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Petri Rautiala

Developments in the evolutionary theory of social interactions

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ABSTRACT

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Altruistic behaviour, which benefits others but harms the actor, can evolve when copies of the underlying genes are transmitted to future generations by related beneficiaries. While we know that the mechanism of '*kin selection*' answers to *how* altruism can evolve, the answers to *why* and *when* it can evolve are still obscure. The first aim of this thesis is to shed light to the evolution of altruism by identifying factors that facilitate or promote it. I find that the conditions under which altruism can evolve follow surprisingly simple principles that are independent of the taxon-specific traits such as fecundity. Further, by analysing the unique aspects of haplodiploid sex determination system (where males are born from unfertilized eggs and females from fertilized eggs) I find that its role in the evolution of altruism might have been prematurely dismissed by recent studies. The second aim is to contribute a new dimension to studies which link mating behaviour with the evolution of altruism. I find that virginity can be an adaptive mating strategy in haplodiploid taxa, and that adaptive virginity affects, and is affected by, the evolution of altruism. According to my analysis haplodiploidy offers a unique adaptive pathway to sociality. The third aim is to predict patterns of genomic imprinting in female soldier development in polyembryonic parasitoid wasps. The function of these soldiers is unknown, and it has been argued that their primary function is either altruistically protect their siblings, or spitefully kill their brothers to make room for sisters. I find that contrasting patterns of genomic imprinting are expected to be found depending on the function of the soldiers. These are empirically testable prediction, which can not only help find the function of these soldiers, but also help test social-evolution theory. The models built in this thesis highlight the need for better incorporation of future expectations, population parameters, and possible epigenetic changes to kin selection models.

Keywords: Alternative mating behaviour; altruism; eusociality; future expectations; genomic imprinting; inclusive fitness; sex ratio conflict.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following four original papers, which will be referred to in the text by their Roman numerals I-IV. I have contributed significantly to planning, modeling and analyses, as well as writing of all articles. The original ideas for Chapters I-III were by me, for Chapter IV by Andy Gardner.

I Rautiala P., Helanterä H. & Puurtinen M. 2014. Unmatedness promotes the evolution of helping more in diplodiploids than in haplodiploids. *The American Naturalist* 184: 318-325. © 2014 by The University of Chicago.

II Rautiala P., Helanterä H., Kokko H. & Puurtinen M. 2016. Linking ecology and life-history to the costs and benefits of altruism. Submitted manuscript.

III Rautiala P., Helanterä H. & Puurtinen M. 2016. The evolutionary dynamics of adaptive virginity, sex-allocation and altruistic helping in haplodiploid animals. Manuscript.

IV Rautiala P. & Gardner A. 2016. Intragenomic conflict over soldier allocation in polyembryonic parasitoid wasps. *The American Naturalist* 187: E106-E115. © 2016 by The University of Chicago.

1 INTRODUCTION

1.1 Simplifications of the questions

> ...legend has it that in a pub one evening Haldane told his friends that he would jump into a river and risk his life to save two brothers, but not one, and that he would jump in to save eight cousins, but not seven.[1]

This quip by J.B.S. Haldane (1892-1964) is well known among researchers studying kin selection. But would he have said the same thing about his sisters that he did about his brothers? And does it matter if these relatives are children, adults, or elderly? These questions are simplifications of the actual questions what I am aiming to answer in this thesis.

1.2 Inclusive fitness, kin selection and altruism

We should also ask why Haldane was ready to sacrifice himself for the sake of relatives in the first place. Altruism, behaviour that benefits others at the actor's expense, was one of Darwin's greatest challenges when formulating the theory of evolution (Darwin 1859, Dugatkin 2007). How could sterile helper castes of ants, bees and wasps have evolved, when the altruists themselves are not passing down sterile helping behaviour? The answer was found after the discovery of genes (chronicled in Dugatkin 2007). Behaviour, or phenotype, is favoured by selection when the underlying genes increase their representation in future generations. The transmission to future generations does not happen solely via ones own offspring, but also through relatives carrying those same genes originating from a common ancestor (Hamilton 1964). This 'gene's eye-view' shifts the unit of selection from the individual to its genes (Hamilton 1964, Dawkins

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1 Lee Alan Dugatkin, 2007

1976). An offspring is just one type of a relative, holding no other special status than carrying the actor's genes with a high probability.

Hamilton's great insight was how individuals do not only gain fitness directly via own offspring, but also indirectly via relatives. The combination of these two is called '*inclusive fitness*'. The conditions for kin selection have become known as the Hamilton's rule, $c < rb$. It states that costly behaviour can evolve when the fitness costs (c) of expressing it are smaller to the actor, than the resulting fitness benefits (b) are to the recipient, weighed by relatedness (r) between them. The relatedness is meant to measure how probable it is that the beneficiaries are carrying the allele(s) which causes the actors costly behaviour, not how much of their genomes are shared. The inequality itself is heuristic, and not meant to be used directly as it is. But when game theory is incorporated into the gene's eye-view (Smith and Price 1973), by following the logic of the Hamilton's rule, we have powerful predictive and explanatory tools. These tools can be used to properly analyse the conditions under which social behaviour can evolve. I have used these tools throughout this thesis. Still, the gene's eye-view is not enough to explain the rationale of sterile helpers. The sterility-genes won't be transmitted to future generations if they are expressed in everyone carrying them! Charlesworth (1978) suggested that this can be mended if the gene(s) in question didn't code strict sterility, but rather the probability of becoming a sterile helper, which then evolved in small steps.

When discussing altruistic behaviour, it is usual to talk about an individual's decision of becoming an altruist. The 'decision' here is semantic, a heuristical way of explaining that the actor's genes are favoured by selection when their expression, the made decision, increases their representation in future generations. It is easier to discuss the benefits of a behaviour to the underlying genes by phrasing this as the actor's 'will', even when it is not a result of a cognitive process.

1.3 Haplodiploidy

The history of inclusive fitness and kin selection is tightly tied to haplodiploid sex determination system. After Hamilton formulated kin selection in his 1964 landmark article, one of the first major applications was to try to explain why eusociality was found, at the time, mainly in the haplodiploid Hymenoptera. And also why the helpers in social Hymenoptera are always females, while in the diplodiploid termites they are mostly a mixture of both sexes. Hamilton's '*haplodiploidy hypothesis*' (Hamilton 1972, West-Eberhard 1975) argued that it was due to the relatedness asymmetries brought by haplodiploidy. In haplodiploid taxa females are diploid and develop from fertilized eggs, and males are haploid and develop from unfertilized eggs. The male's sperm is clonal and it is not used for sons. This makes females more related to their full-sisters than to their brothers or even to their own offspring. Hamilton's argument was that this

predisposes females in haplodiploid taxa to prefer rearing sisters over own offspring.

However, the argument had a major flaw, which even affected how inclusive fitness theory itself was seen (Foster *et al.* 2006). The flaw was that for the female helpers to capitalise on the high sororal relatedness, the helpers would have to discriminate between their brothers and sisters (Trivers and Hare 1976). Otherwise the average relatedness to the individuals they were rearing would be equal to that of their own offspring. Attempts to save the hypothesis followed (chronicled in more detail in Chapters I and II), but currently the haplodiploidy hypothesis has largely fallen out of favour as a mechanism to explain evolution of altruism.

The haplodiploidy hypothesis was so influential and well known that after its gradual falling out of favour, it was argued that the whole idea of inclusive fitness theory was in question (Nowak *et al.* 2010). However, this is not the case. Inclusive fitness theory has been successful in explaining a number of behavioural strategies (reviewed in Abbot *et al.* 2011), one of them being sex allocation theory which is used throughout this thesis.

It is easy to see why haplodiploid sex determination system still attracts attention. Young researchers, like me, who are getting familiar with inclusive fitness usually start by reading about the haplodiploidy hypothesis and why it doesn't hold. No wonder, it is a good exercise. Relatedness is the key to kin selection, and the idea of relatedness asymmetries brought by haplodiploidy puzzles the mind. Maybe there is still something there?

Even more radical relatedness asymmetries can be found in polyembryonic parasitoid wasps. Not only are they haplodiploid, but their eggs proliferate clonally. When the female deposits one male and one female egg into a single host (Strand 1989), the same sex siblings present are clonal. The relatedness between other relatives equals that of any haplodiploid taxa.

1.4 Conflict over sex allocation in haplodiploid taxa

Sex allocation is not the same as sex ratio. Sex allocation represents how energy or resources are used to produce different sexes. Sex ratio represents the proportion of individuals in each sex. When one sex is more expensive to produce than the other, sex allocation does not equal sex ratio. However, for simplicity, in this thesis I am assuming that the sexes cost the same to produce.

The relatedness asymmetries of haplodiploidy are not limited to sibling relations. Out of offspring and siblings, a female of haplodiploid taxa values (in the inclusive fitness sense) her full sister the most, even more than her own son or daughter, and values her brothers the least. From the mother's perspective, she values her sons and daughters equally. Therefore, the population sex allocation remains stable at even when the mothers control the sex allocation of the offspring they produce. But if the female helpers of a social taxa gain control of their sibling brood's sex allocation (Beekman and Ratnieks 2003, Beekman *et al.*

2003), the stable population sex allocation is 75% females (Trivers and Hare 1976). Thus there is a conflict of interest between the mother and her female helpers over the sex allocation in social, monogamous, haplodiploid taxa.

This conflict is magnified in polyembryonic parasitoid wasps. The difference in the valuation of brothers and sisters for a female is even greater than under mere haplodiploidy. This creates even greater pressure for females to manipulate their sibling brood's sex allocation, away from the mother's optimum (Gardner *et al.* 2007). But being parasitoids, the mother is not present while the offspring develop. Her influence on the sex allocation of individuals who emerge from the host ends in the decision to lay both a male and a female egg, or just one of either. The offspring are then free to mediate the conflict, free from their mother's influence.

1.5 Altruism or spite?

In this thesis I am focusing on behaviours that are costly to the actor. When such behaviours are categorized, they are classically defined as interactions between two individuals, A who expresses the behaviour, and B who acts as a recipient (West and Gardner 2010). The rationale of altruistic behaviour, which is harmful for A but beneficial for B, is explained above. But can spiteful behaviour, which harms both A and B, ever evolve? The problem comes from limiting the effects of the behaviour to just the two parties. Spiteful behaviour becomes sensible when a third party, a secondary recipient, C is taken into account (West and Gardner 2010). If the harm A does to B benefits C, then that behaviour can evolve by following the same heuristical rules as altruism: if the benefits are greater as the costs, weighed by the relatedness of the actor A to B and C. However, this time the costs fall on both A and B, and only C benefits. This type of A's behaviour is spiteful towards B and altruistic towards C.

1.6 Genomic imprinting

The conflict of interests between individuals stem from the genetic level (Dawkins 1976). The gene's eye view helps us understand how behaviour is selected for, depending on how that behaviour affects the success of the responsible gene. But what if the expression of the gene responsible for social behaviour can be modified depending if it knows (again, semantics) its own origin? When genes can carry information about their origin, their expression can be modified by taking into account whether they originated from the actor's father or mother. This is called '*genomic imprinting*' (Moore and Haig 1991, Haig 1997, Haig 2000). For example, a female of haplodiploid taxa is related to her brother only through her mother, not through her father, since males are born from unfertilized eggs. Therefore, a gene responsible for behaviour that affects her brothers

can favour different expression levels in the female, depending which parent it originated from. In other words, if the gene knows whether its copies would be affected by the behaviour it codes, it should be either expressed or silenced.

1.7 Aims of this thesis

The first aim of this thesis is to contribute to the existing theory of social evolution by developing new analytical models to identify factors that influence the evolution of altruistic behaviour (Chapters I, II, III). The second aim is to study how virginity can be an adaptive mating strategy in haplodiploid taxa, and how the evolution of virginity affects, and is affected by, the evolution of altruism (Chapter III). The third aim is to predict patterns of genomic imprinting in female soldier development in polyembryonic parasitoid wasps, depending on the function of those soldiers (Chapter IV).

2 METHODS

In all four Chapters I use an inclusive fitness approach (Taylor 1996, Taylor and Frank 1996) to either compare the consequences of different behaviours (Chapters I, II, III), or to find whether selection acts to increase or decrease a trait's value (Chapters I, II, III, IV). The inclusive fitness for the actor is defined by taking into account the effect of the behaviour on the number of different relatives, and their inclusive fitness value to the actor. The relatedness term in the Hamilton's rule is a measure of how valuable the beneficiaries are to the actor. However, when the inclusive fitness is defined properly, both the cost and benefit need to take into account the type of relatives lost or gained by the behaviour. Meaning that the cost, even if it is in the currency of one's own offspring in self-sacrifice, needs to be weighed by their value. As previously said, offspring is just one type of relative.

The value of relatives takes into account how good they are on passing down their genes, and their relatedness to the actor. This is done by first defining each relative's individual reproductive value, which is the probability that a random allele, taken from an infinitely distant future generation, originates from that actor. The individual reproductive value is then multiplied by consanguinity between the actor and the respective relative. Consanguinity represents relatedness, and is the probability that homologous genes drawn randomly from the two actors are identical by descent (Bulmer 1994). While doing these studies, I noticed that the consanguinity values can have another interpretation. Consider that the actor has allele X that is responsible for the behaviour we are analysing. Consanguinity attains the same values as the probability that the respective relative will pass a copy of allele X, originating from a common ancestor, to an offspring. Therefore, by multiplying the individual reproductive value with consanguinity, what we actually have is the probability that a randomly picked distant future allele is in fact allele X, originating from a common ancestor! This is what the behaviour is trying to maximize, allele X's "reproductive value" (Grafen 1999).

3 RESULTS

3.1 In general

In my studies, I found that there are two subjects that seem to underlie, or are linked to, almost all other factors which affect the evolution of altruism. They are the future expectations of the actor (Chapters I, II, III), and sex allocation conflict (Chapters II, III, IV). While the findings of this thesis are highly interconnected, to explain the individual causes consequences of the results, they are presented here in separate Chapters.

I find that the future expectations of the actor have a major effect on behavioural development (Chapters I, II, III). When the expected reproductive success of the actor is lowered, selection can favour alternative strategies. I show how the future expectations are affected by the population sex ratio and the sex of the actor (Chapter II), and costly ecological factors (Chapters I, II, III).

3.2 Chapter I: Unmatedness promotes the evolution of helping more in diplodiploids than in haplodiploids

I show how a constant risk of not finding a mating partner makes the evolution of altruism easier in diplodiploid taxa than in haplodiploid taxa. When there is a risk of remaining a virgin, the future expectations of both males and females of diplodiploid taxa are lowered. This reduces the cost of forgoing their own reproduction, which in turn decreases the requirements for the evolution of altruism, making its evolution easier. The same effect does not arise in haplodiploid taxa, since there the virgin females are able to produce an all-son brood. In haplodiploid taxa, the constant presence of virgins producing all-male broods affects the mated females' sex allocation (Godfray 1990). The mated females compensate for the surplus of males by biasing their produced sex allocation more towards daughters. Daughters are therefore born in broods where the in-

nest relatedness is high, and the inclusive fitness value of an average sibling is greater than of an average offspring (Grafen 1986, Godfray and Grafen 1988, Chapters I, III). This is the 'split sex ratios'-effect (Grafen 1986), which when caused by accidental virgins was the last remaining attempt to save the haplodiploidy hypothesis (Gardner *et al.* 2012, Alpedrinha 2013). Therefore, a risk of remaining a virgin promotes altruism for both diplodiploid males and females, and haplodiploid females. However, I find that the total effect is larger in diplodiploids when all else is equal, providing further theoretical evidence against the haplodiploidy hypothesis.

3.3 Chapter II: Linking ecology and life-history to the costs and benefits of altruism

The goal of Chapter II is to better understand the relation between the cost and the benefit, both realized and required, of altruism, and how specific aspects of ecology and life-history affect this relation. By analysing a population that has reached the environment's carrying capacity, maintaining the population size constant, I find that in such populations that the general fecundity promotes altruism. I come to this conclusion by quantifying the cost of altruism, and finding that the cost does not depend on general fecundity, but rather it is an independent quantity. For helping to be favoured, the increase a helper needs to bring to its mother's fecundity is not a set fraction, but an absolute quantity that is independent of that fecundity and its magnitude. Therefore, I argue that when the mother's fecundity is large to begin with, the required quantity is more easily achieved.

I find that the requirement for helping to be favoured also depends on the population sex ratio. This is because in a stable sized population, the sex ratio defines how much competition individuals encounter, and consequently their reproductive value. If the population sex ratio changes, the expected number of offspring for newly born individuals changes (Fisher 1930, West 2009). Therefore, forgoing own reproduction by becoming a helper is less costly when the population sex ratio is biased to the actor's sex, than it would be otherwise (Gardner and Ross 2013).

Because females of haplodiploid taxa value their sisters over their brother, provided the population sex ratio is not heavily female biased, there is scope for behaviour to evolve which shifts resources from brothers to sisters (Beekman and Ratnieks 2003, Beekman *et al.* 2003). I show that a female can be selected to forgo her own reproduction and kill her brothers to free resources for her sisters, even when there is a transfer loss of the resources, *i.e.* when killing a number of brothers generates a smaller number of sisters. This behaviour is altruistic towards her sisters, but spiteful not only towards her brothers, but also towards her mother. This spiteful behaviour can serve as a starting point for real altruism, by evolving to actually benefit the mother.

The fourth contribution of Chapter II is for the role of haplodiploidy in the evolution of altruism. I show how the same factor that originally toppled the haplodiploidy hypothesis actually results in a new factor that keeps helping promoted.

3.4 Chapter III: The evolutionary dynamics of adaptive virginity, sex-allocation and altruistic helping in haplodiploid animals

I show how virginity can be an adaptive alternative mating strategy in haplodiploid taxa, and how it can offer a unique adaptive pathway to sociality. When mating carries a mortality risk, remaining a virgin and being constrained to produce only sons can be lucrative for a haplodiploid female. I find that virginity can be adaptive when the expected number of offspring, even if they are all sons, is greater when remaining a virgin. Virginity evolves to a point where half of the females choose to mate, and half to remain as virgins. In Chapter I I show that virginity promotes altruism more in diplodiploids than in haplodiploids, all else being equal. However, the fact that virginity can be adaptive, and therefore more common, turns the tables in favour of haplodiploidy allowing for virginity to promote the evolution of helping.

Further, I show how virginity can resolve sex allocation conflict in the favour of the mother in social taxa. If female helpers gain control of the sex allocation, the sex ratio becomes female biased. Under female biased sex ratio, the value of sons is elevated, and by remaining a virgin the mother is free to produce offspring of the more valuable sex. However, virgins produce less offspring than their mated counterparts because having female helpers requires sperm. Although, as shown in Chapter II, the sex ratio manipulation can actually lower the offspring production. I find that virginity can be adaptive even when the increase in offspring production the helpers bring to mated females is relatively large. This effect is magnified when mating carries a cost, and when fecundity before helper influence is large to begin with. Virginity allows the mother to affect her sex allocation. When virginity evolves to such levels where the helpers are selected to manipulate their sibling brood to all-sisters, the probability of remaining a virgin becomes synonymous with sex allocation. By remaining a virgin, a female dedicates her life to production of sons, and by mating to production of daughters. *I.e.* the mother wins the sex allocation conflict and controls it with the decision whether to mate or remain a virgin.

3.5 Chapter IV: Intragenomic conflict over soldier allocation in polyembryonic parasitoid wasps

The function of female soldiers in polyembryonic parasitoid wasps has been under a long debate (Gardner *et al.* 2007). It is unknown whether their purpose is to protect their brothers and sisters, or if they actually spitefully kill their brothers to make room for their sisters, thus mediating a sex allocation conflict (in a similar fashion as in Chapter II). I find that these two purposes are expected to display contrasting patterns of genomic imprinting. If female soldiers benefit the whole brood, genes responsible for soldier development should be silenced when inherited from the father. If female soldiers are mainly killing their brothers, genes responsible for soldier development should be silenced when inherited from the mother. The imprinting directions are switched if soldier development is controlled by genes that inhibit the development.

The derived predictions serve two purposes. First, they can help identify genes responsible for soldier development by limiting the candidates to genes that are affected by genomic imprinting. Second, once the responsible genes and their effect on soldier development are found, the direction of genomic imprinting (Figure 2 in Chapter IV) reveals their primary function.

4 POPULARIZED VIEW OF RESULTS

I now come back to where I started, to J.B.S. Haldane's statement of sacrificing one's self for the sake of relatives. There are no simple answers to the questions I posed, as they are interlinked.

The first question was whether Haldane would sacrifice himself to save the same amount of sisters from drowning than he would brothers, *i.e.* does sex matter? In the results of all four Chapters, the sex of both the altruistic actor (Chapters I, II, IV) and the saved relatives (Chapters I, II, III, IV) play a key part in allowing altruism to evolve. If the actor is of the more common sex, then the cost of self-sacrifice is smaller (Chapters II, III), and saving relatives of the rarer sex is more beneficial (Chapter II). In other words, if there were more males than females in the population, it would take fewer sisters than brothers for Haldane to sacrifice himself. Sex affects the relatedness between the altruist and the ones being saved in haplodiploid taxa (Chapters I, II, IV), and therefore also affects the required number of relatives saved (Chapter II). For example consider if Haldane was a haplodiploid female in a population with an even sex ratio. Again it would take fewer sisters, to whom she is more closely related, than brothers for Haldane to sacrifice herself.

The second question was whether it matters if the drowning relatives are children, adults, or elderly. It is a very human way of thinking that it is better to save a child than an adult. However, that is not necessarily optimal for the genes underlying the saving behaviour. The answer depends on the future expectations of the individuals, which is the primary driver for the results of Chapters I, II and III. A child is about to face the risks of growing up, while an adult has already passed those risks (parallels to Chapter II). Also, the child can face a different level of competition compared to adults that grew in the previous generation, depending how the population dynamics change between the generations (parallels to Chapter I). An elder has already passed the main reproductive phase, and its expected future reproduction is lower than that of an adult. Therefore, by a crude simplification, it is better to save an adult from drowning than a child or an elder. This is because adults have the best expectations of passing down their genes after being saved. Haldane's own status fol-

lows the same principles. He loses most if he was an adult, and would therefore be more reluctant to sacrificing himself than if he was a child or an elder.

According to the results of Chapters II and IV, there are also scenarios where it would actually benefit Haldane to actively drown her (in these scenarios he would be a haplodiploid female) brothers, even if it kills her! This requires other relatives to benefit from this act, for example by freeing resources, so much that the inclusive fitness benefits counter the losses (Chapter II). In the simplest terms, this would be the case when the drowned relatives are less valuable to Haldane than the ones that benefit from the act (Chapters II, IV).

The results of Chapters II and III suggest that dismissing the role of haplodiploidy, both as a sex determination mechanism and as a source of relatedness asymmetries, in the evolution of altruism is premature.

On top of my actual results, the models built in this thesis highlight the need for better incorporation of future expectations, population parameters, and possible epigenetic changes to inclusive fitness models (Chapters I, II, III, IV). I believe that this thesis has contributed to the understanding of social evolution, and I hope the way I have taken into account the future prospects of the actor will have an impact on future inclusive fitness models.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Sosiaalisten vuorovaikutusten evolutiivista teoriaa

Altruismiksi kutsutaan sosiaalista käyttäytymistä, joka on vahingollista toimijalle, mutta hyödyllistä toiminnan kohteena olevalle osapuolelle. Se tarkoittaa siis muiden epäitsekästä auttamista oman edun kustannuksella. Tällainen käyttäytyminen on meille tuttua työläisiä kuhisevista pistiäis- ja termiittipesistä. Mutta miksi steriilit työläiset uurastavat kasvattaakseen siskojaan ja veljiään eivätkä omia jälkeläisiään? Miten altruistinen käyttäytyminen voi levitä valinnan seurauksena, jos sen ilmaiseminen pienentää toimijan kelpoisuutta? Darwin näki tämän kysymyksen mahdollisena uhkana evoluutioteorialleen, sillä steriilien yksilöiden olemassaolo ei tukenut hänen teoriaansa luonnonvalinnasta. Formaali vastaus altruismin kehittymisen mekanismille saatiin geenien löytymisen myötä. Käyttäytymisen taustalla olevien geenien variaatio, joka on valinnan kohteena, siirtyy seuraaviin sukupolviin altruismista hyötyvien sukulaisten kautta. Nämä sukulaiset eivät vain välttämättä itse ilmennä kantamansa käyttäytymisen geenivariantteja.

Pistiäisten ja termiittien ohella auttamiskäyttäytymistä esiintyy esimerkiksi *Anelosimus eximius* -lajin hämähäkeillä, *Kladothrips*-suvun ripsiäisillä sekä *Austroplatypus incompertus* -lajin kovakuoriaisilla. Epäitsekäs auttaminen ei ole kuitenkaan rajoittunut pelkästään selkärangattomiin eläimiin. Useilla lintulajeilla esiintyy tilapäisiä ei-steriilejä auttajia, kun nuoret yksilöt jäävät kotipesäänsä huolehtimaan itseään nuoremmista sisaruksista ennen omia pyrkimyksiä lisääntymiseen. On jopa löydetty sosiaalinen nisäkäslaji, kaljurotta, jolla tilapäisesti steriilit yksilöt huolehtivat sisaruksistaan. Tästä syystä altruismin teoriaa ei ole voitu rajoittaa koskemaan vain hyönteisiä tai edes pelkästään selkärangattomia.

Väitöskirjassani tutkin olosuhteita ja tekijöitä, jotka vaikuttavat altruistisen käyttäytymisen evoluutioon. Kolmea ensimmäistä osatyötä varten rakentamani analyyttiset mallit ottavat huomioon yksilöiden tulevaisuudenodotukset, joilla huomioin olevan merkittävän roolin altruistisen käytöksen hyödyissä ja kustannuksissa. Lisäksi analyysieni keskipisteessä ovat sosiaalisten lajien sukupuolenmääräytymisen mekanismit, joiden tiedetään eroavan esimerkiksi linnuilla ja pistiäisillä. Tässä väitöskirjassa osoitan, miten sukupuolenmääräytymisen mekanismeilla on keskeisiä suoria ja epäsuoria vaikutuksia altruistisen käyttäytymisen hyötyihin ja kustannuksiin ja siten myös kyseisen käyttäytymisen evoluutioon.

Havaitsin väitöskirjatyössäni, että vakaa populaatiokoko poistaa fekunditeetin, eli jälkeläistuoton suuruuden, merkityksen altruistisen käytöksen kustannuksista. Riippumatta populaatiokoon vakaana pitävästä mekanismista, esimerkiksi pesintäpaikkojen rajallisuudesta, nuoret yksilöt odottavat saavansa jälkeläisiä vain sen verran, että edellinen kasvattajasukupolvi korvautuu. Kustannukset ovat siis vakioita fekunditeetin suhteen ja osoitin, että tämän seurauksena myös vaatimukset altruismin kehittymiselle ovat fekunditeetista riip-

pumattomia. Tämä tarkoittaa sitä, että jos korkea fekunditeetti mahdollistaa suuremman auttamalla saavutetun hyödyn, altruismi myös kehittyy helpommin korkean fekunditeetin populaatioissa.

Toinen tämän väitöskirjan päätuloksista on, miten pistiäisistä tuttu sukupuolenmääräytymisen mekanismi antaa mahdollisuuden ainutlaatuiselle naaraiden neitseelliselle lisääntymistavalle, joka on kehittynyt valinnan seurauksena. Mekanismi antaa myös tämän lisääntymistavan kautta mahdollisuuden naarasauttajien kehittymiselle. Pistiäisten naaraat syntyvät hedelmöitetyistä munista ja koiraat hedelmöitymättömistä. Koska koiraiden tuottamiseen ei vaadita siittiösoluja, neitsytnaaraat voivat lisääntyä ja tuottaa pesällisen pelkästään koiraita. Näytän, missä olosuhteissa neitsyt-naaraiden kelpoisuus on paritelleita naaraita suurempi, ja osoitan, miten neitseellinen jälkeläistuotanto voi olla vaihtoehtoinen sopeutuma parittelulle. Lisäksi osoitan tämän sopeutuman antavan pistiäisille ainutlaatuisen reitin altruismin kehittymiselle yhdessä pistiäisten sukupuolenmääräytymisen mekanismin tuomien sukulaisuusepäsymmetrioiden sekä sukupuolijakauman siirtymien kanssa.

Kolmas päätuloksistani antaa ennusteita geenien ilmentymistasoille, jotka ovat vastuussa loispistiäisten naarassotilaiden kehittymisestä. Loispistiäisten naarassotilaiden perimmäinen tarkoitus on epäselvä. Johtavien hypoteesien mukaan sotilaat joko suojelevat koko sisarkatrastaan tai tappavat veljiään tehdäkseen tilaa siskoilleen. Väitöskirjani neljännessä osatyössä näytän, milloin epigeneettiset tekijät hiljentävät äidiltä perityt ja milloin isältä perityt sotilasgeenit. Osoitan sotilaiden perimmäisen tarkoituksen vaikuttavan siihen, kummalta vanhemmalta perityt sotilasgeenit hiljennetään. Ennusteideni pohjalta on mahdollista löytää kandidaatteja geeneille, jotka ovat vastuussa sotilaaksi kehittymisestä. Samojen ennusteiden pohjalta voidaan kokeellisesti selvittää naarassotilaiden perimmäinen tarkoitus, kun vastuussa olevat geenit löydetään kandidaattigeenien joukosta.

REFERENCES

Abbot P. et al. 2011. Inclusive fitness theory and eusociality. *Nature* 471: E1–E4.

Alpedrinha J., West S.A. & Gardner A. 2013. Haplodiploidy and the evolution of eusociality: worker reproduction. *Am. Nat.* 182: 421–438.

Beekman M., Komdeur J. & Ratnieks F.L.W. 2003. Reproductive conflicts in social animals: Who has power? *Trends Ecol. Evol.* 18: 277–282.

Beekman M. & Ratnieks F.L.W. 2003. Power over reproduction in social hymenoptera. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358: 1741–1753.

Bulmer M. 1994. *Theoretical Evolutionary Ecology*. Sinauer Associates, Sunderland, MA.

Charlesworth B. 1978. Some models of evolution of altruistic behavior between siblings. *J. Theor. Biol.* 72: 297–319.

Darwin C. 1859. *On the Origin of Species by Means of Natural Selection*. John Murray, London.

Dawkins R. 1976. *The Selfish Gene*. Oxford University Press, Oxford.

Dugatkin L.A. 2007. Inclusive fitness theory from Darwin to Hamilton. *Genetics* 176: 1375–1380.

Fisher R.A. 1930. *The Genetical Theory of Natural Selection*. The Clarendon Press, Oxford.

Foster K.R., Wenseleers T. & Ratnieks F.L.W. 2006. Kin selection is the key to altruism. *Trends Ecol. Evol.* 21: 57–60.§

Gardner A., Alpedrinha J. & West S.A. 2012. Haplodiploidy and the evolution of eusociality: split sex ratios. *Am. Nat.* 179: 240–256.

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

Gardner A. & Ross L. 2013. Haplodiploidy, sex-ratio adjustment, and eusociality. *Am. Nat.* 181: E60–E67.

Godfray H.C.J. 1990. The causes and consequences of constrained sex allocation in haplodiploid animals. *J. Evol. Biol.* 3: 3–17.

Godfray H.C.J. & Grafen A. 1988. Unmatedness and the evolution of eusociality. *Am. Nat.* 131: 303–305.

Grafen A. 1986. Split sex ratios and the evolutionary origins of eusociality. *J. Theor. Biol.* 122: 95–121.

Grafen A. 1999. Formal Darwinism, the individual-as-maximizing-agent analogy and bet-hedging. *Proc. R. Soc. Lond. B Biol. Sci.* 266: 799–803.

Haig D. 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proc. R. Soc. Lond. B Biol. Sci.* 264: 1657–1662.

Haig D. 2000. The kinship theory of genomic imprinting. *Annu. Rev. Ecol. Syst.* 31: 9–32.

Hamilton W.D. 1964. The genetical evolution of social behavior I & II. *J. Theor. Biol.* 7: 1–52.

Hamilton W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* 3: 193–232.

Moore T. & Haig D. 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet.* 7: 45–49.

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

Smith J.M. & Price G.R. 1973. The logic of animal conflict. *Nature* 246: 15–18.

Taylor P.D. 1996. Inclusive fitness arguments in genetic models of behaviour. *J. Math. Biol.* 34: 654–674.

Taylor P.D. & Frank S.A. 1996. How to make a kin selection model. *J. Theor. Biol.* 180: 27–37.

Trivers R.L. & Hare H. 1976. Haplodiploidy and the evolution of the social insects. *Science* 191: 249–263.

West S.A. 2009. *Sex Allocation*. Princeton University Press, Princeton, NJ.

West S.A. & Gardner A. 2010. Altruism, spite, and greenbeards. *Science* 327: 1341–1344.

West-Eberhard M.J. 1975. The evolution of social behavior by kin selection. *Q. Rev. Biol.* 50: 1–33.

ORIGINAL PAPERS

I

UNMATEDNESS PROMOTES THE EVOLUTION OF HELPING MORE IN DIPLODIPLOIDS THAN IN HAPLODIPLOIDS

by

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Unmatedness Promotes the Evolution of Helping More in Diplodiploids than in Haplodiploids

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abstract: The predominance of haplodiploidy (where males develop from unfertilized haploid eggs and females from fertilized diploid eggs) among eusocial species has inspired a body of research that focuses on the possible role of relatedness asymmetries in the evolution of helping and eusociality. Previous theory has shown that in order for relatedness asymmetries to favor the evolution of helping, there needs to be variation in sex ratios among nests in the population (i.e., split sex ratios). In haplodiploid species, unmated females can produce a brood of all males, and this is considered the most likely mechanism for split sex ratios at the origin of helping. In contrast, in diploidiploids unmatedness means total reproductive failure. We compare the effect of unmatedness on selection for male and female helping in haplodiploids and diplodiploids. We show that in haplodiploids, unmatedness promotes helping in females but not in males within the empirical range. In diplodiploids, unmatedness promotes helping by both sexes, and the effect is stronger than in haplodiploids, all else being equal. Our study highlights the need to consider interactions between ecological and genetic factors in the evolution of helping and eusociality.

Keywords: eusociality, haplodiploidy hypothesis, split sex ratios, virginity.

Introduction

The evolution of nonreproductive castes in eusocial organisms is among the most intriguing questions in evolutionary biology (Bourke 2011). An influential hypothesis suggested that haplodiploid sex determination, where males develop from unfertilized haploid eggs and females from fertilized diploid eggs, could have facilitated the evolution of eusociality due to the relatedness asymmetries arising from this kind of sex determination. The original argument of this "haplodiploidy hypothesis" (Hamilton 1964, 1972) was that because a female is more related to her full sisters ($r = 3/4$, r is life-for-life relatedness in a population with 1 : 1 sex ratio; Hamilton 1972) than to her own sons and daughters ($r = 1/2$), a female could gain higher inclusive fitness by helping her mother to produce more sisters than by producing her own offspring. The haplodiploidy hypothesis was refined by Trivers and Hare (1976), who argued that for the promoting effect to arise from haplodiploidy, the helping behavior needs to be directed to sisters more than their mother would prefer. Their argument was that if helping was directed evenly to sisters and brothers, the benefit of high sister-sister relatedness is canceled out by low sister-brother relatedness ($r = 1/4$), resulting in the same average relatedness to siblings as to own offspring.

Further work however showed that relatedness asymmetries arising from haplodiploidy can indeed promote the evolution of helping, provided that there is variation in sex ratio among nests (i.e., there are "split sex ratios"; Trivers and Hare 1976; Craig 1979, 1980; Grafen 1986). When the potential (female) helper is in a nest with a more female-biased sex ratio than the population average, she values an average sibling more than an average offspring. However, the average sibling is valued more than the average offspring only when the individual reproductive value of males does not counter the benefits of high sister-sister relatedness, that is, when the proportion of males in the population is more than 25%. Although several mechanisms for split sex ratios have been suggested in the literature, it seems that reproduction by unmated females is the only likely mechanism that could have been important at the origin of helping (reviewed in Gardner et al. 2012). In haplodiploid species, unmated females have the option of laying unfertilized eggs, which develop to males. If a fraction of females in the population remain unmated and produce only males, sex ratio in nests of mated females is necessarily more female biased than the population average sex ratio (Godfray and Grafen 1988).

Hamilton (1972) suggested that relatedness asymmetries arising from haplodiploidy could also explain why helpers in eusocial Hymenoptera are always female. Grafen (1986)

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Table 1: Symbol definitions

Symbol	Description
u	Fraction of females that remain unmated
b_{tr}	Benefit threshold for helping to evolve
z_m	Mated female sex ratio (proportion of males)
z_p	Population sex ratio (proportion of males)
c_m	Class reproductive value of males
c_{mf}	Class reproductive value of mated females
c_{uf}	Class reproductive value of unmated females
γ_{sis}	Consanguinity between the focal individual and its sister
γ_{bro}	Consanguinity between the focal individual and its brother
γ_{son}	Consanguinity between the focal individual and its son
γ_{dau}	Consanguinity between the focal individual and its daughter
n	Number of offspring produced by a reproductive female
b	Number of siblings the focal individual gains by helping

later showed that with split sex ratios, potential female helpers do not need to discriminate among brothers and sisters as receivers of the help, which was previously considered necessary for haplodiploidy to promote the evolution of female helping (Trivers and Hare 1976). However, more recent analyses have shown that male workers should evolve as readily as female workers (Craig 1982; Pamilo 1991). Furthermore, eusocial haplodiploid thrips with both male and female helpers have been found (Crespi 1992; Kranz et al. 1999). These findings and new analyses suggest that ecological circumstances and preadaptations such as maternal care are more important in determining the sex of helpers than the genetics of sex determination (Queller and Strassmann 1998; Ross et al. 2013).

In contrast to haplodiploids, in nonclonal diplodiploids the lack of a mate leads to a total reproductive failure for both males and females. This effect of unmatedness must be taken into account when comparing the conditions for the evolution of helping in haplodiploids and diplodiploids. A recent study by Gardner et al. (2012) investigated how unmatedness influences the inclusive fitness valuations a female places on siblings and offspring and how this affects the evolution of helping. Here we compare the effects of unmatedness on the evolution of helping by females and males in haplodiploids and diplodiploids, accounting for both the inclusive fitness valuations of relatives of different classes, and the number of offspring produced by mated and unmated females.

Model and Results

In our model, we compare panmictic, monogamous (Hughes et al. 2008; Boomsma 2009), haplodiploid, and diplodiploid populations where helping has not evolved. Generations are nonoverlapping, so that at the change of season, the parent generation dies and all the offspring disperse to establish their own nests. We are looking at the behavior of an individual born early in the season who can either disperse and establish its own nest in the following season or sacrifice its own reproduction and become a helper at the natal nest, contributing to production of more siblings to the next cohort. The extra siblings produced by helping are assumed to follow the sex ratio determined by the mother.

We assume that, due to some external factor, a constant fraction u of all females remain unmated (all symbols used in the model are given in table 1). A female can remain unmated if she is not able to find a mate, for example, due to low population density (Godfray 1990; Rhainds 2010; Morse 2013). If a female does mate but the mating does not result in fertilization (possible reasons reviewed by Godfray 1990), she may become effectively unmated as remating can be obstructed (reviewed by Godfray 1990; Ode et al. 1997).

In order to analyze selection for helping behavior, we need to define the inclusive fitness valuation an individual places on its siblings and on its own offspring. The inclusive fitness valuation a potential helper places on different types of relatives depends on the consanguinity of the potential helper to those relatives and their individual reproductive values. Consanguinity is the probability that homologous genes drawn randomly from the two actors are identical by descent (Bulmer 1994). In an outbred population, the consanguinities between a haplodiploid female and her brother, sister, son, and daughter are $\gamma_{bro} = 1/4$, $\gamma_{sis} = 3/8$, $\gamma_{son} = 1/2$, and $\gamma_{dau} = 1/4$, respectively. Between a haplodiploid male and his brother, sister, and daughter, consanguinities are $\gamma_{bro} = 1/2$, $\gamma_{sis} = 1/4$, and $\gamma_{dau} = 1/2$, respectively. Between a diplodiploid female (male) and her (his) son, daughter, brother, and sister, consanguinities are all 1/4.

The individual reproductive value represents the relative expected reproductive success of an individual. In our analysis, the population consists of three relevant classes,

males (m), unmated females (uf), and mated females (mf). Class reproductive value is the probability that a random allele from an infinitely distant future generation originates from that class in the current generation. In haplodiploids, the probability for an allele to originate from a male (c_m) equals 1/3 and from either unmated (c_{uf}) or mated female (c_{mf}) totals ($c_{uf} + c_{mf} =$) 2/3. In diplodiploids, the allele originates from a mated female and from a male with the same probability of 1/2 and from an unmated female with a probability of 0. (Bulmer 1994; Taylor 1996) Individual reproductive values are then obtained by dividing the class reproductive value by the proportion of the respective class in the population (Taylor 1996; Taylor and Frank 1996).

We assume that both mated and unmated haplodiploid females produce equal numbers (n) of offspring, but unmated haplodiploid females produce only males (see Ode et al. 1997; Metzger et al. 2008). For ease of notation and comparison, we assume that mated diplodiploid females also produce the same (n) number of offspring. The assumption of identical numbers of offspring produced by reproductive females in both genetic systems is done only for convenience of comparison and does not affect the results or interpretation of the analysis. We further assume that mated females adjust their offspring sex ratio to maximize fitness. In haplodiploids, the mated female sex ratio strategy z_m (proportion of males a mated female produces) is then linked with the population sex ratio z_p (proportion of males in population) by the equation $z_p = u + (1 - u)z_m$. With a constant fraction u of females remaining unmated and producing all-male broods, mated females are selected to produce brood with a female-biased sex ratio. In appendix A, we give the derivation of the convergence-stable mated female sex ratio

$$z_m = \begin{cases} \dfrac{1-2u}{2-2u} & u < \dfrac{1}{2} \\ 0 & u \geq \dfrac{1}{2} \end{cases}, \tag{1}$$

which leads to a population sex ratio (see fig. 1)

$$z_p = \begin{cases} \dfrac{1}{2} & u < \dfrac{1}{2} \\ u & u \geq \dfrac{1}{2} \end{cases}. \tag{2}$$

These ratios are the same as derived by Gardner et al. (2012). For diplodiploids, the convergence-stable sex ratios (z_m and z_p) are 1/2, regardless of u (Fisher 1930).

We study selection for helping by solving how many additional siblings a helper must rear instead of dispersing and producing its own offspring in order for helping to be favored by natural selection. We call this quantity the "benefit threshold" (b_{tr}), and it can be understood as the threshold efficiency of helping, below which helping is selected against and above which helping is selected for. The number of siblings actually gained by helping is denoted with b.

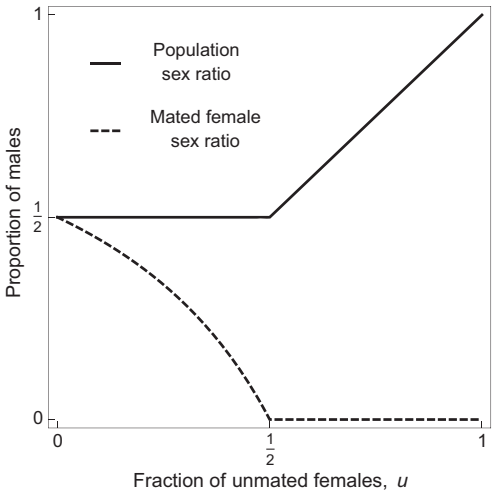

Figure 1: The convergence-stable sex ratios for a haplodiploid population when a constant fraction (u) of females remain unmated and produce all-male broods. The dashed line represents the convergence-stable sex ratio strategy of mated females (eq. [1]), and the solid line represents the population sex ratio (eq. [2]).

Next we derive the inclusive fitness valuations a newly born haplodiploid female and a diplodiploid individual (male or female) place on siblings and on their own offspring. Derivation of valuations a haplodiploid male places on siblings and its own offspring is given in appendix B.

The inclusive fitness valuation functions are composed of three terms, one for each class. Each of these terms is formed by the probability that the relative (sibling or offspring) in question belongs to the respective class, multiplied by consanguinity, and then multiplied by individual reproductive value. For a haplodiploid female and a diplodiploid male or female, the inclusive fitness valuation an individual places on a sibling is

$$\begin{aligned} w_{sib} :=\ & z_m y_{bro} \frac{c_m}{z_p} + u(1 - z_m) y_{sis} \frac{c_{uf}}{u(1 - z_p)} \\ & + (1 - u)(1 - z_m) y_{sis} \frac{c_{mf}}{(1 - u)(1 - z_p)} \\ =\ & \frac{z_m y_{bro} c_m}{z_p} + \frac{(1 - z_m) y_{sis} (c_{uf} + c_{mf})}{1 - z_p}, \end{aligned} \tag{3}$$

where the first term is the inclusive fitness valuation for brothers, the second is for sisters that are expected to remain unmated as adults, and the third is for sisters that are expected to mate.

The inclusive fitness valuation a newly born individual places on an own offspring is

$$\begin{aligned} w_{\text{off}} :&= \frac{z_{\text{p}} y_{\text{son}} c_{\text{m}}}{z_{\text{p}}} + \frac{u(1-z_{\text{p}}) y_{\text{dau}} c_{\text{uf}}}{u(1-z_{\text{p}})} \\ &\quad + \frac{(1-u)(1-z_{\text{p}}) y_{\text{dau}} c_{\text{mf}}}{(1-u)(1-z_{\text{p}})} \\ &= y_{\text{son}} c_{\text{m}} + y_{\text{dau}}(c_{\text{uf}} + c_{\text{mf}}), \end{aligned} \tag{4}$$

where the first term is the inclusive fitness valuation for sons, the second for daughters that are expected to remain unmated as adults, and the third for daughters that are expected to mate.

To take into account the effect of unmatedness on the benefit threshold b_{tr}, we need to weigh the inclusive fitness valuations of the average sibling and the average offspring of a potential helper by their expected numbers. Because both unmated and mated haplodiploid females produce the same amount of offspring, the expected number of offspring for a haplodiploid female is simply n. Fraction u of diplodiploid females do not mate and thus produce zero offspring. Hence, the number of offspring a newly born diplodiploid female expects to have is $(1 - u)n$. Assuming 1 : 1 sex ratio, the expected number of matings for a diplodiploid male is $1 - u$, and the expected number of offspring is $(1 - u)n$, the same as for a diplodiploid female.

Now we can write the inequalities that give conditions for the helping allele to spread in the population and define the benefit thresholds. For a haplodiploid female this inequality is that $bw_{\text{sib}} > nw_{\text{off}}$, which is equivalent with $b > nw_{\text{off}}/w_{\text{sib}} =: b_{\text{tr}}$. After substituting in the sex ratios, consanguinities and reproductive values we obtain the benefit threshold for helping

$$b_{\text{tr}} = \begin{cases} \dfrac{2-2u}{2-u} n & u < \dfrac{1}{2} \\ \dfrac{4}{3(1-u)} n & u \geq \dfrac{1}{2} \end{cases}. \tag{5}$$

The benefit threshold for a haplodiploid male (see app. B for derivation) is

$$b_{\text{tr}} = \begin{cases} n & u < \dfrac{1}{2} \\ 2(1-u)n & u \geq \dfrac{1}{2} \end{cases}. \tag{6}$$

For a diplodiploid male or female, the inclusive fitness inequality condition is that $bw_{\text{sib}} > (1 - u)nw_{\text{off}}$, which is equivalent with $b > (1 - u)nw_{\text{off}}/w_{\text{sib}} =: b_{\text{tr}}$, from which after substituting in the sex ratios, consanguinities, and reproductive values we obtain that

$$b_{\text{tr}} = (1-u)n. \tag{7}$$

To see the full effect of unmatedness on the evolution of helping behavior in haplodiploids and diplodiploids, we compare the benefit thresholds b_{tr} (fig. 2). In both genetic systems, unmatedness promotes helping behavior by females. The evolution of female helping becomes easier as the frequency of unmated females in the population increases in both haplodiploids and diplodiploids. However, for any frequency of unmated females in the population, the benefit threshold b_{tr} is lower for diplodiploids than for haplodiploids. Unmatedness thus makes the evolution of female helping easier in diplodiploids than in haplodiploids.

Looking at the effect of unmatedness on selection for male helping behavior, we see that unmatedness does not promote male helping in haplodiploid species, as long as at least half of the females mate ($u < 1/2$; app. B; see fig. 2). The reason is that the decrease in the expected number of matings with increasing unmatedness is exactly counterbalanced by the more female-biased sex ratio of mated

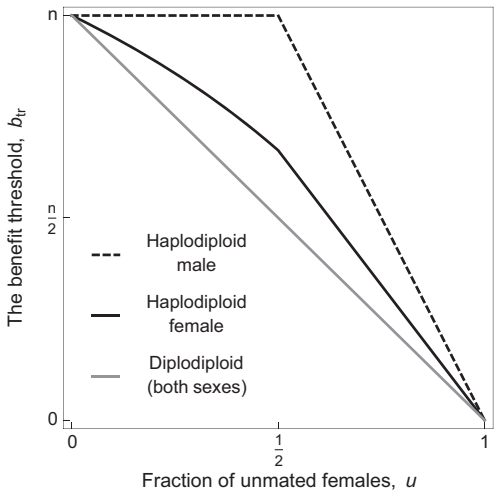

Figure 2: The benefit threshold of helping (b_{tr}) in panmictic populations for different frequencies of unmated females, assuming that mated females are monogamous and optimize their offspring sex ratios. For all frequencies of unmated females, evolution of helping is easier for diplodiploids (solid gray line for both males and females; eq. [7]) than for haplodiploid females (solid black line; eq. [5]) and males (dashed black line; eq. [6]).

females. A more female-biased sex ratio means that per mating, a male sires more offspring (remembering that in haplodiploids males only have daughters). In diplodiploids, on the other hand, unmatedness affects males and females similarly (both unmated males and females have zero reproductive output), and selection on helping behavior is the same for both sexes. When $u = 0$, we also see that without any outside factors, helping should evolve as readily in males as in females in both haplodiploids and in diplodiploids.

Discussion

We have shown that when the effects of unmatedness on relatedness asymmetries and reproductive output are fully taken into account for both haplodiploids and diplodiploids, diplodiploidy may be more favorable to evolution of helping than haplodiploidy. While the existence of unmated females favors the evolution of helping in both systems, the effect is stronger in diplodiploids. Interestingly, the reasons why the benefit threshold decreases with the frequency of unmated females are different in the two genetic systems. In diplodiploids, the benefit threshold decreases when frequency of unmated females increases simply because the decision to disperse carries risk u of complete reproductive failure for females (and for males). In haplodiploids, unmated females produce brood consisting of males only, which results in split sex ratios in the population. The split sex ratios reduce the benefit threshold for females because they are in nests with a more female-biased sex ratio than the population average sex ratio and can thus capitalize on the high sister-sister relatedness.

While population density and structure are important parts of the suite of ecological constraints that underlie the evolution of helping (Emlen 1982; Hatchwell 2009), their impact on resource levels and chances of obtaining a territory or a nest site have been studied more than their effects on possible mating failures. Studies of unmatedness in haplodiploids have mainly concentrated on demonstrating whether mated females shift their offspring sex ratio as a response to the presence of unmated females, as suggested by Godfray (1990). In species where unmatedness is common, mated females do indeed produce female-biased broods. The female-biased sex ratio of mated females seems to be an evolved response to the mean frequency of unmated females in the population over evolutionary time rather than a plastic response to the current mating situation in the population (Ode et al. 1997; Kranz et al. 2000; Metzger et al. 2008).

We have shown that, all else being equal, unmatedness promotes helping more in diplodiploids than in haplodiploids. However, if unmatedness is more common in haplodiploids, then its importance as a driver of helping in haplodiploids would be elevated. As has been pointed out by Godfray (1990), at the incipient stages of social evolution, remaining unmated is not strongly selected against in haplodiploid females (apart from sex ratio selection against all-male broods when population is not at sex ratio equilibrium). Thus, any costs related to mate search or mating itself, such as predation, male harming, and sexually transmitted pathogens, could even select for voluntary virginity in females (Godfray 1990; Guertin et al. 1996). The ability of males (who need to mate in order to gain offspring) to find mates will then be an important factor determining the proportion of virgins. In contrast, remaining unmated is strongly selected against in both sexes in diplodiploids and is thus expected to stay extremely rare. Estimates of frequencies of virgins in haplodiploids range from 0% to 29% (Godfray and Hardy 1992), but many of the estimates come from species where local mating competition and inbreeding prevail. Although unmated females in species with characteristics similar to the ancestors of social Hymenopterans, and our model life-history assumptions, seem to be fairly rare (Gardner et al. 2012), at least one system exists that fits our model assumptions and exhibits high virgin frequencies. In *Kladothrips rugosus*, an outbreeding haplodiploid gall-inducing thrip, 24% of females remain unmated and produce only sons, and the mated females compensate by producing female-biased sex ratio (Kranz et al. 2000). Empirical data on unmatedness, however, is relatively scarce, and the evolution of voluntary virginity and its connection to the evolutionary origins of helping under various life-history and breeding system assumptions clearly merit further study.

A recent analysis by Ross et al. (2013) suggested that instead of ploidy levels, the ecological benefits favoring eusociality (nest defense vs. brood care; Queller and Strassmann 1998) and possible preadaptations (maternal vs. biparental care as an ancestral state; Wade 2001; Linksvayer and Wade 2005; Gardner 2012) could explain the observed patterns in helper sex ratios across taxa. Our analysis however suggests that haplodiploidy, together with unmatedness, could have contributed to the evolution of exclusive female help in outbreeding species like eusocial Hymenoptera by lowering the benefit threshold female helping. In contrast to Hymenoptera, in eusocial haplodiploid thrips both male and female offspring participate in nest defense (Crespi 1992; Kranz et al. 1999). The evolution of both male and female helpers in eusocial thrips may be linked to lack of preadaptations in either sex to soldiering, as suggested by Ross et al. (2013), but also to the high degree of ancestral inbreeding in these species (McLeish et al. 2006), which reduces the importance of relatedness asymmetries

(Chapman et al. 2000). For diplodiploid species, our model predicts equal benefit thresholds for males and females. In nature, diplodiploid species show variable patterns of helper sex, and the patterns may be linked to preadaptions to certain helper tasks (Ross et al. 2013).

In general, our work highlights the need to consider interactions between ecological and genetic factors in the evolution of helping and eusociality. We show that features of mating systems, that on the one hand define the degree of relatedness within families, may also shape the direct costs and benefits relevant to the evolution of helping. Specifically, we suggest that studying the coevolutionary relationship between voluntary virginity and helping behavior may shed light on early stages of eusociality in outbreeding haplodiploid species.

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APPENDIX A

Derivation of Convergence-Stable Sex Ratio

Here we calculate the convergence-stable mated female sex ratio strategy for an infinite haplodiploid population with fixed fraction (u) of unmated females. The model has a class structured population with three classes: males (m), unmated females (uf) and mated females (mf), where only the last can control their offspring sex ratios. The mated females are the balancing class, optimizing their sex ratio z_m to yield maximal fitness (Taylor 1996; Taylor and Frank 1996). The mated female sex ratio determines the population sex ratio z_p. From the perspective of a mutant mated female that follows a sex ratio strategy z in a population of females following z_m, we define the inclusive fitness valuation she places on an average offspring as

$$\begin{aligned} w(z, z_m) &:= \frac{z y_{son} c_m}{u + (1-u) z_m} + \frac{u(1-z) y_{dau} c_{uf}}{u(1-u)(1-z_m)} + \frac{(1-u)(1-z) y_{dau} c_{mf}}{(1-u)(1-u)(1-z_m)} \\ &= \frac{z y_{son} c_m}{u + (1-u) z_m} + \frac{(1-z) y_{dau} (c_{uf} + c_{mf})}{(1-u)(1-z_m)}. \end{aligned} \tag{A1}$$

We can find a candidate for the convergence-stable mated female sex ratio strategy by solving

$$\left. \frac{\partial w(z, z_m)}{\partial z} \right|_{z=z_m} = \frac{1}{6[u + (1-u) z_m]} - \frac{1}{6(1-u)(1-z_m)} = 0, \tag{A2}$$

which gives

$$z_m = \begin{cases} \dfrac{1-2u}{2-2u} & u < \dfrac{1}{2} \\ 0 & u \geq \dfrac{1}{2} \end{cases}. \tag{A3}$$

To see whether this candidate is in fact convergence stable, we need to check whether natural selection favors higher-than-average sex ratio strategies when the population average is lower than the candidate and vice versa. First we look at the case of $u < 1/2$. When the population follows on average a strategy of $z_m < (1 - 2u)/(2 - 2u)$, the partial derivative of w is positive for any z, meaning that natural selection favors any strategy that is greater than z_m. Also when the population follows on average a strategy of $z_m > (1 - 2u)/(2 - 2u)$, the partial derivative of w is negative for any z, meaning that natural selection favors any strategy that is less than z_m. Next we look at the case of $u \geq 1/2$. When the population follows on average a strategy of $z_m > 0$, the partial derivative of w is negative for any z, meaning that natural selection favors any strategy that is less than z_m. This analysis shows that our candidate is the convergence-stable mated female sex ratio strategy (Geritz et al. 1997). This is the same sex ratio as derived by Gardner et al. (2012) and reported by Godfray (1990).

APPENDIX B

The Benefit Threshold for Haplodiploid Males

Here we calculate the threshold benefit for the evolution helping for a haplodiploid male in a population where mated females follow their evolutionarily stable sex ratio strategy. Unlike in diplodiploids, where the inclusive fitness model for females corresponds with the model for males, in haplodiploids the model for males differs from the model for females for two reasons. First, the male can be either a son of an unmated or mated female, meaning that the inclusive fitness valuation of siblings needs to take the probabilities of either into account. Second, since males can only have daughters, the number of offspring a dispersing male expects to have depends on the expected number of matings and on how many daughters a mated female produces.

With a probability of $u/[u + (1 - u)z_m]$ the focal male is a son of an unmated female and has only brothers as siblings, and with the probability of $(1 - u)z_m/[u + (1 - u)z_m]$ the focal male is a son of a mated female and has both sisters and brothers as siblings. Thus the inclusive fitness valuation the focal male places on his average sibling can be written as

$$\begin{aligned} w_{\text{sib}} :&= \frac{(1-u)z_m}{u+(1-u)z_m}\left[\frac{z_m y_{\text{bro}} c_m}{z_p} + \frac{u(1-z_m)y_{\text{sis}}c_{\text{uf}}}{u(1-z_p)} + \frac{(1-u)(1-z_m)y_{\text{sis}}c_{\text{mf}}}{(1-u)(1-z_p)}\right] \\ &\quad + \frac{u}{u+(1-u)z_m}\frac{y_{\text{bro}}c_m}{z_p} \\ &= \frac{[(1-u)z_m^2 + u]y_{\text{bro}}c_m}{z_p^2} + \frac{z_m y_{\text{sis}}(c_{\text{uf}} + c_{\text{mf}})}{z_p}. \end{aligned} \tag{B1}$$

Since males can have only daughters, the inclusive fitness valuation a male places on an offspring of his is

$$w_{\text{off}} := \frac{u y_{\text{dau}} c_{\text{uf}}}{u(1-z_p)} + \frac{(1-u)y_{\text{dau}}c_{\text{mf}}}{(1-u)(1-z_p)} = \frac{y_{\text{dau}}(c_{\text{uf}} + c_{\text{mf}})}{1-z_p}. \tag{B2}$$

The expected number of matings for a male is $(1 - u)(1 - z_p)/z_p$, so the number of offspring (daughters) a male expects to get when dispersing is $n(1 - z_m)(1 - u)(1 - z_p)/z_p$. With that we can write the inequality that gives the condition for the helping gene expressed in a male to spread: $bw_{\text{sib}} > n(1 - z_m)(1 - u)(1 - z_p)w_{\text{off}}/z_p$, which is equivalent with $b > n(1 - z_m)(1 - u)(1 - z_p)w_{\text{off}}/(z_p w_{\text{sib}}) =: b_{\text{tr}}$. Substituting in the consanguinities of the male to his daughter ($y_{\text{dau}} = 1/2$), to his sister ($y_{\text{sis}} = 1/4$) and to his brother ($y_{\text{bro}} = 1/2$), the reproductive values given in the model section and sex ratios calculated in appendix A and model section, we get that for a haplodiploid male the benefit threshold $b_{\text{tr}} = n$ when $u < 1/2$ and $b_{\text{tr}} = 2(1 - u)n$ when $u \geq 1/2$.

Literature Cited

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

Bourke, A. 2011. Principles of social evolution. Oxford University Press, Oxford.

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

Chapman, T. W., B. J. Crespi, B. D. Kranz, and M. P. Schwarz. 2000. High relatedness and inbreeding at the origin of eusociality in gall-inducing thrips. Proceedings of the National Academy of Sciences of the USA 97:1648–1650.

Craig, R. 1979. Parental manipulation, kin selection, and the evolution of altruism. Evolution 33:319.

———. 1980. Sex investment ratios in social hymenoptera. American Naturalist 116:311–323.

———. 1982. Evolution of male workers in the hymenoptera. Journal of Theoretical Biology 94:95–105.

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

Emlen, S. T. 1982. The evolution of helping. I. An ecological constraints model. American Naturalist 119:29–39.

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

Gardner, A. 2012. Evolution of maternal care in diploid and haplodiploid populations. Journal of Evolutionary Biology 25:1479–1486.

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

Geritz, S. A. H., G. Mesze, and J. A. J. Metz. 1997. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evolutionary Ecology 12:35–57.

Godfray, H. C. J. 1990. The causes and consequences of constrained

sex allocation in haplodiploid animals. Journal of Evolutionary Biology 3:3–17.
Godfray, H. C. J., and A. Grafen. 1988. Unmatedness and the evolution of eusociality. American Naturalist 131:303–305.
Godfray, H. C. J., and I. C. W. Hardy. 1992. Sex ratio and virginity in haplodiploid animals. Pages 402–421 *in* D. L. Wrensch and M. A. Ebbert, eds. Evolution and diversity of sex ratio in insect and mites. Chapman & Hall, New York.
Grafen, A. 1986. Split sex ratios and the evolutionary origins of eusociality. Journal of Theoretical Biology 122:95–121.
Guertin, D. S., P. J. Ode, M. R. Strand, and M. F. Antolin. 1996. Host-searching and mating in an outbreeding parasitoid wasp. Ecological Entomology 21:27–33.
Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. Journal of Theoretical Biology 7:17–52.
Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. Annual Review of Ecology and Systematics 3:193–232.
Hatchwell, B. J. 2009. The evolution of cooperative breeding in birds: kinship, dispersal and life history. Philosophical Transactions of the Royal Society B: Biological Sciences 364:3217–3227.
Hughes, W. O. H., B. P. Oldroyd, M. Beekman, and F. L. W. Ratnieks. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213–1216.
Kranz, B. D., M. P. Schwarz, L. C. Giles, and B. J. Crespi. 2000. Split sex ratios and virginity in a gall-inducing thrips. Journal of Evolutionary Biology 13:700–706.
Kranz, B. D., M. P. Schwarz, L. A. Mound, and B. J. Crespi. 1999. Social biology and sex ratios of the eusocial gall-inducing thrips *Kladothrips hamiltoni*. Ecological Entomology 24:432–442.
Linksvayer, T. A., and M. J. Wade. 2005. The evolutionary origin and elaboration of sociality in the aculeate hymenoptera: maternal effects, sib-social effects, and heterochrony. Quarterly Review of Biology 80:317–336.
McLeish, M. J., T. W. Chapman, and B. J. Crespi. 2006. Inbreeding ancestors: the role of sibmating in the social evolution of gall thrips. Journal of Heredity 97:31–38.
Metzger, M., C. Bernstein, and E. Desouhant. 2008. Does constrained oviposition influence offspring sex ratio in the solitary parasitoid wasp *Venturia canescens*? Ecological Entomology 33:167–174.
Morse, D. H. 2013. Reproductive output of a female crab spider: the impacts of mating failure, natural enemies, and resource availability. Entomologia Experimentalis et Applicata 146:141–148.
Ode, P. J., M. F. Antolin, and M. R. Strand. 1997. Constrained oviposition and female-biased sex allocation in a parasitic wasp. Oecologia (Berlin) 109:547–555.
Pamilo, P. 1991. Evolution of the sterile caste. Journal of Theoretical Biology 149:75–95.
Queller, D. C., and J. E. Strassmann. 1998. Kin selection and social insects. BioScience 48:165–175.
Rhainds, M. 2010. Female mating failures in insects. Entomologia Experimentalis et Applicata 136:211–226.
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.
Taylor, P. D. 1996. Inclusive fitness arguments in genetic models of behaviour. Journal of Mathematical Biology 34:654–674.
Taylor, P. D., and S. A. Frank. 1996. How to make a kin selection model. Journal of Theoretical Biology 180:27–37.
Trivers, R., and H. Hare. 1976. Haplodiploidy and the evolution of the social insect. Science 191:249–263.
Wade, M. J. 2001. Maternal effect genes and the evolution of sociality in haplo-diploid organisms. Evolution 55:453–458.

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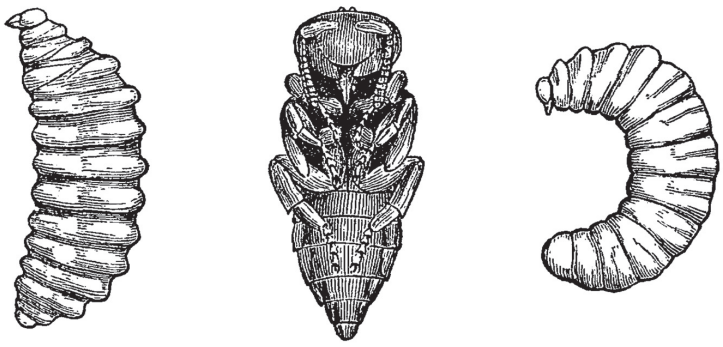

"While the Andrena and Halictus bees, whose habits we now describe, are closely allied in form to the Hive-bee, socially they are the 'mud-sills' of bee society, ranking among the lowest forms of the family of bees, or *Apidæ*. Their burrowing habits ally them with the ants, from whose nests their own burrows can scarcely be distinguished. [...] Moreover they are not social; they have no part in rearing and caring for their young, a fact that lends so much interest to the history of the Hive and Humble-bee." From "The Home of the Bees (Concluded)" by A. S. Packard Jr. (*The American Naturalist*, 1868, 1:596–606).

Correction

The article "Unmatedness promotes the evolution of helping more in diplodiploids than in haplodiploids" (*American Naturalist* 184:318–325) was published with an error in equation (5). The error does not affect any results or conclusions in the article. The authors' corrected equation is shown below:

$$b_{\text{tr}} = \begin{cases} \dfrac{2-2u}{2-u}n, & u < \dfrac{1}{2} \\ \dfrac{4}{3}(1-u)n, & u \geq \dfrac{1}{2} \end{cases}. \tag{5}$$

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II

LINKING ECOLOGY AND LIFE-HISTORY TO THE COSTS AND BENEFITS OF ALTRUISM

by

Petri Rautiala, Heikki Helanterä, Hanna Kokko & Mikael Puurtinen 2016

Submitted manuscript

III

THE EVOLUTIONARY DYNAMICS OF ADAPTIVE VIRGINITY, SEX-ALLOCATION AND ALTRUISTIC HELPING IN HAPLODIPLOID ANIMALS

by

Petri Rautiala, Heikki Helanterä & Mikael Puurtinen 2016

Manuscript

IV

INTRAGENOMIC CONFLICT OVER SOLDIER ALLOCATION IN POLYEMBRYONIC PARASITOID WASPS

by

Petri Rautiala & Andy Gardner 2016

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E-Note

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 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

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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 $1 - d_{\mathrm{f}}$ 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_{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

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

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

$$M = d_{\mathrm{m}} \frac{\bar{N}_{\mathrm{f}}}{\bar{N}_{\mathrm{m}}} + (1 - d_{\mathrm{m}}) \frac{(1 - d_{\mathrm{f}})N_{\mathrm{f}} + d_{\mathrm{f}}\bar{N}_{\mathrm{f}}}{(1 - d_{\mathrm{m}})N_{\mathrm{m}} + d_{\mathrm{m}}\bar{N}_{\mathrm{m}}},$$

where $\bar{N}_{\mathrm{m}}$ and $\bar{N}_{\mathrm{f}}$ 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

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

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

$$\left.\frac{\partial H_{\mathrm{A}}}{\partial z}\right|_{z=\bar{z}} = \left[\frac{(2 - d_{\mathrm{m}})d_{\mathrm{m}}}{\bar{z}} + (d_{\mathrm{f}} - d_{\mathrm{m}})(1 - d_{\mathrm{m}}) - 1\right] Np_{\mathrm{mA}} - 2Np_{\mathrm{fA}} > 0.$$

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.

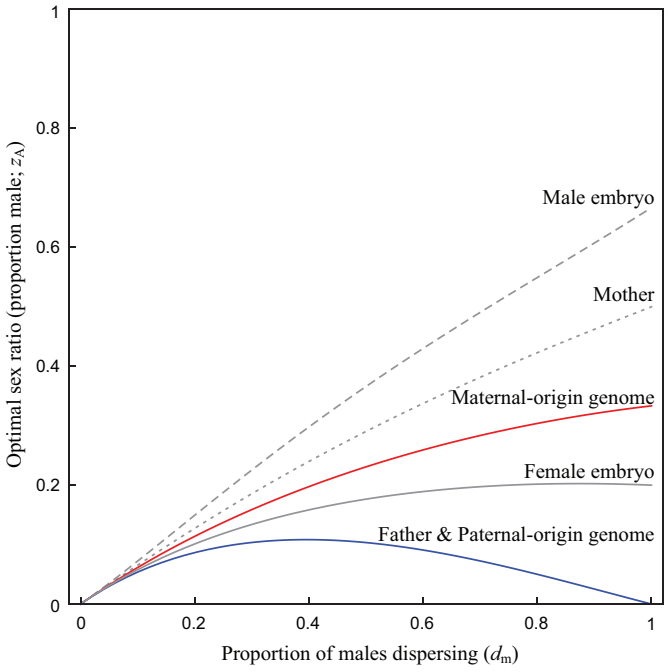

Figure 1: Illustration of the sex-ratio optima z_A. The lines represent the sex-ratio optima with zero female dispersal ($d_f = 0$) with respect to the proportion of males dispersing (d_m). They are calculated from equation (3) by substituting the appropriate consanguinity coefficients given in table 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

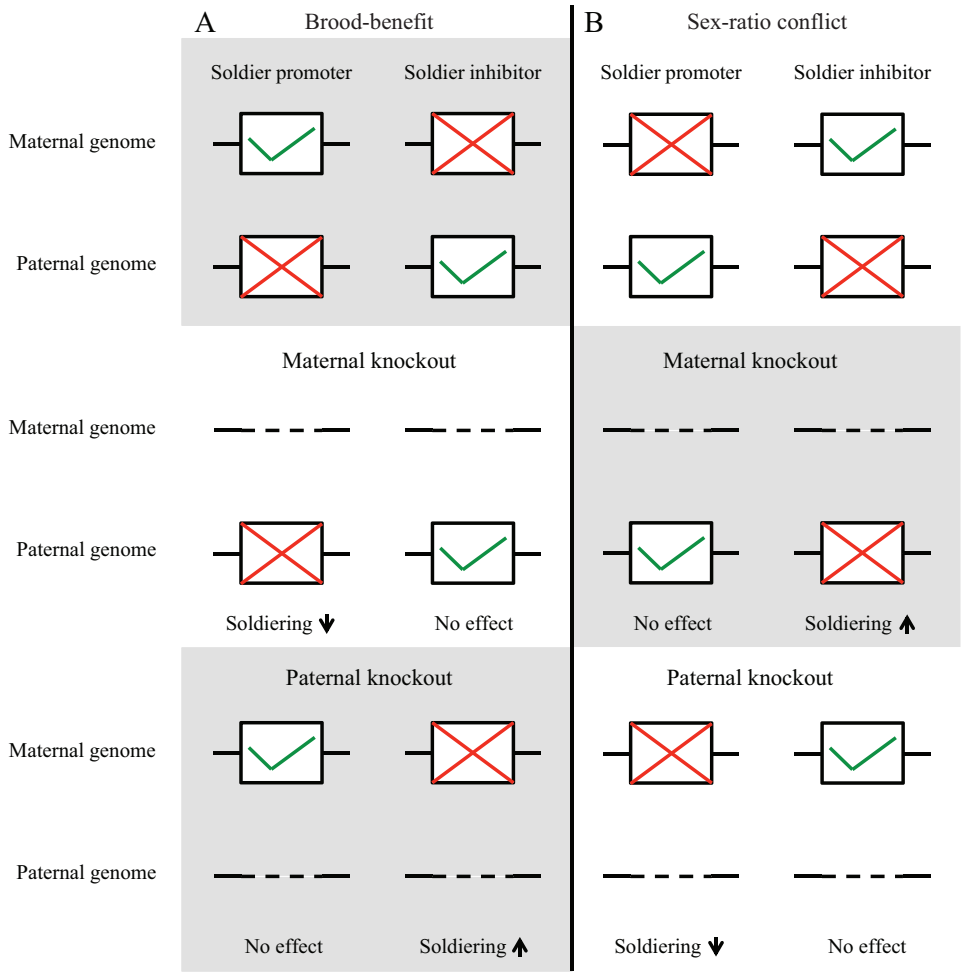

Figure 2: Predictions for patterns of genomic imprinting. Under the brood-benefit hypothesis (*A*), we predict that soldier promoters will be maternally expressed (green) and paternally silenced (red) and that soldier inhibitors will be maternally silenced and paternally expressed. Under the sex-ratio-conflict 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.

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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

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

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.

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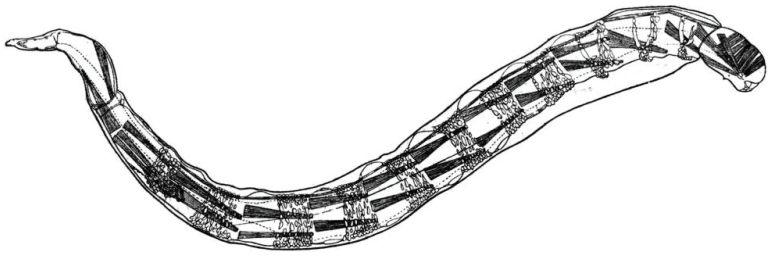

"What, then, is the function of these asexual larvae, whose structure is so unusual, indeed unique, among all known insect larvae?" From "Contribuzioni alla conoscenza biologica degli Imenotteri parassiti I: Biologia del *Litomastix truncatellus* (Dalm.) (2° nota preliminare)" by Filippo Silvestri (*Annali 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).