E-Note

Intragenomic Conflict over Soldier Allocation in Polyembryonic Parasitoid Wasps

Petri Rautiala[1,*] and Andy Gardner[2]

1. Department of Biological and Environmental Science and Centre of Excellence in Biological Interactions, University of Jyvaskyla, PO Box 35, FI-40014 University of Jyvaskyla, Finland; 2. School of Biology, University of St Andrews, St Andrews KY16 9TH, United Kingdom

Submitted June 9, 2015; Accepted September 1, 2015; Electronically published February 18, 2016

abstract: Understanding the selection pressures that have driven the evolution of sterile insect castes has been the focus of decades of intense scientific debate. An amenable empirical test bed for theory on this topic is provided by the sterile-soldier caste of polyembryonic parasitoid wasps. The function of these soldiers has been a source of controversy, with two basic hypotheses emerging: the "brood-benefit" hypothesis that they provide an overall benefit for their siblings and the "sex-ratio-conflict" hypothesis that the soldiers mediate a conflict between brothers and sisters by killing their opposite-sex siblings. Here, we investigate the divergent sex-ratio optima of a female embryo's maternal-origin and paternal-origin genes, to determine the potential for, and direction of, intragenomic conflict over soldiering. We then derive contrasting empirically testable predictions concerning the patterns of genomic imprinting that are expected to arise out of this intragenomic conflict, for the brood-benefit versus the sex-ratio-conflict hypothesis of soldier function.

Keywords: genetic conflict, genomic imprinting, kin selection, parent-of-origin effects, sex allocation, spiteful behavior.

Introduction

Understanding the selection pressures that have driven the evolution of sterile insect castes has been the focus of decades of intense scientific debate (Hamilton 1964, 1972; Wilson 2005; Foster et al. 2006; Boomsma 2007, 2009, 2013; Nowak et al. 2010; Abbot et al. 2011; Gardner et al. 2012; Liao et al. 2015). An amenable empirical test bed for theory on this topic is provided by the sterile-soldier caste of polyembryonic parasitoid wasps of the genus *Copidosoma* (Cruz 1986; Strand 2009). These are wasps that inject their eggs into the bodies of other insects and whose young devour their hosts from the inside before emerging as adults to mate and find new hosts to parasitize. A curious aspect of their biology is that each egg proliferates clonally to give rise to a very large number of genetically identical embryos, which then compete for resources within the host; such polyembryony has arisen independently in four families of the parasitoid Hymenoptera: the Braconidae, Platygastridae, Dryinidae, and Encyrtidae, with *Copidosoma* belonging to the last (Ivanova-Kasas 1972). An even more curious aspect of their biology is that some of these embryos—mostly, but not solely, females—develop precociously as soldier larvae that remain in the interior of the host and do not emerge as reproductive adults.

The function of these soldiers has been a source of controversy, with two basic hypotheses emerging (Gardner et al. 2007*a*). First, the "brood-benefit" hypothesis suggests that their primary function is to provide an overall benefit for their siblings, either by macerating host tissues to facilitate release of nutrients (Silvestri 1906) or, more likely, by attacking the young of other parasitoids that may also be present in the host (Cruz 1981; Strand et al. 1990; Harvey et al. 2000; Giron et al. 2004). Second, the "sex-ratio-conflict" hypothesis suggests that the soldiers' primary function is to mediate a conflict between brothers and sisters over the sex ratio of the reproductive adults that will emerge from the host, by killing their opposite-sex siblings (Godfray 1992; Hardy 1994; Ode and Hunter 2002; Giron et al. 2004). Gardner et al. (2007*a*) provided mathematical analyses of both putative functions and showed that, if individuals of either sex are equally capable of developing and acting as soldiers, then male-biased soldiering is expected under the brood-benefit hypothesis and female-biased soldiering is expected under the sex-ratio-conflict hypothesis, because females value their brothers relatively less than males value their sisters. Accordingly, since female-biased soldiering is observed (Doutt 1947; Grbić et al. 1992; Ode and Strand 1995; Giron et al. 2004; Keasar et al. 2006), Gardner et al's (2007*a*) analysis lends support to the idea that the soldiers have a sex-ratio-conflict function.

However, an alternative explanation for the observed sex bias in soldiering is that the sexes may differ in their intrinsic ability to develop and behave as soldiers (Doutt 1947; Gardner et al. 2007*a*). This view mirrors the more general

* Corresponding author; e-mail: petri.rautiala@gmail.com.

Am. Nat. 2016. Vol. 187, pp. E106–E115.
DOI: 10.1086/685082

understanding of why sterile workers among the social Hymenoptera are always female: although this sex bias was traditionally attributed to relatedness asymmetries arising from haplodiploid inheritance (Hamilton 1964, 1972), more recent empirical analysis instead supports the idea that females are simply better workers, being already equipped with adaptations for nursing young owing to the presence of maternal but not paternal care among the ancestors of this insect group (Ross et al. 2013). Accordingly, the empirically observed female-biased soldiering of polyembryonic parasitoid wasps need not rule out a primarily brood-benefit function for soldiers.

Here, we develop a further set of empirically testable predictions that may be used to discriminate between the brood-benefit and sex-ratio-conflict hypotheses for soldier function and that do not depend on the relative preadaptation of females and males to soldiering. In particular, we follow up on West's (2009, p. 287; see also Wild and West 2009) suggestion that there may be an intragenomic conflict of interests, between a female's maternal-origin and paternal-origin genes, over the decision to develop as a soldier, and that this may drive the evolution of parent-of-origin-specific patterns of gene expression, that is, "genomic imprinting" (Moore and Haig 1991; Haig 1997). We first adapt the mathematical model of Gardner et al. (2007*a*) to investigate the sex-ratio optima of a female's maternal-origin and paternal-origin genes, to ascertain the potential for, and direction of, intragenomic conflict over soldiering. We then derive contrasting predictions as to the patterns of genomic imprinting that are expected to arise out of this intragenomic conflict for the brood-benefit and sex-ratio-conflict hypotheses with regard to soldier function.

Model and Analysis

Basic Model

Following Gardner et al. (2007*a*), whose model focuses mostly on the biology of *Copidosoma floridanum*, we consider that a single foundress wasp injects two eggs—one fertilized (i.e., female) and one unfertilized (i.e., male)—into a parasitized host, with each egg proliferating clonally to give a large number of embryos, such that each embryo is genetically identical to its same-sex broodmates and is related to its opposite-sex broodmates according to the usual brother-sister relationship. Some proportion of female and male embryos develop as soldiers, which modulates the number and sex ratio of the embryos that will successfully emerge from the host as adults. After emerging, a proportion $1 - d_f$ of females and a proportion $1 - d_m$ of males remain close to their host, where they form a mating group, whereas a proportion d_f of females and a proportion d_m of males disperse to other mating groups. Mating then occurs at random within each mating group, after which all males perish and the mated females parasitize the next generation of hosts.

Inclusive Fitness

We take an inclusive-fitness approach to capture the evolutionary interests of each member of the family unit (Hamilton 1964; Gardner and Welch 2011). In particular, we express the inclusive fitness of any actor A as

$$H_A = N_m M p_{mA} + 2N_f p_{fA}, \tag{1}$$

where N_m is the number of males emerging from a focal host, M is the average number of successful matings enjoyed by each of these males, p_{mA} is the consanguinity of each of these males to the actor (i.e., the probability that a gene drawn at random from a male is identical by descent to one drawn at random from the actor, from the same locus; Bulmer 1994), N_f is the number of females emerging from the focal host, p_{fA} is the consanguinity of each of these females to the actor, and the factor 2 reflects that each female has twice the reproductive value of the male with whom she mates, under haplodiploidy (Hamilton 1972). The average number of successful matings per local male may itself be expressed as

$$M = d_m \frac{\bar{N}_f}{\bar{N}_m} + (1 - d_m)\frac{(1 - d_f)N_f + d_f\bar{N}_f}{(1 - d_m)N_m + d_m\bar{N}_m},$$

where $\bar{N}_m$ and $\bar{N}_f$ are the average number of males and females, respectively, emerging from each host in the population.

Intragenomic Conflict over Sex Ratio

Different actors may have different preferences with respect to the sex ratio of the emerging adults, and this disagreement may be investigated by consideration of the inclusive fitness function. Specifically, defining $N = N_f + N_m$ and $z = N_m/N$ and hypothetically assigning the actor full control over the sex ratio z, their inclusive fitness may be written as

$$H_A = Nz\left[d_m\frac{1 - \bar{z}}{\bar{z}} + (1 - d_m)\frac{(1 - d_f)(1 - z) + d_f(1 - \bar{z})}{(1 - d_m)z + d_m\bar{z}}\right]p_{mA} + 2N(1 - z)p_{fA}, \tag{2}$$

where $\bar{z}$ is the population-average sex ratio. The actor prefers a higher-than-population-average sex ratio whenever their marginal inclusive fitness is positive at that population average, that is, when

$$\left.\frac{\partial H_A}{\partial z}\right|_{z=\bar{z}} = \left[\frac{(2 - d_m)d_m}{\bar{z}} + (d_f - d_m)(1 - d_m) - 1\right]Np_{mA} - 2Np_{fA} > 0.$$

Accordingly, setting marginal inclusive fitness equal to 0 and solving for $z = \bar{z} = z_A$ yields the sex-ratio optimum for actor A:

$$z_A = \frac{(2 - d_m)d_m}{1 + (1 - d_m)(d_m - d_f) + 2p_{fA}/p_{mA}}. \quad (3)$$

Different actors may have different sex-ratio optima because they may have different consanguinities to the female and male broods (p_{fA} and p_{mA}, respectively). The coefficients of consanguinity for the different family members are listed in table 1. Equations (3)–(5) of Gardner et al. (2007*a*; see also Gardner et al. 2007*b*) provide the sex-ratio optima from the perspective of a female embryo, a male embryo, and their mother, respectively: these are recovered by substituting the appropriate coefficients of consanguinity into our equation (3) and are illustrated in figure 1.

If all males disperse before mating ($d_m = 1$), then there is full outbreeding and no local mate competition. In this case, mothers prefer an equal sex allocation ($z = 1/2$): although daughters have twice the reproductive value of sons under haplodiploid inheritance, when the sex ratio is unbiased sons are twice as consanguinous to their mothers as daughters, because all of a son's genes derive from his mother, and these two effects exactly cancel to recover the usual equal sex-allocation optimum (Fisher 1930; Hamilton 1967; Gardner 2014). In contrast, female embryos, being clonally related to their sisters, prefer a female-biased sex allocation ($z < 1/2$); male embryos, being clonally related to their brothers, prefer a male-biased sex allocation ($z > 1/2$); and fathers, being entirely unrelated to the sons of their mating partners, prefer all offspring to be female ($z = 0$). If there is incomplete dispersal of males before mating ($0 < d_m < 1$), then this results in local mate competition (i.e., related males competing for mating opportunities), which results in mothers, daughters, and sons all preferring a sex allocation that is relatively female biased (lower z). Moreover, if there is also incomplete dispersal of females before mating ($d_f < 1$), then, because of inbreeding, fathers are related to the sons of their mating partners and accordingly prefer nonzero investment into males ($z > 0$). Finally, in the limit of the complete absence of male dispersal before mating ($d_m \to 0$), local mate competition is complete and all parties prefer vanishingly small investment into males ($z \to 0$; Hamilton 1967).

An intragenomic conflict over sex ratio arises when a female embryo's maternal-origin and paternal-origin genes have different sex-ratio optima. Substituting the appropriate parent-of-origin-specific coefficients of consanguinity (table 1) into equation (3) yields the optima

$$z_{f|M} = \frac{(2 - d_m)d_m}{3 + (1 - d_m)(d_m - d_f)} \quad (4)$$

Table 1: Summary of the consanguinities used in the analysis

Genealogical relationship	Symbol	Value	In terms of model parameters
Mating partners	p_{MP}	$(1 - d_f)(1 - d_m)p_{mf}$	$\frac{(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Male to himself/brother to brother	p_{mm}	1	1
Sister to brother	p_{fm}	$(p_{ff} + p_{MP})/2$	$\frac{1}{4-3(1-d_f)(1-d_m)}$
Female to herself/sister to sister	p_{ff}	$(1 + p_{MP})/2$	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Sister to sister maternal	$p_{ff\|M}$	p_{ff}	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Sister to sister paternal	$p_{ff\|P}$	p_{ff}	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Brother to sister	p_{mf}	p_{fm}	$\frac{1}{4-3(1-d_f)(1-d_m)}$
Brother to sister maternal	$p_{mf\|M}$	p_{ff}	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Brother to sister paternal	$p_{mf\|P}$	p_{MP}	$\frac{(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Daughter to father	p_{fP}	p_{ff}	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Son to father	p_{mP}	p_{MP}	$\frac{(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$
Daughter to mother	p_{fM}	$(p_{ff} + p_{MP})/2$	$\frac{1}{4-3(1-d_f)(1-d_m)}$
Son to mother	p_{mM}	p_{ff}	$\frac{2-(1-d_f)(1-d_m)}{4-3(1-d_f)(1-d_m)}$

Note: Because of the possible inbreeding via failure to disperse, the consanguinity coefficients depend on each other. By using those dependencies, we can solve and express the consanguinity coefficients with the model parameters. The focal dispersing tendencies and the number of emerging males and females are assumed to follow the population average. Therefore, the probability that mating partners are siblings can be simplified to a probability that they both failed to disperse ($(1 - d_f)(1 - d_m)$). The dependencies are presented in the "Value" column, from which the exact values are solved and represented in the last column. In the indices, "f" represents a female embryo, "m" a male embryo, "M" mother and the maternal genome, and "P" father and the paternal genome.

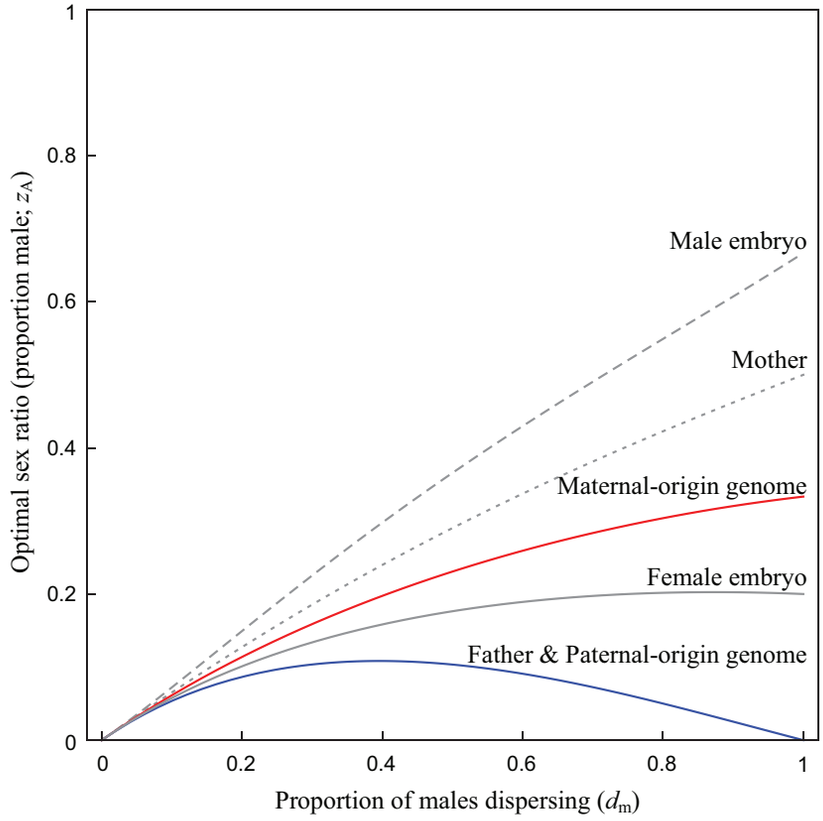


Figure 1: Illustration of the sex-ratio optima z_A. The lines represent the sex-ratio optima with zero female dispersal ($d_f = 0$) with respect to the proportion of males dispersing (d_m). They are calculated from equation (3) by substituting the appropriate consanguinity coefficients given in table 1. Actors whose optima are presented, in order from the top, are the male embryo (dashed gray line), the mother (dotted gray line), the maternal-origin genome of the female embryo (red line), the female embryo as an individual (solid gray line), the paternal-origin genome of the female embryo (blue line), and the father of the brood (same blue line). The sex-ratio optima for the male and female embryos and the mother (gray lines) were previously presented by Gardner et al. (2007*a*, 2007*b*), and they are recovered from our equation (3).

for the female embryo's maternal-origin genes and

$$z_{f|P} = \frac{(1 - d_f)(1 - d_m)(2 - d_m)d_m}{3 + d_f^2(1 - d_m)^2 + (1 - d_m)(2d_m + d_f d_m^2) + d_m^3} \quad (5)$$

for her paternal-origin genes. These distinct sex-ratio optima are illustrated in figure 1.

We find that the female embryo's maternal-origin genes prefer a greater proportion of males among the emerging adults than do the female embryo's paternal-origin genes ($z_{f|M} > z_{f|P}$ for all d_f, $d_m > 0$). This is because the female embryo is more related to her brothers through her mother than through her father, because the entire brood has the same mother but only the female embryos have a father. Note that, although the female embryo's father makes no direct genetic contribution to her brothers, her paternal-origin genes are nevertheless consanguinous with her brother's genes to the extent that the female's mother and father were relatives (i.e., insofar as there is inbreeding). Accordingly, the female's paternal-origin genes need not always favor an entirely female-biased sex ratio. Also note that, while the sex-ratio optimum for the female embryo's maternal-origin genes is distinct from that of her mother's genes, the sex-ratio optimum for the female embryo's paternal-origin genes is exactly the same as that of her father's genes. This is because, while the female embryo's maternal genome is genetically distinct from her mother's genome (the former is a random haploid subset of the latter's diploid set of genes), the female embryo's paternal genome is genetically identical to her father's genome (de novo mutation aside; her father has only a single haploid genome to contribute to each of his daughters). More generally, this point clarifies that conflicts between maternal-origin and paternal-origin genes are conceptually distinct from conflicts between an individual's parents.

Intragenomic Conflict over Soldiering

We now investigate the evolutionary interests of a female's maternal-origin versus paternal-origin genes with respect to soldiering. We consider that the proportion x of female embryos developing as soldiers modulates both the sex ratio z and the number N of adults emerging from the host. The male mating success M is modulated by the number of males and females emerging from the host and therefore by x (and, to be precise, also by the population average $\bar{x}$). Rewriting equation (1) as $H_A(x) = N_m(x)M(x)p_{mA} + 2N_f(x)p_{fA}$ to make this dependency explicit, any actor favors greater-than-population-average female soldiering when

$$\left.\frac{\partial H_A}{\partial x}\right|_{x=\bar{x}} = p_{mA}\left.\frac{\partial (N_m M)}{\partial x}\right|_{x=\bar{x}} + 2p_{fA}\left.\frac{\partial N_f}{\partial x}\right|_{x=\bar{x}} > 0.$$

And so, on the assumption that the focal individual's and population-average probability of developing as a soldier are both at the female's own optimum (i.e., $x = \bar{x} = x_f$, hereafter denoted by an asterisk for ease of presentation) and that this takes an intermediate value (i.e., $0 < x_f < 1$), we may write

$$\left.\frac{\partial (N_m M)}{\partial x}\right|_{*} = -2\frac{p_{ff}}{p_{mf}}\left.\frac{\partial N_f}{\partial x}\right|_{*}. \tag{6}$$

It follows that, from the perspective of the female embryo's maternal-origin genes, the marginal inclusive fitness is

$$\begin{aligned}\left.\frac{\partial H_{f|M}}{\partial x}\right|_{*} &= -2p_{mf|M}\frac{p_{ff}}{p_{mf}}\left.\frac{\partial N_f}{\partial x}\right|_{*} + 2p_{ff|M}\left.\frac{\partial N_f}{\partial x}\right|_{*} \\ &= 2p_{ff}\left(1 - \frac{p_{mf|M}}{p_{mf}}\right)\left.\frac{\partial N_f}{\partial x}\right|_{*},\end{aligned}$$

and, from the perspective of the female embryo's paternal-origin genes, the marginal inclusive fitness is

$$\begin{aligned}\left.\frac{\partial H_{f|P}}{\partial x}\right|_{*} &= -2p_{mf|P}\frac{p_{ff}}{p_{mf}}\left.\frac{\partial N_f}{\partial x}\right|_{*} + 2p_{ff|P}\left.\frac{\partial N_f}{\partial x}\right|_{*} \\ &= 2p_{ff}\left(1 - \frac{p_{mf|P}}{p_{mf}}\right)\left.\frac{\partial N_f}{\partial x}\right|_{*},\end{aligned}$$

where we have made use of the fact that $p_{ff|M} = p_{ff|P} = p_{ff}$, that is, that the consanguinity of a female to herself or to her clonal sister is the same for her maternal-origin and her paternal-origin genes. In the appendix, we show that $(\partial N_f/\partial x)|_* < 0$ under the brood-benefit hypothesis and that $(\partial N_f/\partial x)|_* > 0$ under the sex-ratio-conflict hypothesis. Since $p_{mf|P} < p_{mf} < p_{mf|M}$, it follows that $(\partial H_{f|P}/\partial x)|_* < 0 < (\partial H_{f|M}/\partial x)|_*$ under the brood-benefit hypothesis and $(\partial H_{f|M}/\partial x)|_* < 0 < (\partial H_{f|P}/\partial x)|_*$ under the sex-ratio-conflict hypothesis.

We have found that the female embryo's maternal-origin genes are favored to increase her probability of developing as a soldier under the brood-benefit hypothesis and favored to decrease her probability of developing as a soldier under the sex-ratio-conflict hypothesis, whereas her paternal-origin genes are favored to decrease her probability of developing as a soldier under the brood-benefit hypothesis and favored to increase her probability of developing as a soldier under the sex-ratio-conflict hypothesis. That is, we predict an intragenomic conflict of interest with respect to female soldier development, with a direction that depends on the function of the soldier caste.

Genomic Imprinting

Having ascertained the existence and direction of the conflict of interest between the female embryo's maternal-origin genes and her paternal-origin genes with respect to her probability of developing as a sterile soldier, we now elaborate predictions for patterns of genomic imprinting (fig. 2). For loci whose gene products modulate a female embryo's probability of developing as a soldier, we expect there to be a disagreement between her maternal-origin genes and her paternal-origin genes over the optimal level of gene expression, and, where parent-of-origin-specific gene expression is feasible, we expect such genomic imprinting to evolve as a consequence of this disagreement. According to the "loudest-voice-prevails" principle, the gene that prefers a lower level of expression ultimately silences itself while the gene that prefers a greater level of expression ultimately wins the conflict and expresses at a level corresponding to its optimum (Haig 1996; Úbeda and Haig 2003).

Under the brood-benefit hypothesis, we expect that the female embryo's maternal-origin genes will prefer a greater allocation to soldiering than will her paternal-origin genes. Accordingly, considering loci whose gene products promote soldier development ("soldier promoters"), we expect that her maternal-origin genes will prefer a greater level of gene expression and that her paternal-origin genes will prefer a lower level of gene expression, and so we predict that soldier promoters will be maternally expressed and paternally silenced (fig. 2*A*). And considering loci whose gene products inhibit soldier development ("soldier inhibitors"), we expect that her maternal-origin genes will prefer a lower level of gene expression and that her paternal-origin genes will prefer a greater level of gene expression, and so we predict that soldier inhibitors will be maternally silenced and paternally expressed (fig. 2*A*).

Conversely, under the sex-ratio-conflict hypothesis, we expect that the female embryo's maternal-origin genes will prefer a lower allocation to soldiering than will her paternal-origin genes. Accordingly, considering loci whose gene products promote soldier development, we expect

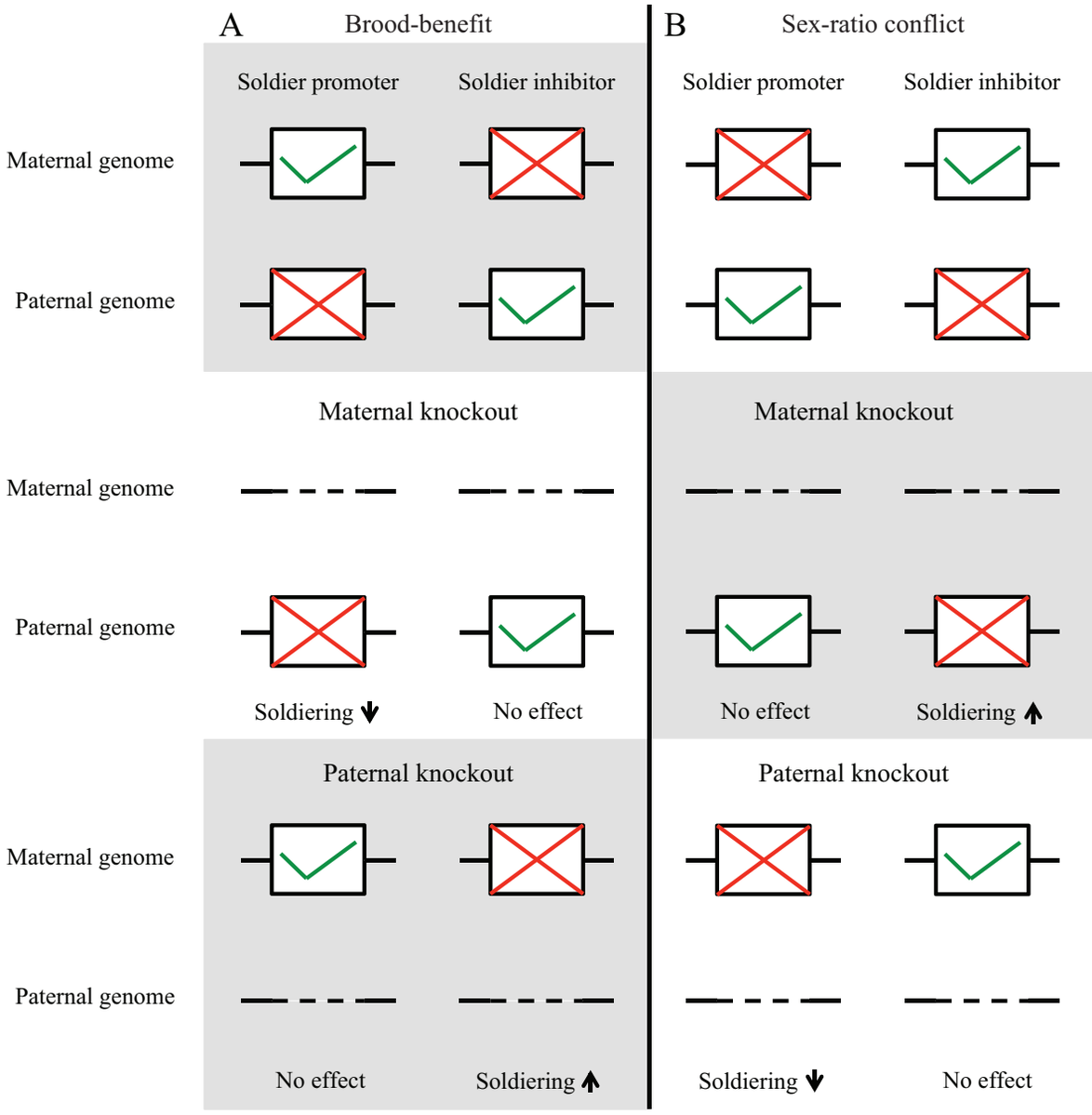


Figure 2: Predictions for patterns of genomic imprinting. Under the brood-benefit hypothesis (*A*), we predict that soldier promoters will be maternally expressed (green) and paternally silenced (red) and that soldier inhibitors will be maternally silenced and paternally expressed. Under the sex-ratio-conflict hypothesis (*B*), the predictions are reversed: the soldier promoters will be maternally silenced and paternally expressed, and the soldier inhibitors will be maternally expressed and paternally silenced. Under both hypotheses, a knockout mutation is expected to reduce soldier development (downward arrow) when the gene is a soldier promoter, enhance soldier development (upward arrow) when it is a soldier inhibitor, and have no effect when the gene is silenced.

that her maternal-origin genes will prefer a lower level of gene expression and that her paternal-origin genes will prefer a greater level of gene expression, and so we predict that soldier promoters will be maternally silenced and paternally expressed (fig. 2*B*). And considering loci whose gene products inhibit soldier development, we expect that her maternal-origin genes will prefer a greater level of gene expression and that her paternal-origin genes will prefer a lower level of gene expression, and so we predict that soldier inhibitors will be maternally expressed and paternally silenced (fig. 2*B*).

Such genomic imprinting is expected to modulate the phenotypic consequences of gene knockouts. A loss-of-function mutation that prevents the affected gene from ex-

pressing a functional gene product is expected to have no impact on the phenotype if that gene is predicted to be silenced anyway. Accordingly, under the brood-benefit hypothesis, a knockout mutation is expected to have no impact on the soldiering phenotype when the gene is a paternal-origin soldier promoter or a maternal-origin soldier inhibitor (fig. 2*A*), but the knockout mutation is expected to reduce soldier development when the gene is a maternal-origin soldier promoter and to enhance soldier development when it is a paternal-origin soldier inhibitor (fig. 2*A*). Conversely, under the sex-ratio-conflict hypothesis, a knockout mutation is expected to have no impact on the soldiering phenotype when the gene is a maternal-origin soldier promoter or a paternal-origin soldier inhibitor (fig. 2*B*), but the knockout mutation is expected to reduce soldier development when it is a paternal-origin soldier promoter and to enhance soldier development when it is a maternal-origin soldier inhibitor (fig. 2*B*).

Discussion

Our analysis concerns the function of the sterile-soldier caste of polyembryonic parasitoid wasps. We have demonstrated that an intragenomic conflict of interest may arise between a female embryo's maternal-origin genes and her paternal-origin genes, ultimately with respect to the sex ratio of the reproductive adults emerging from the parasitized host and more proximately with respect to her own propensity for developing as a sterile soldier. In particular, we have found that, because the female embryo is relatively more related to her brothers through her mother than through her father, her maternal-origin genes prefer the sex ratio to be less female biased than do her paternal-origin genes. Consequently, if the primary function of soldiers is to altruistically benefit the brood overall, such that female soldiers tend to reduce the reproductive success of the female brood and increase the reproductive success of the male brood, then her maternal-origin genes prefer a greater probability of developing as a soldier than do her paternal-origin genes, whereas if the primary function of the soldiers is to spitefully distort the sex ratio in favor of their own sex, at a cost to the overall reproductive success of the brood, such that female soldiers tend to increase the reproductive success of the female brood and decrease the reproductive success of the male brood, then her maternal-origin genes prefer a lower probability of developing as a soldier than do her paternal-origin genes.

Moreover, we have related this intragenomic conflict of interest to patterns of genomic imprinting of loci underpinning soldier development, deriving contrasting predictions that may be used to discriminate between the brood-benefit and sex-ratio-conflict hypotheses for soldier function. In particular, we predict that under the brood-benefit hypothesis, soldier-promoter genes will tend to be maternally expressed and soldier-inhibitor genes will tend to be paternally expressed, whereas under the sex-ratio-conflict hypothesis, soldier-promoter genes will tend to be paternally expressed and soldier-inhibitor genes will tend to be maternally expressed. Furthermore, we have related these patterns of genomic imprinting to predictions for when loss-of-function mutations will have an impact on the phenotype and in which direction, which will further aid empirical discrimination between the brood-benefit and sex-ratio-conflict hypotheses for soldier function. Importantly, the loudest-voice-prevails logic (Haig 1996; Úbeda and Haig 2003) underpinning our predictions of genomic imprinting depends only on the existence and direction—and not the intensity—of intragenomic conflict. Accordingly, our predictions are robust to variation in demographic assumptions concerning, for example, patterns of dispersal that modulate the intensity but not the existence or direction of conflict (cf. Farrell et al. 2015).

Sterile- (or reduced-reproductive-)soldier castes are known from a number of taxa, and—the copidosomatine encyrtids excepted—their primary function is generally understood to be one of brood benefit, for example, nest defense. As the patterns of genomic imprinting predicted by our analysis owe to the basic asymmetry of haplodiploid inheritance (and not the bizarre biology of polyembryony per se), we expect that these predictions will apply widely to female soldiers in many haplodiploid taxa (e.g., eusocial thrips; Crespi 1992). Other asymmetries—such as multiple mating and sex biases in dispersal, mortality, and variance in reproductive success (e.g., Úbeda and Gardner 2012)—have been suggested to drive the evolution of genomic imprinting under diploid inheritance, but here the predicted patterns of imprint are less clear-cut. Accordingly, the scope for genomic imprinting in relation to soldiering in diploids (e.g., eusocial trematodes; Hechinger et al. 2011) represents an avenue for future study.

Parent-of-origin-specific gene expression is well documented in mammals and flowering plants; here, modification of DNA by means of the addition of a methyl group provides a mechanism for regulating gene expression and associated differentiation of cellular tissues, and the differential transmission of methyl modifications via female and male gametes provides the molecular paradigm for parent-of-origin gene effects (Ferguson-Smith 2011). In contrast, the scope for such effects among insects is highly controversial. Previously, the main reason for suspecting that they are unimportant has been the lack of key DNA methylation enzymes in fruit flies (Yan et al. 2014). However, there is now strong evidence of methylation-mediated caste differentiation in the social Hymenoptera (Wang et al. 2006; Kucharski et al. 2008; Herb et al. 2012; Amarasinghe et al. 2014; Yan et al. 2014, 2015), where DNA methylation is widespread (Kronforst et al.

2008), and disruption of DNA methylation has recently been shown to affect sex allocation in the parasitoid wasp *Nasonia vitripennis* (Cook et al. 2015). It is also conceivable that insects could also employ other molecular mechanisms to achieve parent-of-origin-specific gene expression. Moreover, parent-of-origin-specific phenotypic effects have recently been described in relation to social traits of honeybees (Oldroyd et al. 2014), and some retention of parent-of-origin information presumably occurs in those insect taxa in which males routinely eliminate their entire paternal genome during spermatogenesis (Ferguson-Smith 2011; Gardner and Ross 2014).

This study of soldiering in polyembryonic parasitoid wasps has demonstrated that the kinship theory of genomic imprinting may provide a powerful tool for exploring social evolution, not only in terms of understanding the adaptations of genes engaged in intragenomic conflicts but also in terms of elucidating the adaptations of individual organisms. Here, we have highlighted the problem of confounding in comparative analyses, that is, that different populations and different individuals may differ in many respects and rarely for a single explanatory variable. Specifically, Gardner et al. (2007*a*) interpreted an observed sex difference in soldier allocation as a reflection of sex difference in selection pressures and, accordingly, inferred that the function of soldiers lies in sex-ratio conflict as opposed to brood benefit; but it is feasible that a sex difference in inherent soldiering ability is instead responsible for this pattern and that the sex difference in soldiering does not provide any clues as to the soldiers' function. By reframing our comparative analysis at the within-individual level, that is, between a single individual's maternal-origin and paternal-origin genes, we have eliminated the confounding effect of sex differences in inherent soldiering ability (and many other confounds) and have derived a new set of empirically testable predictions for discriminating the function of soldiers.

Moreover, the relative lack of existing data on parent-of-origin-specific patterns of gene expression provides exciting avenues for truly independent tests of social-evolution theory (Queller and Strassmann 2002; Queller 2003; Wild and West 2009). Often, new theoretical developments on the topic of social evolution are put to empirical test using much the same sources of data that have served as inspiration for the theory in the first place: such circularity is inevitable, considering how intensely biological research is focused on a small number of study species. So the possibility of deriving clear-cut predictions about parent-of-origin-specific patterns of gene expression, and the resulting phenotypic effects of gene knockouts, in a taxon for which there is no a priori information about such patterns, represents a rare opportunity for subjecting social-evolution theory to proper empirical evaluation.

Acknowledgments

This work was supported by a European Society for Evolutionary Biology Godfrey Hewitt Mobility Award (P.R.), the Finnish Centre of Excellence in Biological Interactions (P.R.), and a Natural Environment Research Council Independent Research Fellowship (A.G.; grant NE/K009524/1). We would like to thank J. Boomsma, H. Helanterä, A. Micheletti, M. Puurtinen, L. Ross, T. S. Salminen, and J. Wilkins for helpful comments and discussion.

APPENDIX A

FEMALE EMERGENCE UNDER THE BROOD-BENEFIT AND SEX-RATIO-CONFLICT HYPOTHESES

Here we show how the number of emerging females behaves at the female's soldiering optimum under both hypotheses, that is, when $(\partial N_f/\partial x)|_*$ is positive and when it is negative. The consequences of increasing female soldiering can be divided into three effects: (1) increasing female soldiering leads to a partial decrease in the number of emerging females; (2) the action of these extra soldiers increases the number of emerging males under the brood-benefit hypothesis ($(\partial N_m/\partial x)|_* > 0$) and decreases the number of emerging males under the sex-ratio-conflict hypothesis ($(\partial N_m/\partial x)|_* < 0$); (3) more resources are freed up (i.e., from soldier-killed competitors or soldier-macerated host tissue under the brood-benefit hypothesis, from soldier-killed brothers under the sex-ratio-conflict hypothesis, and from soldiers potentially requiring fewer resources to develop than do reproductive-destined larvae under both hypotheses), and this leads to a partial increase in the number of emerging females under both hypotheses. The total change in the number of emerging females, $\partial N_f/\partial x$, is the combined effect of 1 and 3. If effect 2 were null, then this would mean that increasing the female soldiering does not free up any resources, and so effect 3 would also be null; that is, the only consequence of increasing the allocation to female soldiering would be effect 1 and hence fewer emerging females. Therefore, the changes in the number of emerging males ($\partial N_m/\partial x$) and females ($\partial N_f/\partial x$) cannot both be 0 at the same time, especially at the female's soldiering optimum x_f.

Multiplying the male mating success M with the number of emerging males N_m gives the total mating success for the progenitor male egg. This total number can be divided into two components: matings achieved by nondispersing males and matings achieved by dispersing males. Both of these components increase as the number of emerging males increases, the first component because, with more emerging males, the local mating pool has a larger frequency

of focal males competing for an unchanged amount of available matings and the second component because then there are more males pursuing matings outside the focal host, which has an insignificant effect on the male mating success. Therefore, the total mating success of the male egg increases with the number of emerging males ($\partial(N_m M)/\partial N_m > 0$). Increasing the number of emerging females can only increase the total mating success of the male egg, by increasing the number of mating opportunities in the local mating pool ($\partial(N_m M)/\partial N_f \geq 0$).

Rewriting equation (6), using the two-dimensional chain rule, we have

$$\frac{\partial(N_m M)}{\partial N_m}\left|_* \frac{\partial N_m}{\partial x}\right|_* = -\frac{\partial N_f}{\partial x}\left|_* \left(2\frac{p_{ff}}{p_{mf}} + \frac{\partial(N_m M)}{\partial N_f}\right|_*\right). \tag{A1}$$

From the above line of argument—especially effect 2 of increasing female soldiering—we see from equation (A1) that $(\partial N_f/\partial x)|_* < 0$ under the brood-benefit hypothesis and $(\partial N_f/\partial x)|_* > 0$ under the sex-ratio-conflict hypothesis.

Literature Cited

Abbot, P., J. Abe, J. Alcock, S. Alizon, J. A. C. Alpedrinha, M. Andersson, J.-B. Andre, M. van Baalen, et al. 2011. Inclusive fitness theory and eusociality. Nature 471:E1–E4. doi:10.1038/nature09831.

Amarasinghe, H. E., C. I. Clayton, and E. B. Mallon. 2014. Methylation and worker reproduction in the bumble-bee (*Bombus terrestris*). Proceedings of the Royal Society B: Biological Sciences 281: 20132502. doi:10.1098/rspb.2013.2502.

Boomsma, J. J. 2007. Kin selection versus sexual selection: why the ends do not meet. Current Biology 17:R673–R683.

———. 2009. Lifetime monogamy and the evolution of eusociality. Philosophical Transactions of the Royal Society B: Biological Sciences 364:3191–3207.

———. 2013. Beyond promiscuity: mate-choice commitments in social breeding. Philosophical Transactions of the Royal Society B: Biological Sciences 368:20120050. doi:10.1098/rstb.2012.0050.

Bulmer, M. 1994. Theoretical evolutionary ecology. Sinauer, Sunderland, MA.

Cook, N., B. A. Pannebakker, E. Tauber, and D. M. Shuker. 2015. DNA methylation and sex allocation in the parasitoid wasp *Nasonia vitripennis*. American Naturalist 186:513–518. doi:10.1086/682950.

Crespi, B. J. 1992. Eusociality in Australian gall thrips. Nature 359: 724–726.

Cruz, Y. P. 1981. A sterile defender morph in a polyembryonic hymenopterous parasite. Nature 294:446–447.

———. 1986. The defender role of the precocious larvae of *Copidosomopsis tanytmemus* Caltagirone (Encyrtidae, Hymenoptera). Journal of Experimental Zoology 237:309–318.

Doutt, R. L. 1947. Polyembryony in *Copidosoma koehleri* Blanchard. American Naturalist 81:435–453.

Farrell, E. J., F. Úbeda, and A. Gardner. 2015. Intragenomic conflict over dispersal. American Naturalist 186:E61–E71. doi:10.1086/682275.

Ferguson-Smith, A. C. 2011. Genomic imprinting: the emergence of an epigenetic paradigm. Nature Reviews Genetics 12:565–575.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Foster, K. R., T. Wenseleers, and F. L. W. Ratnieks. 2006. Kin selection is the key to altruism. Trends in Ecology and Evolution 21:57–60.

Gardner, A. 2014. Total reproductive value of juvenile females is twice that of juvenile males under X-linkage and haplodiploidy. Journal of Theoretical Biology 359:236–237.

Gardner, A., J. Alpedrinha, and S. A. West. 2012. Haplodiploidy and the evolution of eusociality: split sex ratios. American Naturalist 179:240–256.

Gardner, A., I. C. W. Hardy, P. D. Taylor, and S. A. West. 2007*a*. Spiteful soldiers and sex ratio conflict in polyembryonic parasitoid wasps. American Naturalist 169:519–533.

———. 2007*b*. Correction. American Naturalist 169:837.

Gardner, A., and L. Ross. 2014. Mating ecology explains patterns of genome elimination. Ecology Letters 17:1602–1612.

Gardner, A., and J. J. Welch. 2011. A formal theory of the selfish gene. Journal of Evolutionary Biology 24:1801–1813.

Giron, D., D. W. Dunn, I. C. W. Hardy, and M. R. Strand. 2004. Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. Nature 430:676–679.

Godfray, H. C. J. 1992. Strife among siblings. Nature 360:213–214.

Grbić, M., P. J. Ode, and M. R. Strand. 1992. Sibling rivalry and brood sex ratios in polyembryonic wasps. Nature 360:254–256.

Haig, D. 1996. Placental hormones, genomic imprinting, and maternal-fetal communication. Journal of Evolutionary Biology 9:357–380.

———. 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. Proceedings of the Royal Society B: Biological Sciences 264:1657–1662.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I, II. Journal of Theoretical Biology 7:1–16, 17–52.

———. 1967. Extraordinary sex ratios. Science 156:477–488.

———. 1972. Altruism and related phenomena, mainly in social insects. Annual Review of Ecology and Systematics 3:193–232.

Hardy, I. C. W. 1994. Sex ratio and mating structure in the parasitoid Hymenoptera. Oikos 69:3–20.

Harvey, J. A., L. S. Corley, and M. R. Strand. 2000. Competition induces adaptive shifts in caste ratios of a polyembryonic wasp. Nature 406:183–186.

Hechinger, R. F., A. C. Wood, and A. M. Kuris. 2011. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. Proceedings of the Royal Society B: Biological Sciences 278:656–665.

Herb, B. R., F. Wolschin, K. D. Hansen, M. J. Aryee, B. Langmead, R. Irizarry, G. V. Amdam, and A. P. Feinberg. 2012. Reversible switching between epigenetic states in honeybee behavioral subcastes. Nature Neuroscience 15:1371–1373.

Ivanova-Kasas, O. M. 1972. Polyembryony in insects. Pages 243–271 *in* S. J. Counce and C. H. Waddington, eds. Developmental systems: insects. Vol. 1. Academic Press, New York.

Keasar, T., M. Segoli, R. Barak, S. Steinberg, D. Giron, M. R. Strand, A. Bouskila, and A. R. Harari. 2006. Costs and consequences of superparasitism in the polyembryonic parasitoid *Copidosoma koehleri* (Hymenoptera: Encyrtidae). Ecological Entomology 31:277–283.

Kronforst, M. R., D. C. Gilley, J. E. Strassmann, and D. C. Queller. 2008. DNA methylation is widespread across social Hymenoptera. Current Biology 18:287–288.

Kucharski, R., J. Maleszka, S. Foret, and R. Maleszka. 2008. Nutritional control of reproductive status in honeybees via DNA methylation. Science 319:1827–1830.

Liao, X., S. Rong, and D. C. Queller. 2015. Relatedness, conflict, and the evolution of eusociality. PLoS Biology 13:e1002098. doi:10.1371/journal.pbio.1002098.

Moore, T., and D. Haig. 1991. Genomic imprinting in mammalian development: a parental tug-of-war. Trends in Genetics 7:45–49.

Nowak, M. A., C. E. Tarnita, and E. O. Wilson. 2010. The evolution of eusociality. Nature 466:1057–1062.

Ode, P. J., and M. S. Hunter. 2002. Sex ratios of parasitic Hymenoptera with unusual life histories. Pages 218–234 *in* I. C. W. Hardy, ed. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Ode, P. J., and M. R. Strand. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. Journal of Animal Ecology 64:213–224.

Oldroyd, B. P., M. H. Allsopp, K. M. Roth, E. J. Remnant, R. A. Drewell, and M. Beekman. 2014. A parent-of-origin effect on honeybee worker ovary size. Proceedings of the Royal Society B: Biological Sciences 281:20132388. doi:10.1098/rspb.2013.2388.

Queller, D. C. 2003. Theory of genomic imprinting conflict in social insects. BMC Evolutionary Biology 3:15. doi:10.1186/1471-2148-3-15.

Queller, D. C., and J. E. Strassmann. 2002. The many selves of social insects. Science 296:311–313.

Ross, L., A. Gardner, N. Hardy, and S. A. West. 2013. Ecology, not the genetics of sex determination, determines who helps in eusocial populations. Current Biology 23:2383–2387.

Silvestri, F. 1906. Contribuzioni alla conoscenza biologica degli imenotteri parassiti. I. Biologia del *Litomastix truncatellus* (Dalm.) (secunda nota preliminare). Annali della Regia Scuola Superiore di Agricoltura di Portici 6:3–51.

Strand, M. R. 2009. Polyembryony. Pages 821–825 *in* V. H. Resh and R. T. Cardé, eds. Encyclopedia of insects. 2nd ed. Academic Press, London.

Strand, M. R., J. A. Johnson, and J. D. Culin. 1990. Intrinsic interspecific competition between the polyembryonic parasitoid *Copidosoma floridanum* and solitary endoparasitoid *Microplitis demolitor* in *Pseudoplusia includens*. Entomologia Experimentalis et Applicata 55:275–284.

Úbeda, F., and A. Gardner. 2012. A model for genomic imprinting in the social brain: elders. Evolution 66:1567–1581.

Úbeda, F., and D. Haig. 2003. Dividing the child. Genetica 117:103–110.

Wang, Y., M. Jorda, P. L. Jones, R. Maleszka, X. Ling, H. M. Robertson, C. A. Mizzen, M. A. Peinado, and G. E. Robinson. 2006. Functional CpG methylation system in a social insect. Science 314:645–647.

West, S. A. 2009. Sex allocation. Princeton University Press, Princeton, NJ.

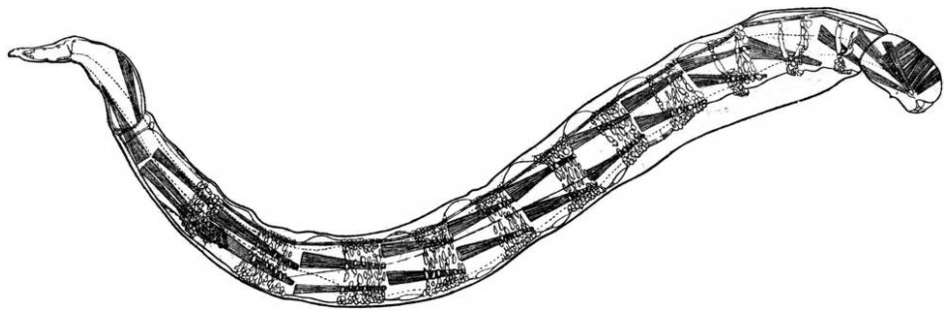
Wild, G., and S. A. West. 2009. Genomic imprinting and sex allocation. American Naturalist 173:E1–E14.

Wilson, E. O. 2005. Kin selection as the key to altruism: its rise and fall. Social Research 72:159–166.

Yan, H., R. Bonasio, D. F. Simola, J. Liebig, S. L. Berger, and D. Reinberg. 2015. DNA methylation in social insects: how epigenetics can control behavior and longevity. Annual Review of Entomology 60:435–452.

Yan, H., D. F. Simola, R. Bonasio, J. Liebig, S. L. Berger, and D. Reinberg. 2014. Eusocial insects as emerging models for behavioural epigenetics. Nature Reviews Genetics 15:677–688.

Associate Editor: William T. Wcislo
Editor: Judith L. Bronstein



"What, then, is the function of these asexual larvae, whose structure is so unusual, indeed unique, among all known insect larvae?" From "Contribuzioni alla conoscenza biologica degli Imenotteri parassiti I: Biologia del *Litomastix truncatellus* (Dalm.) (2° nota preliminare)" by Filippo Silvestri (*Annali della Regia Scuola Superiore di Agricoltura di Portici*, 1906, 6:3–51). Credit: Dipartimento di Agraria, Università degli Studi di Napoli Federico II (image); A. Micheletti (translation).