, although this precaution proved unnecessary because all the lab-reared workers developed as nanitics (small workers often found in queens' first broods; Porter and Tschinkel 1986). Our experiment therefore incidentally suggests that the nanitic phenotype is controlled by a prehatching maternal effect, as opposed to a post-hatching effect such as a queen-specific rearing behavior (because the eggs were rapidly transferred to foster workers obtained from a mature colony). For example, newly mated *L. niger* queens might provision eggs with a hormone that stunts growth, as young harvester ant queens do to prevent their eggs developing into queens (Schwander et al. 2008).

Trait Measurement

Worker ovaries were dissected out, and the degree of ovarian activation was scored on a scale of 1–4 as in Holman et al. (2010*b*), as follows: (1) completely empty, (2) 1–2 very small eggs and/or developing nurse cell material, (3) 1–3 developing eggs in both ovaries or a large egg in one ovary, (4) well-developed eggs in both ovaries. The level of activation of queen ovaries was quantified by counting the number of full-sized eggs present in the ovaries. We also measured head size, scored as the shortest distance between the eyes, from digital photographs taken at ×40 magnification.

The cuticular hydrocarbons (CHCs) of queens and their offspring were analyzed by gas chromatography mass spectrometry (GC-MS) using similar methods to previous studies (Holman et al. 2010*a*, 2010*b*; Holman 2012). Hydrocarbons were extracted from queens by placing the ant in 150 μL of pentane in a 2-mL glass vial for 10 min, then transferring the extract to a 200-μL glass insert and allowing it to evaporate in a laminar flow cabinet. The extract was then diluted in 40 μL of pentane containing an internal standard (icosane; 1 ng μL^{-1}), which was later used to determine the absolute mass of each CHC present in the extract. Two microliters of queen extract was then injected into an Agilent 6890N gas chromatograph coupled to an Agilent 5375 mass spectrometer using an Agilent 7683B autosampler. Workers are much smaller, so they were extracted in 10 μL of pentane (again containing a 1 ng μL^{-1} icosane internal standard) in a 200-μL glass insert for 10 min; 3 μL of this extract was then manually injected into the GC-MS. The GC-MS parameters were the same as in Holman et al. (2010*a*, 2010*b*), and the data were analyzed with MSD Chemstation software. We included a greater number of CHC peaks (46 peaks) than in previous studies (Holman et al. 2010*a*; 2010*b*; Holman 2012) by splitting previously used peaks that contained multiple compounds with similar retention times into separate peaks, in order to improve resolution of the chemical profile. In particular, we avoided grouping hydrocarbons belonging to different functional groups wherever possible, because chemical families may have differing genetic parameters (van Zweden et al. 2010).

The number of worker offspring successfully phenotyped per family was 1–9 (total: 85; mean per family: 2.66 ± 0.41) and the number of families (and queens) was 32. Seven data points (4 workers and 3 queens) were removed from the hydrocarbon mass data set because the internal standards could not be measured clearly. All dissections and chemical analyses were performed in a random order to prevent bias.

Statistical Analysis

Since the mechanisms of hydrocarbon perception are poorly understood (e.g., the extent to which compounds are perceived individually or as blends is largely unknown), we investigated the hydrocarbon data set using two different approaches. First, hydrocarbon peaks were treated individually, using the mass of the peak (as calculated by comparison with the internal standard) as the response. Second, the chemical profile was considered as a blend. To this end, we transformed the raw peak area data by $\ln(A_i/g(A))$, where A_i is the area of the focal hydrocarbon peak and $g(A)$ is the geometric mean of all peak areas for that individual (Aitchison 1986), then reduced it by principal component analysis (PCA). Together, the first three principal components explained 86.7% of the variance in the original data set. We also tested for relationships between hydrocarbon family and certain genetic parameters by assigning the 46 hydrocarbon peaks to one of the following six categories: alkanes, alkenes, 3-methylalkanes, other methylalkanes, dimethylalkanes, and trimethylalkanes.

We quantified the heritability of worker traits, and genetic correlations among them, by comparing worker sibling groups. Heritability and its 95% confidence intervals (CIs) were estimated by bootstrap resampling (10^5 replicates) of the intraclass correlation coefficient (hereafter ICC; Lessells and Boag 1987; Falconer and Mackay 1996). The ICC is probably an overestimate of both broad-sense heritability (i.e., the proportion of phenotypic variance explained by additive and nonadditive genetic effects) and narrow-sense heritability (the proportion of phenotypic variance explained by additive genetic effects) because it potentially encompasses prefostering maternal effects, common environmental effects, dominance, and epistasis in addition to additive genetic variance. In particular, hydrocarbons are transferred among cohabiting ants (van Zweden et al. 2010; Bos et al. 2011), which should increase the chemical similarity within families. The ICC is also potentially biased downward, because the workers in our study are siblings rather than clones. The ICC is therefore likely to be positively correlated with genetic variance but should not be equated with it.

Pearson product moment correlations among family trait means were used to estimate the genetic correlation among worker traits. Although these measures might also over- or underestimate the true genetic correlation for similar reasons and their magnitudes should be treated with caution, it is improbable that the signs of significant Pearson correlations are opposite to those of the true genetic correlation (Agrawal et al. 2001; Astles et al. 2006). For example, any significant positive trait correlations provide good evidence for a positive genetic correlation, but the magnitude of the genetic correlation may be higher or lower than the reported value.

In comparisons of worker and queen trait values, we believe it is most biologically relevant to consider traits expressed in different castes as separate traits (or as the same trait expressed in a different environment), especially because the characters in this study show strongly caste-biased expression. We therefore regard trait covariance between queens and their worker offspring as an intercaste genetic correlation, similar to the cross-sex genetic correlation that is often used when comparing a trait expressed in males and females (Poissant et al. 2010). We estimated the intercaste genetic correlation as the Pearson product moment correlation between mean offspring and maternal trait values. This method might underestimate the true genetic correlation by a factor of two because mothers share only half their genes with their daughters; however, prefostering maternal effects could inflate (or even deflate) the estimate. We therefore present the Pearson correlations as an estimate of the true genetic correlations (as in Agrawal et al. 2001), illustrating their likely signs.

We present an effect size statistic and its 95% confidence intervals for all genetic estimates, illustrating the range of estimates that is consistent with the data. Inspection of the confidence intervals provides a measure of certainty in whether or not the true value might be zero, obviating the need to correct P values for multiple testing (see Nakagawa 2004; Nakagawa and Cuthill 2007). These intervals aid in the interpretation of both negative results (confidence in a negative result grows as the upper and lower bounds tend to zero) and positive results (confidence in a positive result grows as the upper and lower bounds move away from zero).

Results

Phenotypic Correlations among Queen Traits

The number of eggs in queens' ovaries was not significantly related to head size ($r = -0.31$, 95% CIs $= -0.59$ to 0.05, $t_{30} = 1.76$, $P = .09$). In contrast to expectations based on previous work (Holman et al. 2010*a*; Holman 2012), queen hydrocarbons were not significantly correlated with ovary development, whether peaks were treated individually or by principal component analysis (all $P >$ .053, df = 30). The number of queens sampled was substantially lower than in previous work focusing on queens only (Holman et al. 2010*a*; Holman 2012) and we used a different fertility measure, which may explain the discrepancy.

Intraclass Correlation Coefficients of Worker Traits

The intraclass correlation coefficient was estimated as 0.36 (95% CIs: 0.14–0.60) for worker ovary development and 0.50 for worker head size (0.24–0.78) and was high for the first three principal components of the hydrocarbon data (PC1: 0.63, 0.44–0.79; PC2: 0.64, 0.43–0.79; PC3: 0.84, 0.76–0.91), suggesting that direct genetic effects, indirect sibling effects (e.g., sibling-to-sibling hydrocarbon transfer) and prefostering maternal effects together explain substantial variation in these traits. When considering the masses of individual hydrocarbon peaks, we found some evidence that the chain length of a hydrocarbon predicted its ICC. There was a marginally nonsignificant negative relationship between chain length and the ICC (fig. A1; table A1, available online; $t_{44} = 1.86$, $P = .069$). If this effect is genuine, it implies that long-chained hydrocarbons are less easily transferred among siblings sharing a nest, have a weaker genetic component, and/or are less strongly affected by prefostering maternal effects.

Linear alkanes also had a significantly lower ICC than all other hydrocarbon families (table A1; planned contrast: $t_{44} = 2.06$, $P = .046$), consistent with a previous finding that linear alkanes have lower broad-sense heritability and are less readily transferred among nestmates than other types of hydrocarbons in *Formica rufibarbis* ants (van Zweden et al. 2010).

Genetic Correlations among Worker Traits

There was a significantly positive correlation between the family mean trait values for worker head size and ovary development ($r = 0.26$, 95% CIs = 0.036–0.45, $t_{77} = 2.32$, $P = .023$), implying that worker body size and fertility are genetically correlated.

The family mean values of hydrocarbon PC1, PC2, and PC3 were all uncorrelated with ovary development (all $P_{30} > .35$). However, the masses of seven individual peaks were positively correlated with ovaries (table A2, available online): these included 3-MeC$_{27}$ and 3-MeC$_{29}$. The queen pheromone 3-MeC$_{31}$ was not significantly related to ovary development, though the statistically supported range of effect sizes was predominantly positive, suggesting that we have low confidence in the null hypothesis ($r = 0.21$, 95% CIs = −0.05–0.60, $t_{29} = 1.76$, $P = .09$). The other significant peaks were dimethylalkanes and trimethylalkanes (table A2). There was also a negative relationship between the chain length of a hydrocarbon and the genetic correlation between that hydrocarbon and ovary development: in other words, shorter hydrocarbons showed evidence of having a more positive genetic correlation with ovaries (fig. 1; linear regression: $t_{44} = 4.25$, $P = .0001$). Additionally, the estimated genetic correlations with ovaries were significantly higher for 3-methylalkanes than for alkanes (contrast: $t_{40} = 2.15$, $P = .038$) and other types of methylalkanes ($t_{40} = 2.17$, $P = .036$), and borderline nonsignificantly higher than for alkenes ($t_{40} = 1.99$, $P = .053$). Lastly, the signs of 45/46 of the estimated correlations between individual hydrocarbons and ovaries were positive (the exception was C$_{35:1}$; table A2), further suggesting that the production of eggs and hydrocarbons are genetically correlated.

Intercaste Genetic Correlations for the Same Trait

The number of eggs in a queen's ovaries predicted the mean ovarian development of her worker offspring (fig. 2*A*; $r = 0.39$; 95% CIs = 0.05–0.65, $t_{30} = 2.34$, $P = .026$). This result is consistent with a positive intercaste genetic correlation for ovary development and/or a prefostering maternal effect. However, we note that queen egg number was unrelated to worker size (see below), ruling out the plausible possibility that fertile queens made larger workers via a maternal effect (Schwander et al. 2008), in turn increasing worker fertility (because larger workers are more fertile; see above).

Queen head size did not significantly predict mean offspring head size, consistent with a weak or nonexistent intercaste genetic correlation for head size ($r = 0.21$, 95% CIs = −0.15–0.51, $t_{30} = 1.17$, $P = .25$).

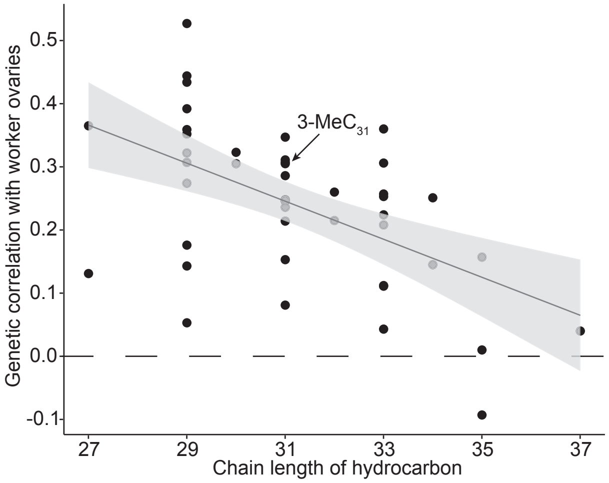

Figure 1: Chain length of a hydrocarbon significantly predicted the strength of its genetic correlation with fertility (estimated by comparing 32 worker sibling groups). Almost all hydrocarbons were positively genetically correlated with ovaries, though these correlations were not statistically significant for the majority of hydrocarbons. The gray area shows 95% confidence limits on the regression line.

The hydrocarbon profile of queens (as measured by PCA) did not significantly predict that of their worker offspring. However, inspection of the effect size 95% CIs suggests that we cannot confidently rule out some degree of parent-offspring similarity. Although neither result was statistically significant, queens with high values of PC2 tended to produce workers with low values of PC2 ($r = -0.31$; 95% CIs = −0.59–0.05, $t_{30} = 1.76$, $P = .088$), while maternal and offspring values were positively related for PC3 ($r = 0.33$; 95% CIs = −0.025–0.60, $t_{30} = 1.89$, $P = .068$). PC2 described the relative abundance of hydrocarbons previously found to be overexpressed by queens (Holman et al. 2010*b*) and augmented by juvenile hormone treatment (Holman 2012), namely linear alkanes, 3-methylalkanes, and the alkene C$_{31:1}$, while PC3 described the relative abundance of long-chained hydrocarbons (table A3, available online). We therefore found a nonsignificant trend for queens with particularly queen-like hydrocarbons to produce workers with especially worker-like hydrocarbons and for queens with long-chain hydrocarbons to produce workers that also had long-chain hydrocarbons.

When analyzing the mass of individual CHC peaks, only two peaks were significantly positively correlated in mothers and their offspring; these were 7,11,15-triMeC$_{31}$ and C$_{35:1}$ (table A4, available online). However, the estimated intercaste genetic correlations were significantly higher for

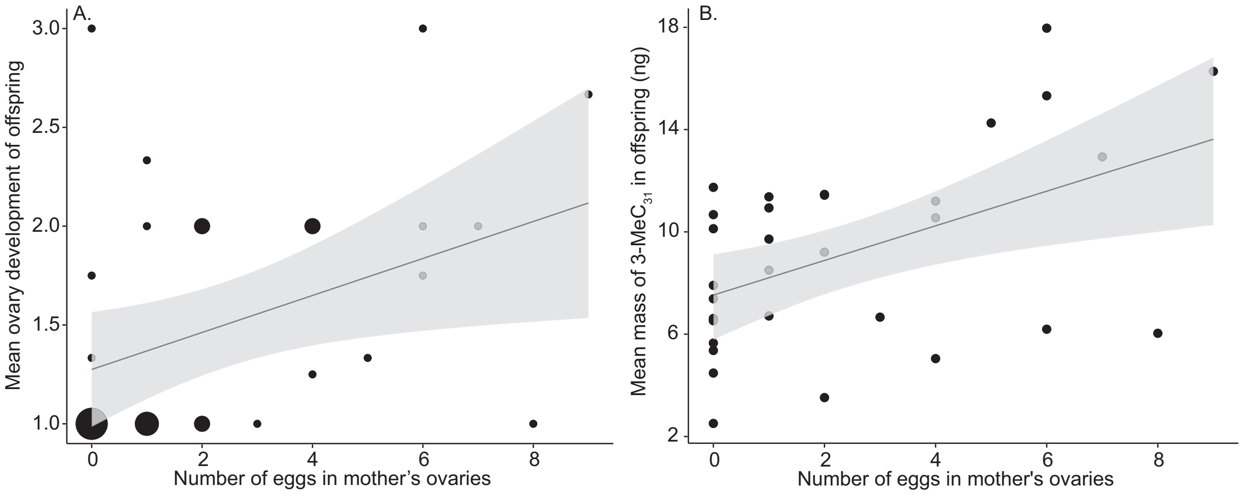

Figure 2: Queens with many eggs in their ovaries produced workers with significantly more well-developed ovaries (*A*) and a higher mean mass of the queen pheromone 3-MeC$_{31}$ on the cuticle (*B*), consistent with a positive intercaste genetic correlation for fertility and genetic links between fertility and queen pheromone production, respectively ($n = 32$ families). The large points in *A* show overplotting, with points of increasing size representing 1, 2, 4, and 7 data points, respectively.

3-methylalkanes and alkenes relative to alkanes and dimethylalkanes (table A4; contrasts: all $p_{40} < 0.025$), again consistent with differences in the heritabilities of different hydrocarbon families.

Intercaste Genetic Correlations among Different Traits

Queen egg number did not predict worker head size, though there was a nonsignificant trend for a positive correlation ($r = 0.34$, 95% CIs = −0.011–0.61, $t_{30} = 1.97$, $P = .058$). For the reciprocal comparison, queen head size and worker ovarian development, there was no relationship ($r = -0.11$, 95% CIs = −0.44–0.25, $t_{30} = 0.61$, $P = .54$).

The number of eggs in queens' ovaries was significantly positively correlated with the mass of eight cuticular hydrocarbon peaks in their worker offspring (table A5, available online). Of these, the queen pheromone 3-MeC$_{31}$ showed an especially strong correlation (fig. 2*B*; $r = 0.49$, 95% CIs = 0.16–0.72, $t_{30} = 3.00$, $P = .005$), suggesting a genetic correlation between fertility in queens and queen pheromone production in workers. The other peaks were 7,11-MeC$_{29}$, 3,9- and 3,11-diMeC$_{29}$, and five trimethylalkanes. The signs of 45/46 of the estimated correlations were positive (the exception was again C$_{35:1}$; table A5), providing further evidence that the production of eggs and hydrocarbons is genetically correlated. In a similar fashion to the worker data (cf. figs. 1, 3), the carbon chain length of a hydrocarbon was a significant predictor of its genetic correlation with ovaries (fig. 3; linear effect of chain length: $t_{43} = 2.29$, $P = .027$), though this time there was also a significant quadratic effect of chain length ($t_{43} = 2.41$, $P = .021$). This result suggests that hydrocarbons of intermediate chain length are more positively genetically correlated with fertility than are long- and short-chained hydrocarbons.

If we instead estimated the genetic correlations between the mean trait value for worker ovaries and the mass of

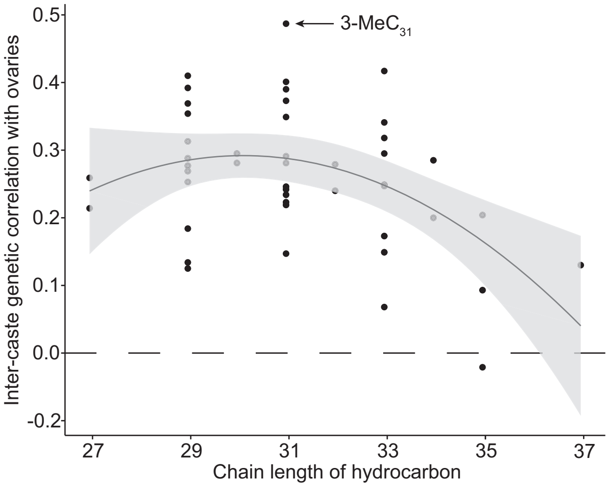

Figure 3: There was a significant, nonlinear relationship between the chain length of a hydrocarbon and its intercaste genetic correlation with ovary activation (as measured by comparing the number of eggs in queens' ovaries with worker hydrocarbon mass; $n = 32$ families). Almost all hydrocarbons showed a positive genetic correlation with ovaries, and the queen pheromone 3-MeC$_{31}$ had a particularly strongly positive genetic correlation.

each CHC produced by the mother queen, only one correlation was significantly different from zero (table A6, available online). Maternal mass of 5,9,13-triMeC_{33} was negatively correlated with worker ovary development ($r = -0.37$, 95% CIs $= -0.64$ to -0.19, $t_{29} = 2.15$, $P = .04$).

Discussion

The data provide evidence that fertility and hydrocarbon production are heritable but not genetically independent in the ant *Lasius niger*. These genetic relationships may impose evolutionary constraints on the evolution of both fertility signaling and queen-worker dimorphism and thereby contribute to the maintenance of honest signaling and worker reproduction.

We found evidence for a significant genetic correlation between ovary activation and the 3-methylalkanes 3-MeC_{27} and 3-MeC_{29} when comparing worker families; there was also a nonsignificant trend for a positive genetic correlation between ovaries and the queen pheromone 3-MeC_{31}. Additionally, we found a strong, significant intercaste genetic correlation between the mass of the queen pheromone 3-MeC_{31} and ovary development when comparing maternal fertility with offspring 3-MeC_{31} production. Together, these findings suggest that 3-MeC_{31} and other 3-methylalkanes signal a genetic tendency for high fertility, in addition to advertising an individual's current fertility level as found previously (Holman et al. 2010*a*).

A genetic correlation between queen pheromone production and fertility could constrain the evolution of dishonest signaling. If queens were under directional selection to produce more queen pheromone (West-Eberhard 1983), for example, to reduce their chance of being executed (Sommer and Hölldobler 1995; Holman et al. 2010*a*; Wurm et al. 2010), solicit a great share of workers' attention (Hannonen et al. 2002), or more effectively inhibit worker reproduction (Holman et al. 2010*b*), any response to selection for increased queen pheromone production would produce a correlated response in queen fertility. The link between fertility and the pheromone would therefore be maintained, preserving signal honesty, and continued exaggeration of the pheromone would be limited by concurrent selection on fertility (Blows and Hoffmann 2005).

Our hypothesis that genetic constraints may maintain signal honesty over evolutionary time is applicable to many other taxa and signaling systems. For example, sexually selected acoustic signals are strongly genetically correlated with body size in zebra finches (Forstmeier et al. 2009) and hissing cockroaches (Clark and Moore 1995), suggesting that many polymorphic loci affecting song or hissing also affect size. Therefore, these signals cannot easily evolve without a corresponding change in size, preserving signal honesty. Likewise, Emlen et al. (2012) showed experimentally that rhinoceros beetles' horns are especially sensitive to perturbations of the insulin/IGF pathway, making them strong predictors of overall condition. They present the intriguing hypothesis that the proximate mechanism by which horn size was exaggerated in ancestral small-horned beetles, namely elevated sensitivity of the horn to growth factors, now ensures its honesty across the population. This hypothesis also implies that there should be little genetic variation for horn size that is independent of condition, ensuring that condition and horn size remain correlated over evolutionary time. A final example is provided by green beards, which are loci that produce three traits: a signal, a preference for that signal, and a social trait (reviewed in Gardner and West 2010). The "beard" signal is an honest indicator of the social trait because a single locus produces both traits, that is, there is a perfect genetic correlation. Populations of green beards are therefore thought to be stable unless "false beards" that produce the signal but do not perform the social behavior are able to evolve (Gardner and West 2010). Therefore, genetic constraints that prevent the decoupling of the beard and the social trait are essential for long-term honesty. As in the rhinoceros beetle example, such a constraint may arise from the proximate mechanism. Two known green beard loci in microorganisms produce cell adhesion proteins that are able to bind to the same proteins expressed on other cells (Queller et al. 2003; Smukalla et al. 2008). Therefore, producing the signal (the adhesion protein) necessitates that the bearer performs the social behavior (binding), ensuring honesty.

The comparatively strong genetic link between 3-methylalkanes and ovaries may also explain why 3-MeC_{31} is a queen pheromone in *L. niger*, as opposed to some other hydrocarbon. Tinbergen (1952) suggested that signals represent "ritualized" forms of preexisting cues associated with a given behavior or trait. If some hydrocarbons were better predictors of fertility than others at the origin of sociality, for example, because their synthesis was especially affected by changes in reproductive physiology, ancestral workers might have evolved to respond to these hydrocarbons when performing behaviors that are only beneficial in the presence of a fertile relative (e.g., deciding whether to remain sterile). Senders might then be selected to exaggerate the cue in order to increase the receiver's response, causing the cue to become a signal (by definition). The current data are consistent with the ritualization hypothesis because the strong genetic correlations between 3-methylalkanes and fertility suggest that these traits are especially genetically or physiologically related. Moreover, Holman (2012) found that juvenile hormone treatment affected both fertility and the production of the queen-like hydrocarbons C_{27}, C_{29}, 3-MeC_{29}, and 3-MeC_{31}, suggesting either causal links between the production of eggs and the expression of cuticular hydrocarbons or common endocrine regulation of both traits (Fan et al. 2002; Blomquist and Bagnères 2010).

We also found that the genetic correlation with ovaries was highest for hydrocarbons with short or intermediate chain length, both when contrasting worker families and when comparing queens with their offspring. There was also a marginally nonsignificant relationship between the relative amount of long-chain hydrocarbons produced by queens and their offspring. These results are consistent with the presence of additive genetic variation for the elongase enzymes that control hydrocarbon chain length (Blomquist and Bagnères 2010) and imply genetic links between elongase enzymes and fertility. If shorter-chained hydrocarbons were indeed the most genetically linked to fertility across taxa, we might expect queens to have shorter hydrocarbons than workers in most ant species. There is mixed support for this prediction: queens possess more relatively short-chained hydrocarbons than workers in the ants *Camponotus floridanus* (Endler et al. 2004) and *Linepithema humile* (de Biseau et al. 2004), but long-chain hydrocarbons denote higher fertility in *Harpegnathos saltator* (Liebig et al. 2000), and mean chain length increases during reproductive development in queen *L. niger* (Holman et al. 2010*a*). A negative genetic correlation between fertility and hydrocarbon chain length could also influence the evolution of chain length; Kwan and Rundle (2010) found that desiccation-stressed *Drosophila melanogaster* populations evolved longer hydrocarbons (suggesting that long hydrocarbons are better for desiccation resistance), and our results imply that selection on fecundity might be one factor favoring short hydrocarbons in benign conditions.

An unanticipated but important result of the study was that we found evidence for an intercaste genetic correlation for ovary development. This result suggests a possible constraint on the evolution of queen reproductive output: selection for increased queen fertility would likely be accompanied by an increase in worker fertility, which is thought to be detrimental to queen and colony productivity (reviewed in Wenseleers et al. 2004). Our data therefore suggest the existence of a "caste load," comparable to the "gender load" imposed by intralocus sexual conflict (Prasad et al. 2007; Connallon et al. 2010; Innocenti and Morrow 2010), in which a trait cannot be simultaneously optimized in queens and workers because of intercaste genetic correlations and caste-specific selection pressures. Various authors have proposed that caste-biased gene expression should evolve in order to weaken the intercaste genetic correlation for traits in which selection differs between castes (West-Eberhard 1996; Gadagkar 1997; Linksvayer and Wade 2005), but our data suggest that selection has not achieved total "genetic release" (Gadagkar 1997) of queen and worker fertility in *L. niger*. Hints at the extent of genetic release in *L. niger* were provided by a transcriptomic study, which found that 16/369 of the examined transcripts showed caste-specific expression (Graff et al. 2007). Queens had more of two transcripts putatively associated with oogenesis, though these transcripts were also present in workers (e.g., 11 times lower for a vitellogenin homolog).

The observed intercaste genetic correlation for ovary development also supports the hypothesis that worker reproduction may partly result from maladaptation, in contrast to the widely accepted but largely untested hypothesis that fertile workers are selfish "cheaters" (Smith et al. 2009) maintained at a given level by selection (Wenseleers et al. 2004). Using a theoretical model, Van Dyken et al. (2011) showed that a sizeable number of cheaters (e.g., reproductive workers) can exist at mutation-selection balance in a cooperative system even when cheating lowers the cheater's inclusive fitness. In light of our data, we propose a related but distinct hypothesis, that worker reproduction is maintained because it cannot be eliminated without causing a costly decline in queen fecundity. That is, alleles that cause a maladaptive increase in fertility when expressed in workers might experience net positive selection if they have sufficiently beneficial effects on queen fertility. Genetic variation along the putative multivariate axis of selection (i.e., higher queen fertility and lower worker fertility) might well be exhausted (Blows and Hoffmann 2005), especially because intragroup relatedness is high in many social insects (including our study species; Corley and Fjerdingstad 2011), strengthening the response to selection on worker traits (see Van Dyken et al. 2011). The proximate and ultimate causes of worker reproduction remain an exciting topic for future work. Empirical work should attempt to evaluate the strength and generality of the caste load in advanced and primitively eusocial species. Theoretical models could benefit by incorporating (evolvable) genetic links between queen and worker traits, and by considering nonadaptive explanations for patterns of reproductive skew in animal societies.

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Worker tends to *Lasius niger* queen about to lift off in search of a new colony. Photo by Stuart Hogton; www.hogton.com.