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COSTS AND CONSTRAINTS CONSPIRE TO PRODUCE HONEST SIGNALING: INSIGHTS FROM AN ANT QUEEN PHEROMONE

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Signal costs and evolutionary constraints have both been proposed as ultimate explanations for the ubiquity of honest signaling, but the interface between these two factors is unclear. Here, I propose a pluralistic interpretation, and use game theory to demonstrate that evolutionary constraints determine whether signals evolve to be costly or cheap. Specifically, when the costs or benefits of signaling are strongly influenced by the sender's quality, low-cost signals evolve. The model reaffirms that cheap and costly signals can both be honest, and predicts that expensive signals should have more positive allometric slopes than cheap ones. The new framework is applied to an experimental study of an ant queen pheromone that honestly signals fecundity. Juvenile hormone was found to have opposing, dose-dependent effects on pheromone production and fecundity and was fatal at high doses, indicating that endocrine-mediated trade-offs preclude dishonesty. Several lines of evidence suggest that the realized cost of pheromone production may be nontrivial, and the antagonistic effects of juvenile hormone indicate the presence of significant evolutionary constraints. I conclude that the honesty of queen pheromones and other signals is likely enforced by both the cost of dishonesty and a suite of evolutionary constraints.

KEY WORDS: **Cuticular hydrocarbons, fertility signal, handicap, index, *Lasius niger*.**

A biological signal may be defined as an evolved action or structure that increases the sender's fitness by eliciting a response in one or more receivers (Maynard Smith and Harper 1995). The majority of biological signals are thought to transfer reliable information; that is, the presence or intensity of the signal correlates with some other trait (Searcy and Nowicki 2005). The prevailing explanation for honest signaling is that dishonesty is prohibitively costly (the handicap principle; Zahavi 1975; Grafen 1990; Zahavi and Zahavi 1997). A proposed alternative is that signals are inexpensive, but are kept honest by evolutionary and mechanical constraints (e.g., Maynard Smith and Harper 1995; Lappin et al. 2006; Forstmeier et al. 2009; Castellano and Cermelli 2010; Számadó 2011). Maynard Smith and Harper (1995; 2003) use the term "index" to describe signals that have no strategic costs (i.e., costs over and above the minimum necessary for signal transmission), but are nevertheless honest because there is an unbreakable, causal link between the signal and the quality it advertises. Putative indices include vocalizations of some mammals (Reby and McComb 2003; Charlton et al. 2011), anurans (Searcy and Nowicki 2005), and birds (Forstmeier et al. 2009) that signal body size to conspecifics. Certain acoustic properties of a call (e.g., formant dispersion) are thought to be limited by the size of the

caller's vocal apparatus (Reby and McComb 2003; Charlton et al. 2011), such that genetic constraints (e.g., a lack of evolvability for throat size) and mechanical constraints (physical limits imposed by throat dimensions) might enforce the link between the signal and body size.

A key difference between the handicap principle and the index hypothesis is the presence or absence of strategic costs (Maynard Smith and Harper 2003), and researchers have attempted to classify signals as either handicaps or indices based on their apparent costs and constraints. However, this dichotomous view obscures the interdependence of costs and constraints in the evolution of honest signaling. Signal costs ensure honesty by providing each signaler with its own optimum signaling level, determined by the individual-specific cost and benefit functions of signaling (e.g., Grafen 1990; Getty 1998; Kotiaho 2001; Lachmann et al. 2001; Számadó 2011). For costs to ensure that a signal honestly predicts some quality of the signaler, theory has shown that high-quality signalers must receive greater marginal fitness returns from investment in signaling (Grafen 1990; Getty 1998); for example, they must produce a better signal or suffer lower costs for a given investment. However, such quality-dependent signaling is conceptually indistinct from the index hypothesis, which posits that signals are honest because they are constrained by the sender's quality. I will argue that handicaps and indices are facets of the same process, and that costs and constraints work together to produce a continuum of honest signal types rather than a dichotomy.

Using a game theoretical model, I place signaling costs and constraints in a common framework. The model assumes that investment in signaling has costs, namely a reduction in another fitness trait, and benefits, namely an improved signal that provides fitness returns through interactions with receivers. The model also specifies a continuous range of signal constraints. I define signal constraints as the degree to which a sender's quality limits the range of signal strengths it can produce and/or affects the costliness of a given level of signaling. Under this definition, investment in highly constrained signals produces rapidly diminishing returns in signal strength and/or rapidly increasing costs for other fitness traits for a sender of any given quality. For example, many acoustic signalers can control the pitch of their calls by contracting muscles that change the size and shape of the vocal apparatus (Searcy and Nowicki 2005; Charlton et al. 2011), but the change in pitch with increasing effort should rapidly saturate and become limited by the caller's body size (high constraints). By contrast, in animals in which male mating success increases linearly with song duration (e.g., Collins et al. 1994; Holzer et al. 2003), males of all qualities might be theoretically capable of producing a large range of song lengths (low constraints). The strength of such constraints will be shown to profoundly affect signal evolution.

The model is followed by experiments on a recently discovered ant queen pheromone, which investigate the relative importance of costs and constraints in a signaling system in which there is a long-running debate over the ultimate factors ensuring honesty (Keller and Nonacs 1993; Heinze and d'Ettorre 2009).

The Model

Individuals are assumed to vary in some quality q ($0 \leq q \leq 1$), a trait that receivers cannot directly observe, but which they would be selected to respond to preferentially if they could (as in Grafen 1990); q may be thought of as genetic quality, condition, fighting ability, or capacity to provide parental care. Individuals must divide their resources between a signaling trait s or to some other fitness trait t ($0 \leq t \leq 1$), which could represent survival probability or fecundity, by choosing a signaling strategy x ($0 \leq x \leq 1$). Higher values of x represent greater investment in signaling. Quality q may affect how x translates into s and t, for example, if high-quality individuals produce better signals for a given investment or are better able to tolerate signaling costs.

We may write the strength of the signal of an individual of quality q playing strategy x as

$$s(x, q) = (1 + c_s q)\, x^{\left(\frac{b_s}{1+k_s q}\right)}, \tag{1}$$

where b_s ($b_s > 0$) determines the shape of the relationship between x and s (Fig. 1A). The parameters c_s and k_s represent constraints: their magnitude determines the extent to which an individual's quality affects its signal. c_s determines the effect of q on the maximum signal that an individual can produce (when $x = 1$), while k_s determines the effect of q on the shape of the relationship between x and s (Fig. 1A). When $c_s > 0$, high-quality individuals produce a stronger signal for any given investment, while $k_s > 0$ means that high-quality individuals produce a signal that is closer to their personal maximum for any intermediate investment (i.e., $0 < x < 1$).

The other fitness trait t declines with x from its maximum value of 1 via the function

$$t(x, q) = 1 - x^{b_t(1+k_t q)}, \tag{2}$$

where b_t ($b_t > 0$) determines the shape of the relationship between x and t (Fig. 1B). As before, the parameter k_t represents constraints affecting the extent to which q influences the shape of the relationship between x and t (Fig. 1B). When $k_t > 0$, high-quality individuals lose a lower proportion of trait t for any intermediate investment than do low-quality individuals.

I assume that fitness returns from s and t are multiplicative, which forces all individuals to signal, and precludes them from reducing trait t to zero. I additionally assume that the fitness increase provided by signaling is equal to $(s/\bar{s})^r$, where s is the focal individual's signal, $\bar{s}$ is the population mean signal, and r is a scaling factor ($r > 0$). Large r means that the fitness returns from

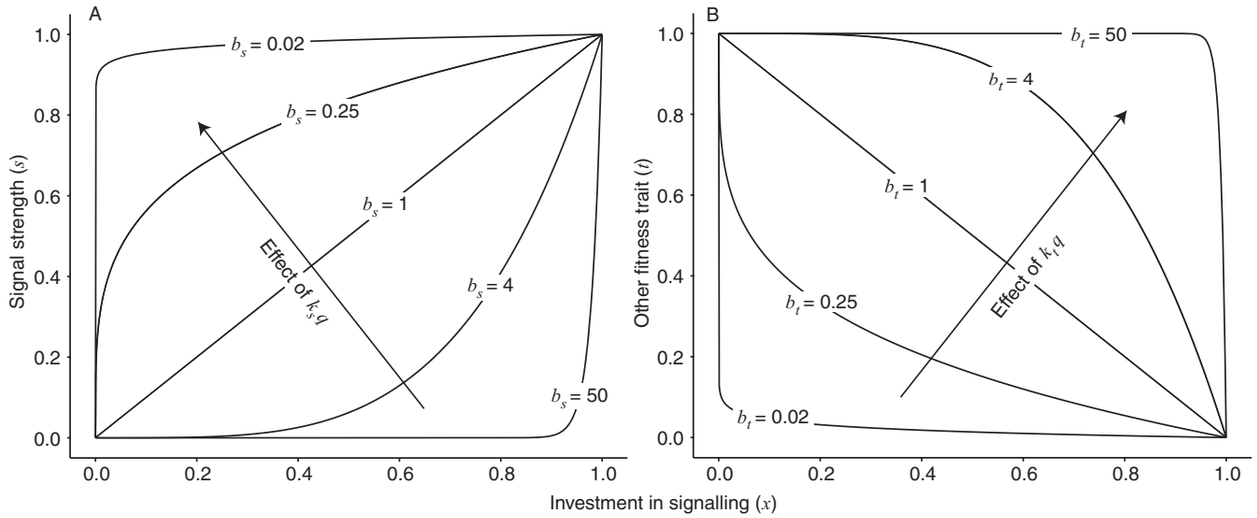

Figure 1. **Investment in signaling (x) may yield a variety of returns and costs, and may depend on the quality of the signaler (q). (A) The parameter b_s determines whether investment in signaling yields decreasing, linear, or increasing returns in the strength of the signal s. The arrow shows the effect of q on the shape of the relationship between x and s when $k_s > 0$. The figure assumes $c_s = 0$; when $c_s > 0$, signal strength runs from 0 at $x = 0$ to $1 + c_s q$ at $x = 1$. (B) The parameter b_t determines whether investment in signaling produces decreasing, linear, or increasing reductions in the other fitness trait t. The arrow shows the effect of q on the shape of the relationship between x and t when $k_t > 0$.**

signaling accelerate with increasing $s/\bar{s}$, small r denotes decelerating fitness returns, and $r = 1$ means linear fitness returns. For simplicity I assume that fitness increases linearly with t. To find the evolutionarily stable investment in signaling for an individual of quality q, I consider the fitness of a mutant of quality q playing strategy x in a population with mean signal strength $\bar{s}$, which is given by

$$w(x, q) = t(x, q) * \left(\frac{s(x, q)}{\bar{s}}\right)^r. \quad (3)$$

A fitness maximum was found by setting $\partial w/\partial x = 0$ (investigation of the second derivative confirmed that this was a maximum). Solving for x gives the evolutionarily stable strategy (ESS), $x^*(q)$:

$$x^*(q) = \left(\frac{b_s r}{b_s r + b_t(1 + k_s q)(1 + k_t q)}\right)^{\frac{1}{b_t(1+k_t q)}}. \quad (4)$$

FACTORS AFFECTING THE OPTIMAL INVESTMENT IN SIGNALING

Inspection of equation (4) reveals that high b_s, b_t, k_t, and r select for greater investment in signaling, while high k_s selects for lower investment in signaling. The shapes of the benefit and cost functions of signaling are therefore key predictors of the ESS (see also Lachmann et al. 2001): when small investments in signaling yield comparatively weak returns (high b_s), individuals are selected to invest more resources in signaling. When the cost of signaling is minimal for small investments (high b_t), higher investment is favored.

Additionally, the ESS is affected by the dependence of $s(x, q)$ and $t(x, q)$ on quality, which I define as signal constraints. When high-quality individuals can produce a signal close to their personal maximum with minimal investment (high k_s), the optimal investment in signaling is reduced, especially for high-quality individuals. When high-quality individuals can invest relatively more in signaling before experiencing a large cost (high k_t), the optimal investment in signaling is elevated, particularly in individuals of higher quality. The ESS was independent of c_s, because this parameter affects the magnitude but not the shape of $s(x, q)$. If both k_s and k_t equal zero, q does not affect the ESS; that is, if the shape of the cost and benefit functions are not constrained by quality, all individuals play the same signaling strategy (this does not imply a breakdown of honest signaling provided that $c_s > 0$; see below).

The relationship between $x^*(q)$ and q can be either positive or negative, depending on the values of k_s and k_t. High k_s means that high-quality individuals can produce a strong signal with minimal effort, so q negatively affects $x^*(q)$; high k_t means that they can signal more with less reduction in t, so q positively affects $x^*(q)$. The positive effect of r shows that individuals are selected to invest more in signaling when the fitness returns from increased signaling do not rapidly saturate. Lastly, the ESS is independent of the population average signal, $\bar{s}$, under the present assumption that fitness increases by a function of $s/\bar{s}$.

THE STRATEGIC COST OF SIGNALING OPTIMALLY

We may define the strategic cost of signaling optimally as $1 - t(x^*, q)$, that is, the proportion of trait t that is lost due to playing strategy $x^*(q)$. After simplifying, the strategic cost is equal to

$$\frac{b_s r}{b_s r + b_t (1 + k_s q)(1 + k_t q)}. \quad (5)$$

The strategic cost approaches zero as b_s tends to zero (Fig. 2A), showing that signals evolve to be cheap when signalers can maximize their signal with a small investment. By contrast, the strategic cost approaches zero as b_t tends to infinity, showing that cheap signals evolve when signalers can invest a lot in signaling without impairing other fitness traits (Fig. 2A). The constraint parameters k_s and k_t both negatively affect the evolved strategic cost and are associated with q in equation (5), showing that signals evolve to be cheaper (especially for individuals of higher quality) when the benefits or costs of investing in signaling are strongly constrained by quality. When only those with strong signals receive significant fitness returns (high r), signaling evolves to be more expensive. Additionally, the partial derivative of equation (5) with respect to q is always negative assuming $k_s, k_t > 0$, showing that high-quality signalers always pay lower costs when signaling is constrained by quality.

HONESTY AND THE RELATIONSHIP BETWEEN QUALITY AND SIGNAL STRENGTH

We may define an honest signaling system as one in which senders are selected to produce a signal that is monotonically related to their quality (similar to the strict definition in Kokko 1997). To investigate when this condition is fulfilled, we consider the relationship between sender quality and signal strength at the ESS, $s(x^*(q), q)$. Inspection of the partial derivative of this function with respect to q revealed that it is always positive, indicating a constant increase in s with q at the ESS, provided that at least one of c_s, k_s, and k_t is greater than zero (if all equal zero, the partial derivative is zero). In other words, honest signaling is guaranteed provided that constraints ensure that high-quality signalers are capable of producing superior signals, can produce equivalent signals for less investment, and/or receive a smaller reduction in other fitness traits for any given investment. The model therefore recapitulates previous results that honest signaling requires that high-quality individuals receive higher marginal fitness returns from investment in signaling (e.g., Grafen 1990; Getty 1998). Furthermore, because signals are always honest when at least one of c_s, k_s, and $k_t > 0$, this result demonstrates that cheap and costly signals (and strongly and weakly constrained signals) are "equally honest." Because constraints linking quality to signal production or costs are required for honest signaling under the present assumptions, this result suggests that constraints affect most honest signals; a likely exception is the case of signals between individuals with common fitness interests, such as close relatives (Maynard Smith 1991).

The model can also be used to predict the shape of the relationship between q and s at the ESS, which is analogous to the allometry of the signal in the special case in which q is equivalent to or correlated with body size. The second partial derivative of $s(x^*(q), q)$ with respect to q is complicated, so I investigated signal allometry numerically. After setting the values of b_s, b_t, and r to random values between 10^{-3} and 20, and c_s, k_s, and k_t between 0 and 20, I determined the relative signal strength of individuals with $q = 0, 0.01, 0.02 \ldots 1$ at the ESS for that parameter space, then found the allometric slope by regressing $\log(s)$ on $\log(q)$ ($n = 10^5$ replicates). Signal strength s was scaled to run between 0 and 1 (i.e., was expressed relative to the signals of the worst and best possible individuals in that parameter space) via the transformation $(s(x^*(q), q) - s(x^*(0), 0)) / (s(x^*(1), 1) - s(x^*(0), 0))$. I then plotted each parameter against the allometric slope to test its effect on the evolution of signal allometry (Fig. S1), and simultaneously calculated the strategic cost at the ESS (for an individual with $q = 0.5$) to test whether it correlated with the allometric slope.

Strongly negative allometry only occurred when c_s was close to zero, showing that low-quality signalers must be capable of matching the signals of high-quality individuals for negative allometry to evolve (Fig. S1). Strong positive allometry was found when b_t and r were low, suggesting that positive allometry will evolve when investment in signaling causes a sharp reduction in other fitness traits, and when the fitness returns from signaling rapidly saturate with $s/\bar{s}$ (Fig. S1). The latter result suggests that if increases in signal strength yield diminishing fitness returns, only the best individuals are selected to produce strong signals, because they can do so most efficiently. Negative allometry did not evolve when k_s was close to 0, further supporting a link between signal constraints and allometry, while b_s and k_t had comparatively weak effects (Fig. S1).

Interestingly, the allometry of a signal correlated with its cost: costly signals tended to have positive allometry, while signals with a cost close to zero typically showed negative allometry or isometry (Fig. 2B). Figure 2B assumes $r = 1$; the allometric prediction also held for values of r less than one (Fig. S2), but the relationship between signal cost and allometry grew weaker as r increased, because high r values produced signals with an allometric slope close to 1 in most parameter spaces (Fig. S2). The model therefore suggests that signal allometry provides an indirect measure of the realized strategic cost of signals, a characteristic that cannot be measured empirically because of the difficulty of distinguishing strategic costs from the basic costs required to transmit the signal (see Számadó 2011).

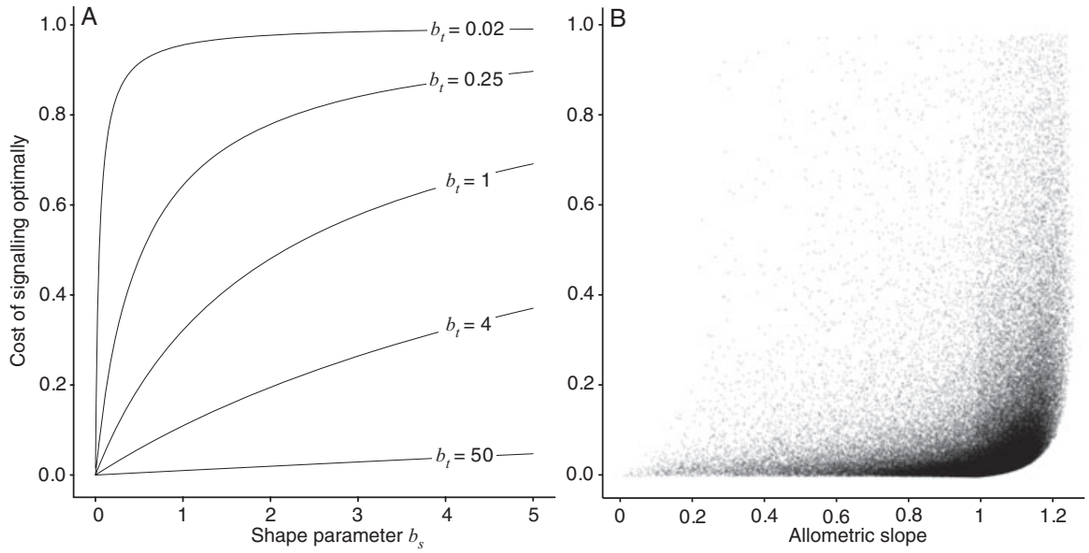

Figure 2. Evolutionary constraints determine whether a signal evolves to be costly or cheap at equilibrium, and signal allometry predicts signal costs. (A) Strategic signaling costs at the ESS are lower when individuals can maximize their signal at low investment levels (low b_s). Costs are higher when small investments in signaling cause large decreases in the other fitness trait t (low b_t). The figure shows the cost of signaling for an individual of quality $q = 0.5$, and assumes $k_s = k_t = r = 1$. (B) Signals with high strategic costs are more likely to be positively allometric than cheaper signals. Each of the 10^5 points represents a random parameter space generated by drawing random values of b_s, b_t, c_s, k_s, and k_t, assuming $r = 1$.

BROADER IMPLICATIONS OF THE MODEL

The model predicts that signals with negligible strategic cost evolve when the costs and benefits of signaling are tightly linked to the sender's quality (high k_s and k_t). Costly signals evolve when signal strength is less strictly limited by the individual's quality. Signaling can be both honest and virtually cost-free provided that the costs or benefits of investment in signaling are sufficiently strongly linked to the quality being signaled by evolutionary or mechanical constraints, consistent with the original formulation of the index hypothesis (Maynard Smith and Harper 1995, 2003). Costs and constraints therefore work together to ensure honest signaling: the cost that senders must pay when behaving optimally depends on the strength of constraints acting on the signal.

The results also show that evolutionary constraints precluding adaptations that improve the efficacy of signaling (i.e., those that change b_s and b_t in the direction of the arrows in Fig. 1) are required for costly honest signaling to persist over evolutionary time. In the absence of any such constraints, adaptations that increase b_s or reduce b_t should continually fix until the cost of signaling is negligible. For example, the honesty of some sexual signals has been proposed to be enforced by the costly levels of testosterone required for their production (e.g., Folstad and Karter 1992). This hypothesis implicitly invokes evolutionary constraints, because mutants that produced an equivalently strong signal for a smaller investment (e.g., by changing the sensitivity of signal production to testosterone) or that could better tolerate the costs of signaling (e.g., through tolerance of the hormone's deleterious effects) would spread, unless prevented from doing so by evolutionary constraints such as antagonistic pleiotropy (types of evolutionary constraint are reviewed in Arnold 1992).

The shape of the relationship between q and s at the ESS was predicted to vary between cheap and expensive signals, with the former predicted to be largely negatively allometric or isometric, and the latter positively allometric, assuming that q is related to body size. This prediction has substantial empirical support. A study of male *Crotaphytus* lizards found that the size of the white patch on the major jaw adductor muscle complex, a putatively low-cost, highly constrained signal used to intimidate rivals during gaping displays, is linearly related to bite force (Lappin et al. 2006). Similarly, *Plexippus* jumping spiders have color patches that are thought to highlight the spider's condition (Taylor et al. 2000); the width of the patch is linearly related to time since feeding, as predicted from the signal's apparently low cost and mechanistic links with the quality being signaled. Studies of sexually selected ornaments and weapons provide good evidence that high-cost signals tend to show positive allometry: conspicuous and putatively costly animal signals such as antlers, fiddler crab claws, earwig forceps, and Diopsid eye stalks are often positively allometric (reviewed in Kodric-Brown et al. 2006; Bonduriansky 2007), while putatively cheap sexually selected traits tend not to

be positively allometric (Bonduriansky 2007). Additionally, earwig species with relatively large forceps were also found to have more positive forceps allometry (Simmons and Tomkins 1996). The large diversity of allometric slopes among sexual signals has yet to be fully explained (Bonduriansky 2007); differences in the quality dependence of the benefits and costs of signaling provide a possible ultimate explanation.

Because constraints on signal evolution influence signal costs, they may also indirectly affect population viability. Signaling systems with high strategic costs may represent a "tragedy of the commons" (Rankin et al. 2007); the evolution of very costly signals is favored by individual-level selection in some parameter spaces, but costly signals can increase the risk of extinction (Kokko and Brooks 2003). The tragedy arises because population viability likely depends more on nonsignaling traits (t) than signals (s), but individual fitness depends on both. Population-level selection against costly signals may therefore have influenced observed signal diversity by favoring signals that are tightly constrained by the sender's quality.

A Case Study: Social Insect Queen Pheromones

Queen pheromones are chemical signals produced by queens and other reproductives that affect the behavior and/or physiology of other colony members, and are thought to be ubiquitous in social insects (Monnin 2006; Le Conte and Hefetz 2008; Heinze and d'Ettorre 2009; Holman et al. 2010b). Because the fitness interests of colony members are often incompletely aligned, queens might evolve pheromones that benefit their own fitness at the expense of their nestmates. For example, queens in multiqueen societies may be selected to produce more pheromone to solicit a greater share of the workers' attention (West-Eberhard 1983). Workers should evolve resistance to signals that lower their fitness, for example, by inducing them to remain sterile and care for an unproductive queen, which led to the prediction that queen pheromones should honestly signal queens' fecundity or overall quality (reviewed in Keller and Nonacs 1993; Heinze and d'Ettorre 2009). Essentially all data have supported this prediction: queen pheromones (and queen-specific chemicals that might be pheromones) correlate with fecundity and other quality measures across the eusocial insects (Sledge et al. 2001; Hannonen et al. 2002; Dietemann et al. 2003; Cuvillier-Hot et al. 2004a; d'Ettorre et al. 2004; Strauss et al. 2008; Holman et al. 2010a,b; Matsuura et al. 2010). However, the ultimate factors that keep queen pheromones honest are largely unknown (Heinze and d'Ettorre 2009).

Fertility-signaling queen pheromones could be costly, for example, because pheromone production consumes limiting resources such as energy or dietary amino acids (Blomquist and Bagnères 2010), necessitates costly social interactions (Cuvillier-Hot et al. 2004b; Dapporto et al. 2007; Smith et al. 2009), or involves toxicity either from the pheromone itself (Zahavi and Zahavi 1997) or its precursors or regulatory hormones. Queen pheromone production could also be constrained: for example, pheromone synthesis might be linked to oogenesis by shared genetic or physiological networks. A third possibility is that dishonest signaling by queens would not be selectively favored because of high relatedness and other common fitness interests within colonies (e.g., Maynard Smith 1991; Bergstrom and Lachmann 1998; Mitri et al. 2011). This hypothesis may hold for specific cases but is unlikely to be universally true. Within-colony relatedness can be low, especially in multiqueen colonies (e.g., Adams and Balas 1999; Helanterä et al. 2009; Holman et al. 2010a), and surplus queens may be actively killed by workers, putatively based on the quantity of pheromones they produce (West-Eberhard 1983; Adams and Balas 1999; Holman et al. 2010a; Wurm et al. 2010).

The only experimentally identified ant queen primer pheromone (i.e., a pheromone affecting the recipient's physiology) is 3-methylhentriacontane (3-MeC_{31}), a cuticular hydrocarbon that inhibits ovarian activation and aggressive behavior in workers of the black garden ant *Lasius niger* (Holman et al. 2010b). Consistent with a function as an honest signal of quality and reproductive potential, the proportion of 3-MeC_{31} in the chemical profile increases with fecundity and reproductive maturity in queens, is much higher in queens than workers, and is reduced following immune challenge unlike all other hydrocarbons (Holman et al. 2010ab). Workers also appear to choose queens based on this hydrocarbon: some colonies are co-founded by unrelated queens, but the first workers kill all but one queen, preferentially targeting those with lower amounts of 3-MeC_{31} (Holman et al. 2010a).

Here, I search for costs and constraints associated with the *L. niger* queen pheromone using experiments with juvenile hormone III (JH). JH affects chemical fertility signals in honey bees (Malka et al. 2009) and burying beetles (Haberer et al. 2010), and also influences oogenesis (Robinson and Vargo 1997) and survival (Tibbetts and Banan 2010) in hymenopterans. I therefore hypothesized that JH might mediate a trade-off between pheromone production and egg production and/or survival in *L. niger*. In this case, JH titer would represent a mechanism by which investment in signaling (x) is adjusted, the hydrocarbon profile would be the signal (s), and egg production and survival would be traits that are traded off against signaling (t). High-quality queens might be better able to tolerate the costs of JH (k_t) or produce signals that are differentially affected by JH treatment (c_s and k_s). The experiments test if queen pheromone production has significant endocrine-mediated costs in terms of fecundity and survival, and whether the costs and benefits of adjusting JH titer are strongly

constrained by the quality of the queen (as measured by body mass and pre-experimental fecundity).

Methods

Recently mated, wingless queen *L. niger* were collected during a mating flight in Copenhagen, Denmark on 20 July 2011. The queens were weighed to the nearest 0.1 mg (giving a measure of fat reserves accrued in the natal colony) and then housed individually in plastic cylinders (26 × 38 mm) and given a ball of moist cotton wool, but no food, mimicking natural claustral colony founding (Holman et al. 2010a).

A total of 120 queens were randomly and equally divided among four treatments. All ants were allowed to oviposit for 240 ± 2 h after mating, at which point all eggs were removed and counted (providing a measure of queens' fecundity prior to experimental manipulation). The queens were then given a single 2 μl topical application of treatment solution, pipetted onto the ventral surface of the gaster. The control group received acetone (Sigma-Aldrich, http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=179124|SIAL&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC) only, while the other groups received "low" (0.05 μg/μl), "medium" (0.5 μg/μl), and "high" (5 μg/μl) doses of JH III (Sigma-Aldrich) dissolved in acetone. After a further 240 h, the number of eggs laid by each queen was recorded, and the queens were frozen for later chemical analysis. Queen cuticular hydrocarbons were extracted with pentane and then analyzed by gas chromatography-mass spectrometry (GC-MS) as described in Holman et al. (2010a,b). Nine queens died prematurely, and were excluded.

A second group of 120 queens from the same mating flight were also randomly divided among four JH treatments. These queens were also given a 2μl topical application of an acetone solution (containing either 0, 0.05, 0.5, or 5 μg/μl JH) 10 days after mating, and again on days 18, 26, and 39 post-mating. The ants were checked daily, and deaths were recorded to assess the effect of JH treatment on survival. The experiment was terminated 49 days postmating.

All data were collected and analyzed blind to treatment. Statistics were performed in R 2.13.1 (www.r-project.org); generalized linear models (GLMs) were used when nonnormal errors were present, treatment levels were compared using contrasts, and minimum adequate models were fit by sequentially removing nonsignificant terms. Where present, overdispersion was corrected for using quasi-likelihood estimation. Hydrocarbon data were transformed (Aitchison 1986; Holman et al. 2010a) prior to principal component analysis (PCA). The allometric slope linking queen mass and pheromone production was estimated from the log-transformed data using reduced major axis regression, jack-knifing over cases (Bohonak and van der Linde 2004).

Results

EFFECT OF JH ON EGG PRODUCTION AND CUTICULAR HYDROCARBONS

Low and medium doses of JH stimulated queens to lay more eggs relative to the control, while high doses inhibited oviposition (Fig. 3A). A model containing both a linear and quadratic term for JH concentration provided the best fit of the data (GLM with quasi-Poisson errors; linear effect: $t_{108} = 6.06$, $P < 0.0001$, quadratic effect: $t_{108} = 6.60$, $P < 0.0001$). Two quality measures, the number of eggs laid by queens prior to experimental manipulation and their mass at the time of collection (as well as all interaction terms), did not significantly affect the number of eggs laid after treatment (all $P > 0.59$).

A single principal component (PC1) explained 79.4% of variation in the cuticular hydrocarbon profile. The seven hydrocarbons with the highest loadings on PC1 were C_{27}, C_{29}, 3-MeC_{31}, C_{31}, 3-MeC_{29}, $C_{33:1}$, and $C_{31:1}$ in that order, which are the same seven hydrocarbons previously found to be significantly more abundant in the chemical profiles of queens than workers (Holman et al. 2010b). Higher values of PC1 therefore indicate a more queen-like chemical profile. PC1 was significantly positively related to the concentration of JH applied (Fig. 3B; $R^2 = 0.19$; $t_{109} = 4.98$, $P < 0.0001$), suggesting that JH increased the relative abundance of queen-like hydrocarbons in a dose-dependent manner. The quadratic effect of JH treatment, queen mass, the number of eggs laid prior to treatment, and all interaction terms were not significant predictors of PC1 (all $P > 0.35$). To quantify the effect of JH on individual cuticular hydrocarbons, univariate tests (GLMs with quasi-binomial errors) were performed on GC-MS peaks with strong loadings on PC1, using the proportion of the peak in the total profile as the response. The queen pheromone 3-MeC_{31} increased with the concentration of JH applied ($t_{108} = 3.41$, $P = 0.0009$), and was also positively related to queen mass ($t_{108} = 2.78$, $P = 0.007$); there was no interaction between these predictors and no significant quadratic effect of JH ($P > 0.12$). C_{27}, C_{29}, and 3-MeC_{29} were also positively correlated with JH dose, and two worker-like peaks containing dimethylalkanes were substantially reduced (Table S1).

To quantify the shape of the trade-off between queen-like hydrocarbons (as measured by PC1) and egg production, I tested how these traits co-varied across JH treatments in a linear model. There was a significant negative relationship ($t_{108} = 3.98$, $P = 0.0001$), and also a significant positive quadratic term ($t_{108} = 2.61$, $P = 0.010$). A similar model of the relationship between the queen pheromone 3-MeC_{31} and egg production produced the same results (Fig. 4): there was a negative relationship ($t_{108} = 3.42$, $P = 0.0008$) with a positive quadratic term ($t_{108} = 2.10$, $P = 0.038$). These results suggest a nonlinear, endocrine-mediated trade-off between fertility and queen pheromone production.

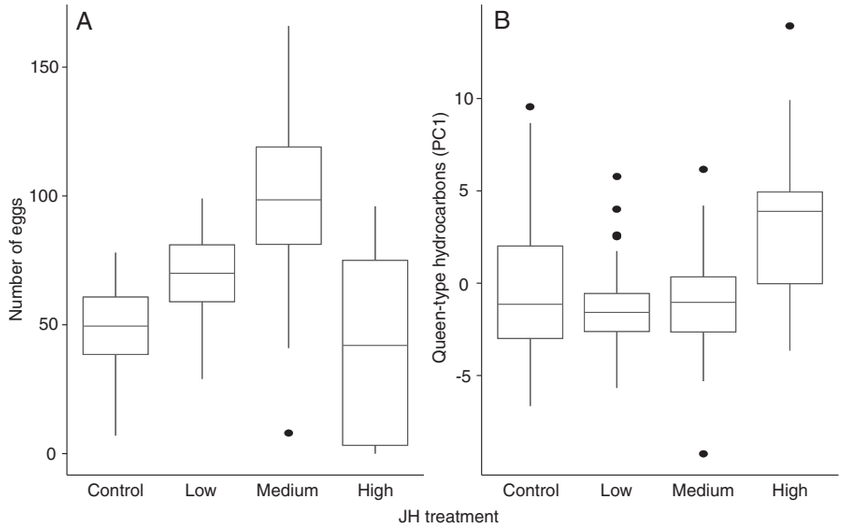

Figure 3. Effects of juvenile hormone (JH) on oviposition and cuticular hydrocarbons in queen *Lasius niger* ($n = 111$). (A) JH treatment stimulated egg production at low (0.05μg/μl) and medium (0.5μg/μl) doses, and had a weak inhibitory effect at high (5μg/μl) doses. (B) High doses of JH increased the relative abundance of queen-type cuticular hydrocarbons in the chemical profile (shown here by PC1; 79.4% explained variation), while low and medium doses had either no effect or a small negative effect.

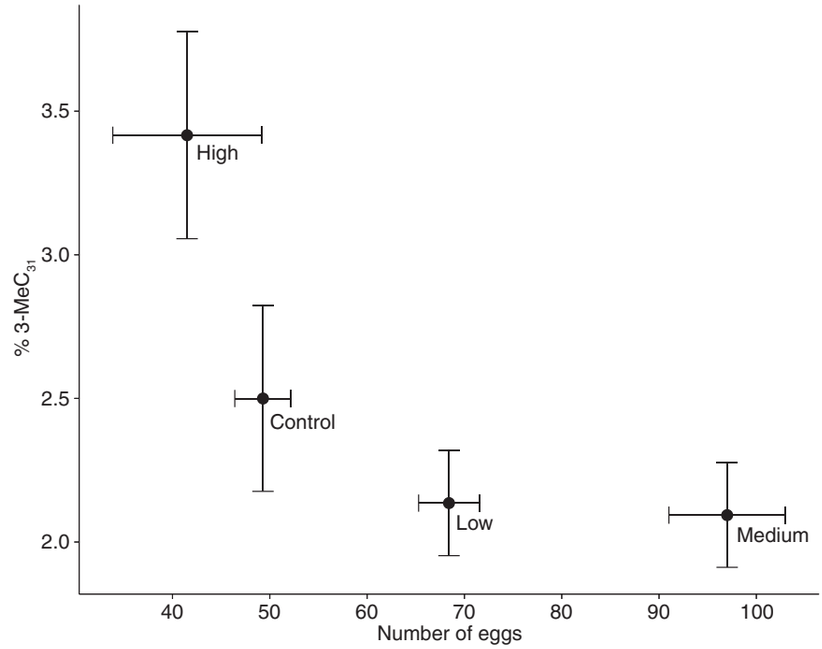

Figure 4. The number of eggs produced by queens in each juvenile hormone treatment was negatively and nonlinearly related to the proportion of the queen pheromone 3-MeC_{31} present in the chemical profile. The figure shows means and standard errors ($n = 111$).

The relationship between body mass and the proportion of the hydrocarbon profile composed of 3-MeC_{31} suggested negative allometry: the allometric slope was 0.18, with bootstrapped 95% confidence intervals of 0.15–0.22 ($n = 111$).

EFFECT OF JH ON QUEEN SURVIVAL IN INCIPIENT COLONIES

Queens treated with the high dose of JH had greatly reduced survival, while the other treatment groups largely survived the observation period (Fig. 5). Survival analysis (treating JH dose

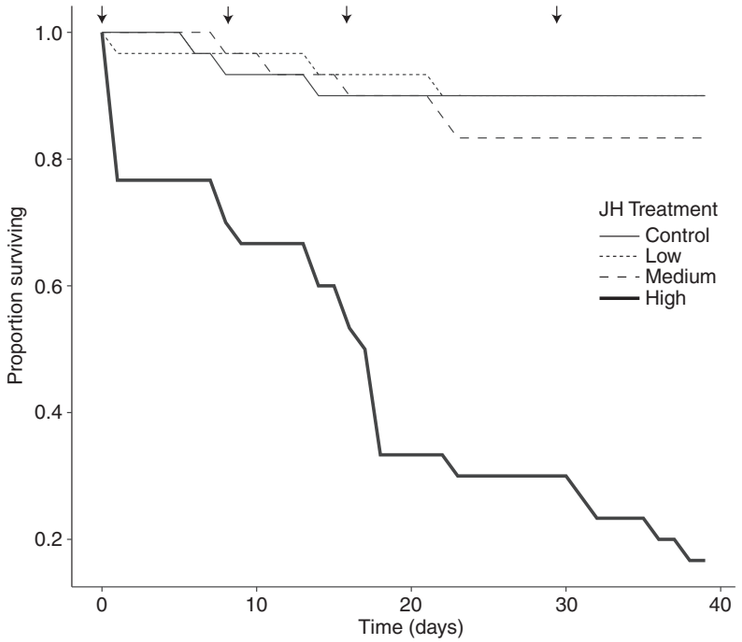

Figure 5. Treatment with high doses of juvenile hormone greatly reduced queen survival, but lower doses had no detectable effects on mortality. Arrows mark the days on which treatment solutions were applied ($n = 120$).

as a fixed factor) revealed that the survival of control queens was near identical to that of low-JH queens (Cox's proportional hazard regression; $z = 0.001$, $P = 1$), similar to medium-JH queens ($z = 0.74$, $P = 0.46$), but much higher than high-JH queens ($z = 4.27$, $P < 0.0001$). These data suggest that there is a range in which queens can modulate their JH titer without compromising short-term survival, but that larger increases are fatal.

Discussion

JH was found to mediate a trade-off between egg production and the composition of the cuticular hydrocarbon profile. Specifically, doses of JH that produced high fertility reduced the representation of queen-like hydrocarbons, including the queen pheromone 3-MeC_{31}. High doses of JH also greatly reduced survival. The trade-off between fertility and signaling was nonlinear, suggesting that the effect of investment in signaling (x) on the signal (s) and/or egg production (t) must also be nonlinear. The results also suggest that queens could increase their fecundity by slightly elevating JH titer (without no apparent survival cost), but that this would reduce their pheromone signal. Therefore, signaling may have a nontrivial strategic cost, in the form of a JH titer that is suboptimal for other fitness traits such as fecundity. The number of workers reared by incipient colonies is thought to be a key determinant of colony success (Bernasconi and Strassmann 1999), implying that increased fecundity would be beneficial.

Although heavier queens produced more 3-MeC_{31} (this study) and 3-MeC_{31} correlates with fertility (Holman et al. 2010a), suggesting that signaling honestly advertises quality and therefore that c_s, k_s, and/or k_t are positive, the experiment found no direct evidence that JH has quality-dependent effects, using either body mass or pre-experiment fecundity as quality measures. There was no detectable interaction between quality and the effects of JH treatment on fecundity or pheromone production, consistent with relatively low c_s, k_s, and k_t. Pheromone production was strongly negatively allometric, implying that c_s is low. The experiment therefore suggests that the effects of JH on signal strength and signal costs are not strongly constrained by queen quality. The model predicts that relatively unconstrained signals should evolve to be more costly, again suggesting that signaling may have a nontrivial cost.

Significant evolutionary constraints are likely present on the evolution of JH titer. In addition to survival and fertility, JH affects growth, immunity, and susceptibility to oxidative stress (e.g., Robinson and Vargo 1997; Rolff and Siva-Jothy 2002; Amdam et al. 2007; Corona et al. 2007). Because JH affects so many traits simultaneously, queens might require multiple adaptations to evolve tolerance to higher levels, meaning that b_t cannot easily evolve. It is less clear why queens could not evolve to produce more pheromone without elevating JH (evolving b_s), but the most likely explanation is resource trade-offs. JH inhibits production of eggs and the yolk glycolipoprotein vitellogenin (Cuvillier-Hot et al. 2004b; Corona et al. 2007), which might free up resources

for pheromone production. Eggs are coated with 3-MeC_{31} and other queen-like hydrocarbons (Holman et al. 2010b) and are provisioned with hydrocarbons by lipophorin carrier molecules including vitellogenin (Fan et al. 2002), so the inhibition of oogenesis may make more 3-MeC_{31} available for transport to the cuticle. Also, synthesis of 3-methylalkanes requires the amino acids valine, methionine, and isoleucine, unlike synthesis of other hydrocarbons (Blomquist and Bagnères 2010); these amino acids must be sequestered from food and are also used in the production of vitellogenin (Bonasio et al. 2010).

JH had both positive and negative effects on fecundity, depending on dose. JH has been reported to stimulate oviposition in some hymenopteran insects, for example, *Polistes* wasps, *Bombus* bees, and *Solenopsis* ants (reviewed in Robinson and Vargo 1997), and inhibit it in others, for example, *Apis mellifera* (Malka et al. 2009), *L. niger* (Sommer and Hölldobler 1995), and *Streblognathus peetersi* ants (Cuvillier-Hot et al. 2004b), although some experiments used only a single concentration. The present results show that JH can have opposing effects on fecundity at different doses, suggesting that endocrine regulation of fertility is more complex than currently appreciated.

Costs and constraints associated with JH may be a general mechanism for the maintenance of honesty in hymenopteran queen pheromones. In queenless *A. mellifera* workers, JH application inhibited ovarian activation and induced production of a more queen-like chemical profile relative to fertile controls (Malka et al. 2009). By contrast, a study of *S. peetersi* ants found that reproductive inhibition with JH caused the hydrocarbon profile of reproductives to become more similar to that of infertile workers (Cuvillier-Hot et al. 2004a). However, the JH-treated reproductives were also aggressively immobilized by their nestmates, likely reducing their condition, which may explain the discrepancy with the present study. JH treatment also lowered the survival of *P. dominulus* wasps, but queens and individuals with patchy facial coloration (thought to signal quality) were more likely to survive (Tibbetts and Banan 2010), suggesting that JH has quality-dependent costs.

In summary, I argue that costs and constraints work together to maintain honest signaling. Signals that are strongly constrained by the quality of the sender evolve to be cheap and are more likely to have low allometric slopes, while less constrained signals should be expensive and positively allometric. Ant queen pheromones appear to carry a nontrivial signaling cost in the form of a JH titer that lowers fecundity, and queen quality had no detectable effect on response to elevated JH, suggesting low constraints. However, queen pheromone and egg production are likely genetically and physiologically linked via a shared dependence on JH and vitellogenesis, suggesting that the evolution of "dishonest" queens that overproduce 3-MeC_{31} without increasing fecundity may be precluded by genetic constraints.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Effect of the model's parameters on the allometric slope of the relationship between q and s at the ESS.
Figure S2. Signals with high strategic costs are more likely to be positively allometric than cheaper signals.
Table S1. Effects of JH treatment on the proportion of the chemical profile made up of each cuticular hydrocarbon.

Supporting Information may be found in the online version of this article.

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