# Intragenomic conflict over soldier allocation in polyembryonic parasitoid wasps

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**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 testbed 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 sex-ratio-conflict hypotheses of soldier function.

## 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; Abbott et al 2011; Gardner et al 2012; Liao et al 2015). An amenable empirical testbed 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 latter (Ivanova-Kasas 1972). An even curiouser aspect of their biology is that some of these embryos - mostly, but not solely, females - develop precociously as soldier larvae that patrol 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 2007a). 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 & Hunter 2002; Giron et al 2004). Gardner et al (2007a) 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; Grbic et al 1992; Ode & Strand 1995; Giron et al 2004; Keasar et al 2006), Gardner et al's (2007a) 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 2007a). This view mirrors the more general 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 upon the relative preadaptation of females and males to soldiering. In particular, we follow up on West's (2009, p287; see also Wild & 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, i.e. "genomic imprinting" (Moore & Haig 1991; Haig 1997). We first adapt the mathematical model of Gardner et al (2007a) 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 versus sex-ratio-conflict hypotheses with regards to soldier function.

## Model and analysis

*Basic model* - Following Gardner et al (2007a), whose model focuses mostly upon the biology of *Copidosoma floridanum*, we consider that a single foundress wasp injects two eggs - one fertilised (i.e. female) and one unfertilised (i.e. male) - into a parasitised host, with each egg proliferating clonally to give a large number of embryos, such that each embryo is genetically identical to its same-sex brood mates and is related to its opposite-sex brood mates 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 $1-d_{\mathrm{f}}$ of females and a proportion $1-d_{\mathrm{m}}$ of males remain close to their host, where they form a mating group, whereas a proportion $d_{\mathrm{f}}$ of females and a proportion $d_{\mathrm{m}}$ 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. In particular, we express the inclusive fitness of any actor A as:

$$H_{\mathrm{A}} = N_{\mathrm{m}} M p_{\mathrm{mA}} + 2N_{\mathrm{f}} p_{\mathrm{fA}}, \quad (1)$$

where: $N_{\mathrm{m}}$ is the number of males emerging from a focal host; $M$ is the average number of successful matings enjoyed by each of these males; $p_{\mathrm{mA}}$ 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 with one drawn at random from the actor, from the same locus; Bulmer 1994); $N_{\mathrm{f}}$ is the number of females emerging from the focal host; $p_{\mathrm{fA}}$ 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 $M = d_{\mathrm{m}}(\overline{N}_{\mathrm{f}}/\overline{N}_{\mathrm{m}}) + (1 - d_{\mathrm{m}})\,[(1 - d_{\mathrm{f}})N_{\mathrm{f}} + d_{\mathrm{f}}\overline{N}_{\mathrm{f}}]/[(1 - d_{\mathrm{m}})N_{\mathrm{m}} + d_{\mathrm{m}}\overline{N}_{\mathrm{m}}]$, where $\overline{N}_{\mathrm{m}}$ and $\overline{N}_{\mathrm{f}}$ 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 $N = N_{\mathrm{f}} + N_{\mathrm{m}}$ and $z = N_{\mathrm{m}}/N$, and hypothetically assigning the actor full control over the sex ratio $z$, their inclusive fitness may be written as:

$$H_{\mathrm{A}} = Nz\left[d_{\mathrm{m}}\frac{1-\bar{z}}{\bar{z}} + (1-d_{\mathrm{m}})\frac{(1-d_{\mathrm{f}})(1-z) + d_{\mathrm{f}}(1-\bar{z})}{(1-d_{\mathrm{m}})z + d_{\mathrm{m}}\bar{z}}\right]p_{\mathrm{mA}} + 2N(1-z)p_{\mathrm{fA}}, \quad (2)$$

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, i.e. when $\partial H_A/\partial z_{|\mathrm{z}=\bar{\mathrm{z}}} = [(2 - d_{\mathrm{m}})d_{\mathrm{m}}/\bar{z} + (d_{\mathrm{f}} - d_{\mathrm{m}})(1 - d_{\mathrm{m}}) - 1]Np_{\mathrm{mA}} - 2Np_{\mathrm{fA}} > 0$. Accordingly, setting marginal inclusive fitness equal to zero, and solving for $z = \bar{z} = z_A$, yields the sex ratio optimum for actor A:

$$z_{\mathrm{A}} = \frac{(2 - d_{\mathrm{m}})d_{\mathrm{m}}}{1 + (1 - d_{\mathrm{m}})(d_{\mathrm{m}} - d_{\mathrm{f}}) + 2p_{\mathrm{fA}}/p_{\mathrm{mA}}}. \quad (3)$$

Different actors may have different sex ratio optima because they may have different consanguinities to the female and male brood ($p_{\mathrm{fA}}$ & $p_{\mathrm{mA}}$). The coefficients of consanguinity for the different family members are listed in table 1. Equations (3)-(5) of Gardner et al (2007a; see also Gardner et al 2007b) 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 equation (3), above, and are illustrated in figure 1.

If all males disperse prior to mating ($d_{\mathrm{m}} = 1$), then there is full outbreeding and no local mate competition. In this case, mothers prefer an equal sex allocation ($z = ½$): 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 than are daughters, owing to all of the son's genes deriving 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 ($z < ½$); male embryos, being clonally related to their brothers, prefer a male-biased sex allocation ($z > ½$); 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 prior to 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 prior to mating ($d_f < 1$) then, owing to 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 prior to 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:

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

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, on account of the entire brood having the same mother but only the female embryos having a

159 father. Note that, although the female embryo's father makes no direct genetic contribution to her
160 brothers, her paternal-origin genes are nevertheless consanguinous with her brother's genes to the extent
161 that the female's mother and father were relatives (i.e. insofar as there is inbreeding). Accordingly, the
162 female's paternal-origin genes need not always favour an entirely female-biased sex ratio. Also note
163 that, whilst the sex-ratio optimum for the female embryo's maternal-origin genes is distinct from that of
164 her mother's genes, the sex-ratio optimum for the female embryo's paternal-origin genes is exactly the
165 same as that of her father's genes. This is because, whilst the female embryo's maternal genome is
166 genetically distinct from her mother's genome (the former is a random haploid subset of of the latter's
167 diploid set of genes), the female embryo's paternal genome is genetically identical to her father's
168 genome (*de novo* mutation aside; her father has only a single haploid genome to contribute to each of his
169 daughters). More generally, this point clarifies that conflicts between maternal-origin versus paternal-
170 origin genes are conceptually distinct from conflicts between an individual's parents.
171
172 *Intragenomic conflict over soldiering* - We now investigate the evolutionary interests of a female's
173 maternal-origin versus paternal-origin genes with respect to soldiering. We consider that the proportion
174 $x$ of female embryos developing as soldiers modulates both the sex ratio $z$ and the number $N$ of adults
175 emerging from the host. The male mating success $M$ is modulated by the number of males and females
176 emerging from the host, and therefore by $x$ (and, to be precise, also by the population average $\bar{x}$).
177 Rewriting equation (1) as $H_{\mathrm{A}}(x) = N_{\mathrm{m}}(x)M(x)p_{\mathrm{mA}} + 2N_{\mathrm{f}}(x)p_{\mathrm{fA}}$, to make this dependency explicit,
178 any actor favours a greater than the population average female soldiering when $\partial H_{\mathrm{A}}/\partial x|_{x=\bar{x}} =$
179 $p_{\mathrm{mA}}\partial(N_{\mathrm{m}}M)/\partial x|_{x=\bar{x}} + 2p_{\mathrm{fA}}\partial N_{\mathrm{f}}/\partial x|_{x=\bar{x}} > 0$. And so, upon the assumption that the focal individual's
180 and population-average probability of developing as a soldier are both at the female's own optimum (i.e.
181 $x = \bar{x} = x_{\mathrm{f}}$ which is hereafter abbreviated "*" for ease of presentation), and that this takes an
182 intermediate value (i.e. $0 < x_f < 1$), we may write

183 $$\left.\frac{\partial(N_{\mathrm{m}}M)}{\partial x}\right|_{*} = -2\frac{p_{\mathrm{ff}}}{p_{\mathrm{mf}}}\left.\frac{\partial N_{\mathrm{f}}}{\partial x}\right|_{*}. \quad (6)$$

184 It follows that, from the perspective of the female embryo's maternal-origin genes, the marginal
185 inclusive fitness is $\partial H_{\mathrm{f|M}}/\partial x|_{*} = -2p_{\mathrm{mf|M}}(p_{\mathrm{ff}}/p_{\mathrm{mf}})\partial N_{\mathrm{f}}/\partial x|_{*} + 2p_{\mathrm{ff|M}}\partial N_{\mathrm{f}}/\partial x|_{*}$
186 $= -2\left(p_{\mathrm{mf|M}}/p_{\mathrm{mf}} - 1\right)p_{\mathrm{ff}}\partial N_{\mathrm{f}}/\partial x|_{*}$ and, from the perspective of the female embryo's paternal-origin
187 genes, the marginal inclusive fitness is $\partial H_{\mathrm{f|P}}/\partial x|_{*} = -2p_{\mathrm{mf|P}}(p_{\mathrm{ff}}/p_{\mathrm{mf}})\partial N_{\mathrm{f}}/\partial x|_{*} + 2p_{\mathrm{ff|p}}\partial N_{\mathrm{f}}/\partial x|_{*} =$
188 $-2\left(p_{\mathrm{mf|P}}/p_{\mathrm{mf}} - 1\right)p_{\mathrm{ff}}\partial N_{\mathrm{f}}/\partial x|_{*}$, where we have made use of the fact that $p_{\mathrm{ff|M}} = p_{\mathrm{ff|P}} = p_{\mathrm{ff}}$, i.e. the
189 consanguinity of a female to herself or to her clonal sister is the same for her maternal-origin and her
190 paternal-origin genes. In the appendix, found online, we show that $\partial N_{\mathrm{f}}/\partial x|_{*} < 0$ under the brood-
191 benefit hypothesis and that $\partial N_{\mathrm{f}}/\partial x|_{*} > 0$ under the sex-ratio-conflict hypothesis. Since $p_{\mathrm{mf|P}} < p_{\mathrm{mf}} <$
192 $p_{\mathrm{mf|M}}$, it follows that $\left.\partial H_{\mathrm{f|P}}/\partial x\right|_{*} < 0 < \left.\partial H_{\mathrm{f|M}}/\partial x\right|_{*}$ under the brood-benefit hypothesis, and
193 $\left.\partial H_{\mathrm{f|M}}/\partial x\right|_{*} < 0 < \left.\partial H_{\mathrm{f|P}}/\partial x\right|_{*}$ under the sex-ratio-conflict hypothesis.

194 We have found that the female embryo's maternal-origin genes are favoured to increase her
195 probability of developing as a soldier under the brood-benefit hypothesis and they are favoured to
196 decrease her probability of developing as a soldier under the sex-ratio-conflict hypothesis, whereas her
197 paternal-origin genes are favoured to decrease her probability of developing as a soldier under the
198 brood-benefit hypothesis and they are favoured to increase her probability of developing as a soldier
199 under the sex-ratio-conflict hypothesis. That is, we predict an intragenomic conflict of interests with
200 respect to female soldier development, with a direction that depends on the function of the soldier caste.
201
202 *Genomic imprinting* - Having ascertained the existence and direction of the conflict of interest between
203 the female embryo's maternal-origin genes and her paternal-origin genes with respect to her probability
204 of developing as a sterile soldier, we now elaborate predictions for patterns of genomic imprinting
205 (figure 2). For loci whose gene products modulate a female embryo's probability of developing as a
206 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 whilst 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 & Haig 2003).

Under the brood-benefit hypothesis, we expect 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 her maternal-origin genes will prefer a greater level of gene expression and her paternal-origin genes will prefer a lower level of gene expression, and so we predict soldier promoters will be maternally-expressed and paternally-silenced (figure 2A). And considering loci whose gene products inhibit soldier development ("soldier inhibitors") we expect her maternal-origin genes will prefer a lower level of gene expression and her paternal-origin genes will prefer a greater level of gene expression, and so we predict soldier inhibitors will be maternally-silenced and paternally-expressed (figure 2A).

Conversely, under the sex-ratio-conflict hypothesis, we expect 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 her maternal-origin genes will prefer a lower level of gene expression and her paternal-origin genes will prefer a greater level of gene expression, and so we predict soldier promoters will be maternally-silenced and paternally-expressed (figure 2B). And considering loci whose gene products inhibit soldier development, we expect her maternal-origin genes will prefer a greater level of gene expression and her paternal-origin genes will prefer a lower level of gene expression, and so we predict soldier inhibitors will be maternally-expressed and paternally-silenced (figure 2B).

Such genomic imprinting is expected to modulate the phenotypic consequences of gene knockouts. A loss-of-function mutation that prevents the affected gene from expressing 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 (figure 2A); 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 (figure 2A). 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 (figure 2B); 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 (figure 2B).

## 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 parasitised host, and more proximately with respect to her own propensity for developing as a sterile soldier. In particular, we have found that, owing to the female embryo being 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 favour of their own sex, at a cost to the overall

257 reproductive success of the brood, such that female soldiers tend to increase the reproductive success of
258 the female brood and decrease the reproductive success of the male brood, then her maternal-origin
259 genes prefer a lower probability of developing as a soldier than do her paternal-origin genes.
260 Moreover, we have related this intragenomic conflict of interests to patterns of genomic
261 imprinting of loci underpinning soldier development, deriving contrasting predictions that may be used
262 to discriminate between the brood-benefit versus sex-ratio-conflict hypotheses for soldier function. In
263 particular, we predict that: under the brood-benefit hypothesis, soldier-promoter genes will tend to be
264 maternally-expressed and soldier-inhibitor genes will tend to be paternally-expressed; whereas under the
265 sex-ratio-conflict hypothesis, soldier-promoter genes will tend to be paternally-expressed and soldier-
266 inhibitor genes will tend to be maternally-expressed. Furthermore, we have related these patterns of
267 genomic imprinting to predictions for when loss-of-function mutations will have an impact on the
268 phenotype, and in which direction, which will further aid empirical discrimination between the brood-
269 benefit and sex-ratio-conflict hypotheses for soldier function. Importantly, the "loudest voice prevails"
270 (Haig 1996; Úbeda & Haig 2003) logic underpinning our predictions of genomic imprinting depend
271 only upon the existence and direction – and not the intensity – of intragenomic conflict. Accordingly,
272 our predictions are robust to variation in demographic assumptions concerning, for example, patterns of
273 dispersal that modulate the intensity but not the existence or direction of conflict (cf Farrell et al 2015).
274 Sterile (or reduced-reproductive) soldier castes are known from a number of taxa and – the
275 copidosomatine encyrtids excepted – their primary function is generally understood to be one of brood-
276 benefit, e.g. nest defence. As the patterns of genomic imprinting predicted by the present analysis owe to
277 the basic asymmetry of haplodiploid inheritance (and not the bizarre biology of polyembryony per se),
278 we expect that these predictions will apply widely to female soldiers in many haplodiploid taxa (e.g.
279 eusocial thrips; Crespi 1992). Other asymmetries – such as multiple mating and sex-biases in dispersal,
280 mortality and variance in reproductive success (e.g. Úbeda & Gardner 2012) – have been suggested to
281 drive the evolution of genomic imprinting under diploid inheritance, but here the predicted patterns of

282 imprint are less clear cut. Accordingly, the scope for genomic imprinting in relation to soldiering in
283 diploids (e.g. eusocial trematodes; Hechinger et al 2011) represents an avenue for future study.
284 Parent-of-origin-specific gene expression is well documented in mammals and flowering plants:
285 here, modification of DNA by means of the addition of a methyl group provides a mechanism for
286 regulating gene expression and associated differentiation of cellular tissues, and the differential
287 transmission of methyl modifications via female and male gametes provides the molecular paradigm for
288 parent-of-origin gene effects (Ferguson-Smith 2011). In contrast, the scope for such effects among
289 insects is highly controversial. Previously, the main reason for suspecting that they are unimportant has
290 been the lack of key DNA methylation enzymes in fruit flies (Yan et al 2014). However, there is now
291 strong evidence of methylation-mediated caste differentiation in the social Hymenoptera (Wang et al
292 2006; Kucharski et al 2008; Herb et al 2012; Amarasignhe et al 2014; Yan et al 2014, 2015), where
293 DNA methylation is widespread (Kronforst et al 2008), and disruption of DNA methylation has recently
294 been shown to affect sex allocation in the parasitoid wasp *Nasonia vitripennis* (Cook et al 2015). It is
295 also conceivable that insects could also employ other molecular mechanisms to achieve parent-of-
296 origin-specific gene expression. Moreover, parent-of-origin-specific phenotypic effects have recently
297 been described in relation to social traits of honeybees (Oldroyd et al 2014), and some retention of
298 parent-of-origin information presumably occurs in those insect taxa in which males routinely eliminate
299 their entire paternal genome during spermatogenesis (Ferguson-Smith 2011; Gardner & Ross 2014).
300 The present study of soldiering in polyembryonic parasitoid wasps has demonstrated that the
301 kinship theory of genomic imprinting may provide a powerful tool for exploring social evolution, not
302 only in terms of understanding the adaptations of genes engaged in intragenomic conflicts but also in
303 terms of elucidating the adaptations of individual organisms. Here, we have highlighted the problem of
304 confounding in comparative analyses, i.e. that different populations and different individuals may differ
305 in many respects, and rarely for a single explanatory variable. Specifically: Gardner et al (2007a)
306 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, i.e. 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 & Strassmann 2002; Queller 2003; Wild & 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 into a small number of study species. So the possibility for deriving clear-cut predictions about parent-of-origin-specific patterns of gene expression, and 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.

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498 **Tables**

499

| Genealogical relationship | Symbol | Value | In terms of model parameters |
|---|---|---|---|
| Mating partners | $p_{\mathrm{MP}}$ | $(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})p_{\mathrm{mf}}$ | $\frac{(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Male to himself/ brother to brother | $p_{\mathrm{mm}}$ | 1 | 1 |
| Sister to brother | $p_{\mathrm{fm}}$ | $\frac{p_{\mathrm{ff}}+p_{\mathrm{MP}}}{2}$ | $\frac{1}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Female to herself/ sister to sister | $p_{\mathrm{ff}}$ | $\frac{1+p_{\mathrm{MP}}}{2}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Sister to sister maternal | $p_{\mathrm{ff\|M}}$ | $p_{\mathrm{ff}}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Sister to sister paternal | $p_{\mathrm{ff\|P}}$ | $p_{\mathrm{ff}}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Brother to sister | $p_{\mathrm{mf}}$ | $p_{\mathrm{fm}}$ | $\frac{1}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Brother to sister maternal | $p_{\mathrm{mf\|M}}$ | $p_{\mathrm{ff}}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |

| | | | |
|---|---|---|---|
| Brother to sister paternal | $p_{\mathrm{mf\|P}}$ | $p_{\mathrm{MP}}$ | $\frac{(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Daughter to father | $p_{\mathrm{fP}}$ | $p_{\mathrm{ff}}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Son to father | $p_{\mathrm{mP}}$ | $p_{\mathrm{MP}}$ | $\frac{(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Daughter to mother | $p_{\mathrm{fM}}$ | $\frac{p_{\mathrm{ff}}+p_{\mathrm{MP}}}{2}$ | $\frac{1}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |
| Son to mother | $p_{\mathrm{mM}}$ | $p_{\mathrm{ff}}$ | $\frac{2-(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}{4-3(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})}$ |

**Table 1:** *Summary of the consanguinities used in the analysis* - Because of the possible inbreeding via failure to disperse, the consanguinity coefficients depend on each other. By using those dependences 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 ($(1-d_{\mathrm{f}})(1-d_{\mathrm{m}})$). The dependancies are presented in the 'Value' column, from which the exact values are solved and represented in the 'In terms of model parameters' column. In the indices f represents female embryo, m male embryo, M mother and maternal genome, and P father and paternal genome.

## Figure Legends

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

**Figure 2:** *Predictions for patterns of genomic imprinting* - Under the brood-benefit hypothesis (column 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 (column B) the predictions are reversed: the soldier promoters will be maternally-silenced and paternally-expressed, and the soldier inhibitors maternally-expressed and paternally-silenced. Under both hypotheses, a knockout mutation is expected to reduce soldier development (arrow down) when the gene is a soldier promoter, enhance soldier development (arrow up) when it is a soldier inhibitor, and have no effect when the gene is silenced.

## Online appendix

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

Multiplying the male mating success $M$ with the number of emerging males $N_{\mathrm{m}}$ gives the total mating success for the progenitor male egg. This total number can be divided into two components: matings achieved by non-dispersing 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; 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.

556 Therefore, the total mating success of the male egg increases with the number of emerging males
557 ($\partial(N_{\mathrm{m}}M)/\partial N_{\mathrm{m}} > 0$). Increasing the number of emerging females can only increase the total mating
558 success of the male egg, by increasing the number of mating opportunities in the local mating pool
559 ($\partial(N_{\mathrm{m}}M)/\partial N_{\mathrm{f}} \geq 0$).

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

561 $$\left.\frac{\partial(N_{\mathrm{m}}M)}{\partial N_{\mathrm{m}}}\right|_{*} \left.\frac{\partial N_{\mathrm{m}}}{\partial x}\right|_{*} = -\left.\frac{\partial N_{\mathrm{f}}}{\partial x}\right|_{*} \left(2\frac{p_{\mathrm{ff}}}{p_{\mathrm{mf}}} + \left.\frac{\partial(N_{\mathrm{m}}M)}{\partial N_{\mathrm{f}}}\right|_{*}\right). \quad \text{(A1)}$$

562 From the above line of argument – especially the effect (ii) of increasing female soldiering – we see
563 from equation (A1) that $\partial N_{\mathrm{f}}/\partial x|_{*} < 0$ under the brood-benefit hypothesis and $\partial N_{\mathrm{f}}/\partial x|_{*} > 0$ under the
564 sex-ratio-conflict hypothesis.

Figure 1

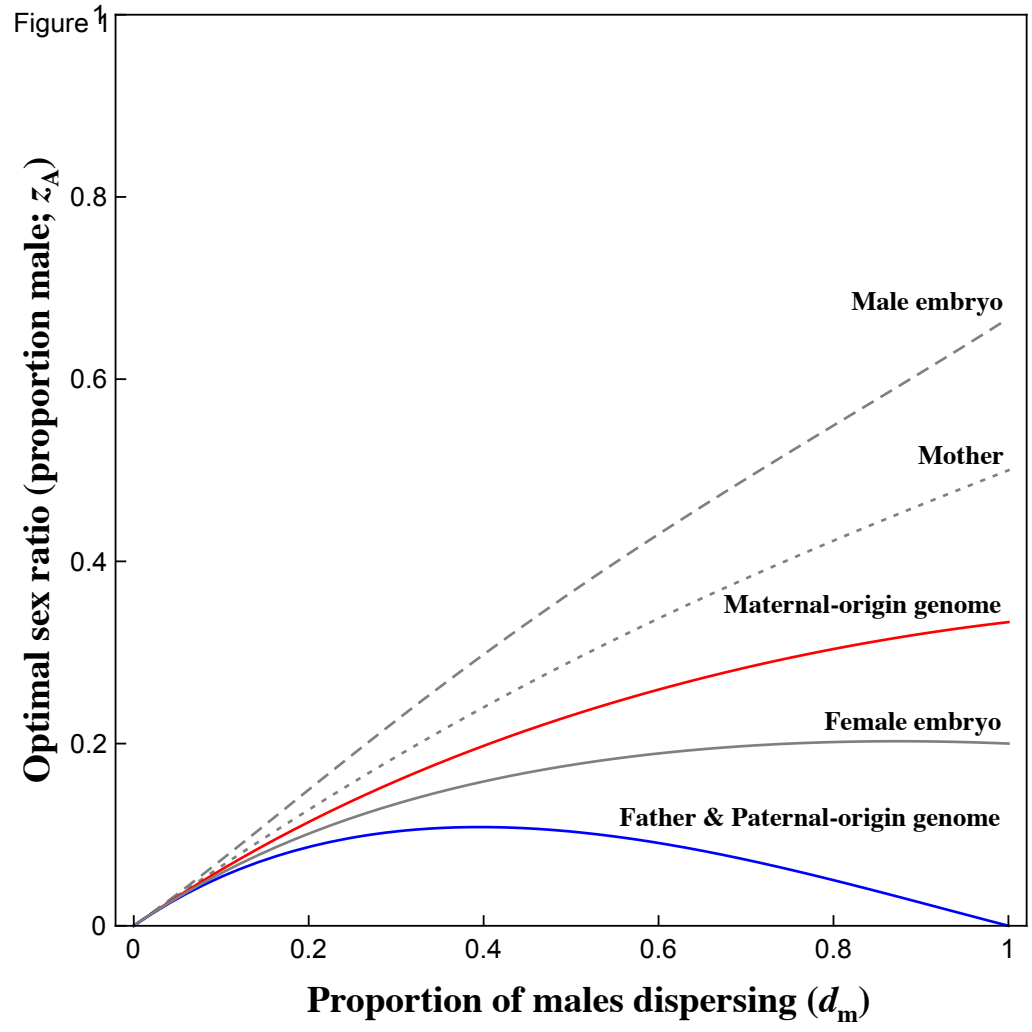
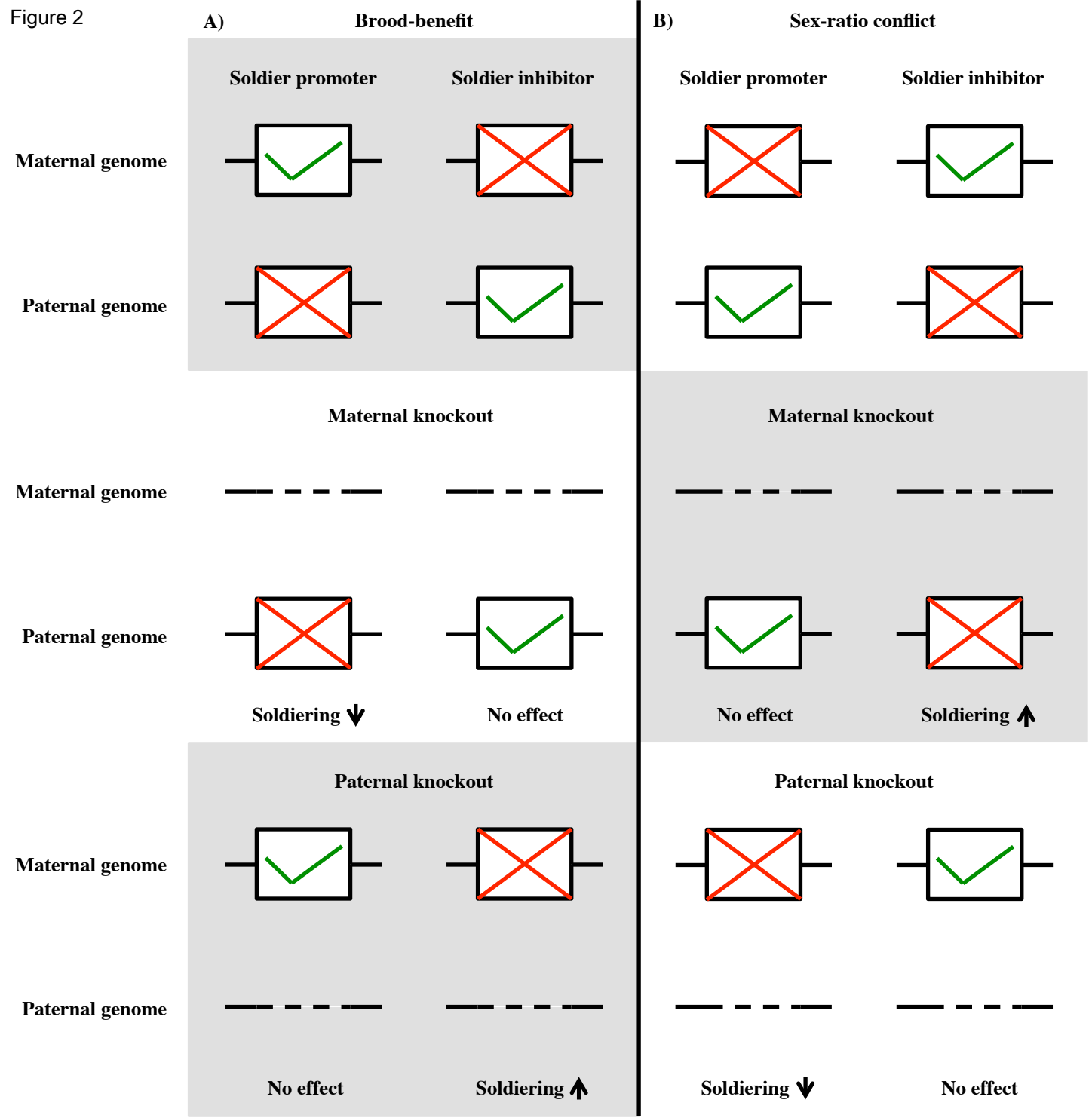

Optimal sex ratio (proportion male; $z_A$)
0
0.2
0.4
0.6
0.8
1
Male embryo
Mother
Maternal-origin genome
Female embryo
Father & Paternal-origin genome
0
0.2
0.4
0.6
0.8
1
Proportion of males dispersing ($d_m$)

Figure 2


A)
Brood-benefit
B)
Sex-ratio conflict
Soldier promoter
Soldier inhibitor
Soldier promoter
Soldier inhibitor
Maternal genome
Paternal genome
Maternal knockout
Maternal genome
Paternal genome
Soldiering
No effect
Maternal knockout
No effect
Soldiering
Paternal knockout
Maternal genome
Paternal genome
No effect
Soldiering
Paternal knockout
Soldiering
No effect