

A review of the consequences of complementary sex determination and diploid male production on mating failures in the Hymenoptera

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Abstract

Complementary sex determination is the ancestral sex-determination mechanism in the Hymenoptera. Under this system, diploid individuals develop into females if they are heterozygous at an autosomal sex-determining locus or loci, whereas haploid individuals develop into males because they are hemizygous at the sex-determining locus or loci. However, diploid males can still arise from fertilized eggs if such individuals are homozygous at the sex-determining locus or loci. Diploid males are often viable but sire few daughters, thereby representing a substantial genetic load in hymenopteran populations. Here, we review the effects of complementary sex determination and diploid male production from the perspective of female hymenopterans. Because female hymenopterans need not mate to produce haploid sons, complementary sex determination can cause special forms of mating failures by preventing some females from controlling the sex ratio of their brood and producing the desired number of daughters. Under some circumstances, complementary sex determination can cause complete mating failure by preventing females from producing daughters altogether. Although we outline serious gaps of knowledge in the field, the data at hand suggest that diploid male production can substantially increase mating failures in small populations of economically and ecologically important hymenopterans.

Introduction

The insect order Hymenoptera is comprised of sawflies, wasps, ants, and bees with several taxa of immense ecological and economic relevance. Bees, for example, provide indispensable pollination services in both natural and agricultural settings (Michener, 2000). Ants, through their sheer biomass, play major roles in the ecology of tropical and temperate ecosystems (Wilson, 1987). Wasps are important parasitoids of several damaging insect pests, and play a very useful role in biological control and integrated pest management (Clausen, 1978; Heimpel & Mills, 2008). Hymenopteran insects are characterized by haplodiploidy or male-haplodiploidy, which results from arrhenotokous parthenogenesis whereby unfertilized eggs develop into haploid males and fertilized eggs develop into diploid females (Crozier, 1977, 1985). Haplodiploidy allows

female hymenopterans to directly control the primary sex ratio of their brood by simply controlling the ratio of unfertilized to fertilized eggs – a phenomenon of great relevance to the biology of these fascinating insects (Hamilton, 1964a,b; Meunier et al., 2008).

However, not all fertilized eggs develop into diploid females. Diploid males were first observed in *Habrobracon* (= *Bracon*) species (Whiting, 1943), and were later found in more than eighty hymenopteran species (van Wilgenburg et al., 2006; Heimpel & de Boer, 2008). The observation of diploid males in *Habrobracon* spp. led Whiting (1943) to propose that sex was determined by the complementary actions of alleles at a single hypervariable autosomal locus: heterozygous and hemizygous individuals develop into diploid females and haploid males, respectively, whereas homozygous individuals develop into diploid males (Figure 1A). A similar model posits that sex is determined by the complementary action of alleles at multiple loci (multiple-locus complementary sex determination, ml-CSD) (reviewed by Cook, 1993).

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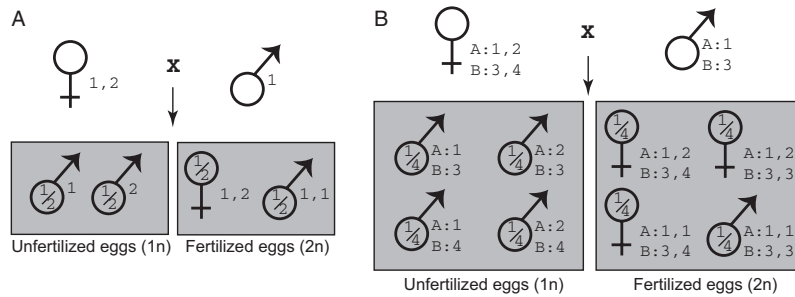


Figure 1 The consequences of a matched mating with complementary sex determination (CSD). (A) With single-locus (sl)-CSD, a mating between two adults sharing the same sex allele will result in 50% of the diploid progeny being homozygous at the sex-determining locus (i.e., diploid males). (B) With multiple-locus (ml)-CSD, assuming two unlinked loci, only 25% of the progeny from a matched mating will be homozygous at both sex-determining loci and will develop into diploid males.

Under ml-CSD, as observed in the wasp *Cotesia vestalis* (Haliday) (de Boer et al., 2008), homozygotes at all sex-determining loci develop into diploid males (Figure 1B).

Single-locus CSD has been experimentally validated in many Hymenoptera, and the sex-determination locus has been genetically identified in the honey bee, *Apis mellifera* L. The sex-determining gene, *csd* in honey bees (Beye et al., 2003), sends the primary sex-determining signal, that leads to sex-specific splicing of *feminizer* – a gene that is homologous to *Drosophila melanogaster* Meigen's *transformer* (Hasselmann et al., 2008). The *feminizer* male-specific splice form contains a premature stop codon and does not result in a functional product, but the female-specific splice form encodes a functional protein that is hypothesized to act on the conserved *doublesex* gene, which in turn leads to sex-specific differentiation (Hasselmann et al., 2008; Gempe et al., 2009). This mechanism presumably acts in a similar way in other species with CSD, although Hasselmann et al. (2008) did not detect *csd* outside of *Apis*, suggesting that different genes may be responsible for initiating the primary sex-determination signal in other species.

Complementary sex determination is common in the Hymenoptera, although some species lack CSD. For example, the Chalcididae (Dobson & Tanouye, 1998) and species within Figitidae, Braconidae, Scelionidae, and Bethyridae (van Wilgenburg et al., 2006) have been found to lack CSD. Furthermore, diploid males can be produced in some species that lack CSD, such as in *Nasonia* spp. (Dobson & Tanouye, 1998), albeit at very low frequencies. However, by mapping the mode of sex determination on a phylogeny of the Hymenoptera, Asplen et al. (2009) determined that CSD is the ancestral trait, although it is unclear whether sl (single-locus)-CSD preceded ml-CSD, or vice versa (van Wilgenburg et al., 2006; Asplen et al., 2009).

Population genetics of complementary sex determination

The frequency of diploid male production is mostly a function of the number of sex-determining alleles segregating in the population (Figure 2; Adams et al., 1977; Owen & Packer, 1994; Cook & Crozier, 1995; Zayed, 2009). Mutation introduces novel allelic variation at the complementary sex locus (or loci), and individuals carrying rare sex-determining alleles will have higher fitness because they are less likely to participate in a matched mating, where the two mating adults share the same sex-determining allele (Figure 1). As a result, the sex-determining locus experiences strong negative frequency-dependent selection that acts to homogenize allele frequencies in the population. However, there are limits to the maximum number of sex alleles that can be maintained in a finite population. As the number of alleles increases, the equilibrium allele frequency decreases proportionally. For k alleles, the equilibrium allele frequency is $1/k$ (Adams et al., 1977; Owen & Packer, 1994; Zayed, 2009). Eventually, the number of sex alleles reaches a mutation-selection-drift equilibrium where the effects of mutation and negative frequency-dependent selection are offset by random sampling effects (Yokoyama & Nei, 1979). All other factors being equal, populations with larger effective sizes (N_e) are expected to maintain more sex-determining alleles than those with smaller N_e (Figure 2; Yokoyama & Nei, 1979; Cornuet, 1980; Zayed, 2009).

In random-mating populations, diploid males arise as a function of the equilibrium allele frequency at the sex-determination locus: with k alleles, each at frequency $1/k$, the expected frequency of homozygosity is $k(1/k)^2 = 1/k$. In other words, $1/k$ of all the fertilized eggs in a random-mating population with sl-CSD will develop into diploid males. Therefore, in random-mating populations, the frequency of diploid male production is a function of the

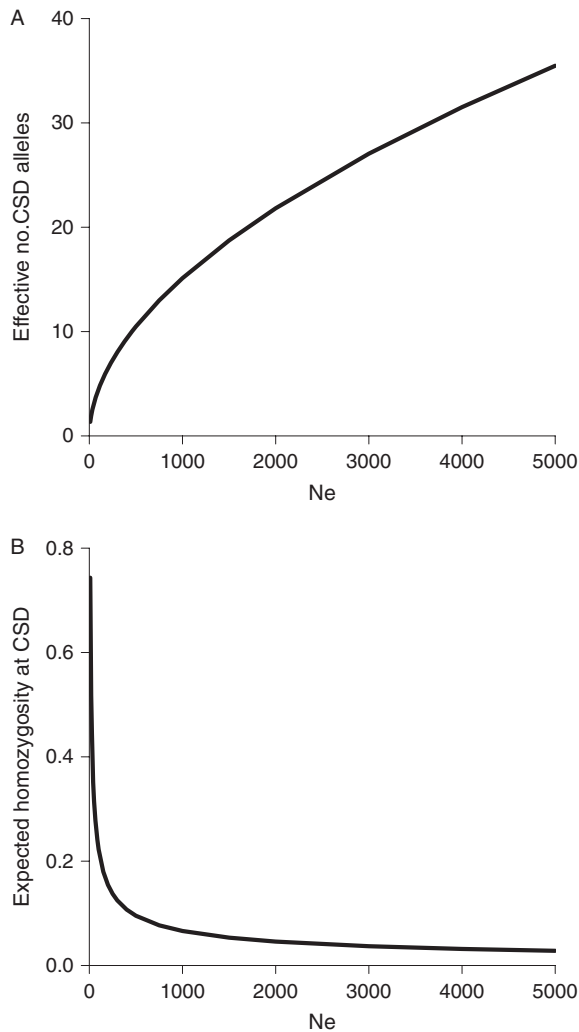


Figure 2 (A) Smaller hymenopteran populations (effective population size; N_e) with single-locus complementary sex determination (sl-CSD) maintain fewer alleles at the sex-determining locus when compared with larger populations. (B) Assuming random mating, the expected frequency of homozygous individuals at the sex-determination locus, which develop into diploid males, is higher in smaller populations. The expected number of sex-determining alleles was estimated following Cornuet (1980), and assuming a mutation rate of 10^{-6} .

number of sex-determining alleles, which is in turn controlled by effective population size (Figure 2B).

In populations that inbreed in a mating-system sense, the frequency of diploid male production is more influenced by the inbreeding coefficient rather than the number of sex-determining alleles. For example, consider the progeny of an unmatched singly mated female. Half of all possible matings between sibs will be matched at the sex locus, and will result in the production of diploid males. Diploid

male production is thus expected to be highest in small populations, as well as in populations that inbreed in a mating-system sense (Cook & Crozier, 1995; van Wilgenburg et al., 2006; Heimpel & de Boer, 2008; Zayed, 2009). Species with life-history traits that promote inbreeding are expected to have evolved adaptations to reduce the negative impacts of diploid male production, or eliminate CSD altogether (van Wilgenburg et al., 2006; Heimpel & de Boer, 2008). Indeed, it has been hypothesized that the duplication of sex-determination loci can drastically reduce the genetic load associated with inbreeding (de Boer et al., 2008). Further, species with CSD have been hypothesized to have the capacity to detect the sex-determination alleles of their mates and/or to avoid matched matings (van Wilgenburg et al., 2006). Although, some hymenopterans have general behaviours that reduce the potential of mating with closely related individuals, such as protandry, post-natal dispersal, and nestmate recognition (Plowright & Pallett, 1979; Foster, 1992), there is no evidence supporting that hymenopterans signal their allelic composition at CSD loci. As such, diploid males can still be produced at high frequencies in randomly mating populations that are depauperate at the sex-determination locus or loci (Ross & Fletcher, 1986; Buttermore et al., 1998; Zayed et al., 2007).

Diploid male production can have drastic effects on small populations with CSD because diploid males often have reduced fertility (Cook & Crozier, 1995; Wu et al., 2003; Zayed, 2004; Zayed & Packer, 2005; Hein et al., 2009). From the population's perspective, diploid males represent failed attempts at female production. High levels of diploid male production reduce both the number of breeding females, and their net reproductive output, which in turn reduces the intrinsic growth rate of populations, and can theoretically bring about extinction in small isolated populations (Zayed, 2004; Zayed & Packer, 2005; Hein et al., 2009; Whitehorn et al., 2009). Although the effects of CSD are often discussed from a population's perspective, here we shift our focus and reinterpret the effects of CSD from a female's perspective.

Because of haplodiploidy, female hymenopterans only need to mate to produce female progeny. If mating is solely a means for female hymenopterans to obtain sperm for fertilized eggs, then it is easy to envision how CSD can cause special forms of 'mating failures', here defined by the compromised reproductive potential of females mated to matched-haploid males and diploid males (Figure 3). First, consider a female that is singly mated to an unmatched-haploid male; 100% of her fertilized eggs will develop into daughters (Figure 3A). Now consider a female participating in a matched mating: half of her diploid progeny, all intended to be daughters, will now

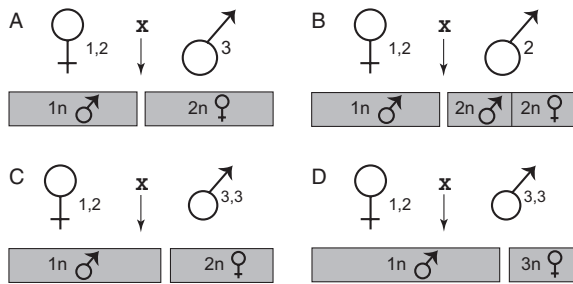


Figure 3 Complementary sex determination (CSD) and female mating failures in the Hymenoptera. (A) Mating failures are benchmarked against a female mated to an unmatched-haploid male. All of the diploid progeny from unmatched matings develop into daughters. (B) A matched mating constitutes a partial-mating failure because females produce 50% fewer daughters assuming single-locus (sl)-CSD. (C) In two species, diploid males can produce reduced haploid sperm and are capable of siring diploid daughters. Nevertheless, females mated to fertile diploid males experience partial-mating failures because they produced fewer daughters relative to females mated to haploid males. (D) In most species, effectively sterile diploid males severely limit the productivity of their mates by constraining them to producing haploid males or triploid daughters, thereby constituting a complete mating failure. Grey boxes represent theoretical ratios of haploid, diploid, and triploid offspring.

develop into diploid sons (Figure 3B), resulting in a partial-mating failure relative to the unmatched mating described previously. Finally, consider a female mated to a diploid male; she is mostly constrained to producing haploid sons, or relatively fewer daughters, thereby constituting complete or partial-mating failures (Figure 3C–D). Here, we present a review of the role of CSD and diploid male production in female mating failure in the Hymenoptera. We highly recommend two recent reviews on CSD (van Wilgenburg et al., 2006; Heimpel & de Boer, 2008) as an introduction to our study.

Complementary sex determination: one or many loci?

Although the presence of diploid males, as detected using genetic markers or cytological methods, provides support for CSD, it does not indicate if sex is determined at one or many loci. Breeding experiments are necessary to differentiate between sl-CSD and ml-CSD (e.g., Butcher et al., 2000). These experiments are often initiated with a mother mated to her haploid son. Under sl-CSD, 50% of the diploid progeny from such matings will develop into diploid males (Figure 3B). However, assuming multiple variable sex loci, a lower proportion of diploid males is expected (de Boer et al., 2008). Following the first round of

inbreeding, regular sib-matings will gradually increase the proportion of diploid males under ml-CSD, but not under sl-CSD (de Boer et al., 2008). Of course, it is important to take into account population history before formally ruling out ml-CSD; small population size, linkage disequilibrium, and non-random mating can erode allelic diversity at the majority of sex-determining loci, and can ultimately result into the collapse of ml-CSD into sl-CSD (de Boer et al., 2008).

Of the 83 species known to produce diploid males (Table S1) only 24 species (28.9%) are confirmed via inbreeding experiments to have sl-CSD (Figure 4); only a single species, *C. vestalis*, has experimentally confirmed ml-CSD (de Boer et al., 2008; but see Naito et al., 2000). The remaining studies, which document diploid males in 59 species, were not formally designed to distinguish between sl- and ml-CSD. However, these studies often implicitly assume sl-CSD because: (1) ml-CSD is considered rare and phylogenetically restricted in the Hymenoptera, and (2) high levels of diploid male production in natural populations are apparently inconsistent with ml-CSD given the lower chance of homozygosity at multiple sex-determining loci (Cook & Crozier, 1995). These assumptions should not be taken unreservedly, as very few studies explicitly test for ml-CSD, and because ml-CSD

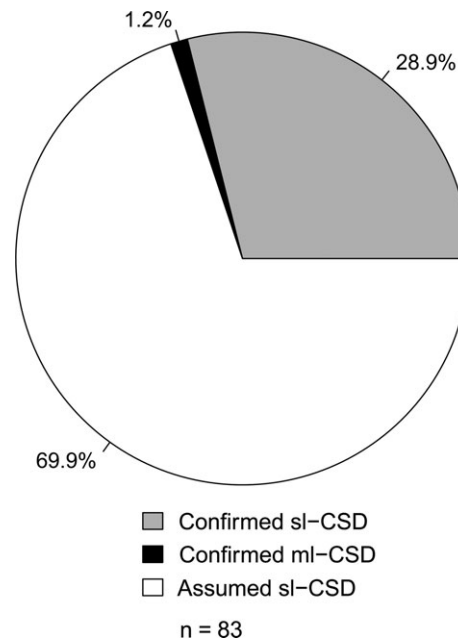


Figure 4 The prevalence of single-locus complementary sex determination (sl-CSD) and multiple-locus (ml)-CSD in the Hymenoptera. Single-locus CSD and ml-CSD have been confirmed using inbreeding experiments in less than half of the species where diploid males have been reported.

systems can collapse into sl-CSD systems (de Boer et al., 2008). More studies are clearly needed to better understand the prevalence of ml-CSD in the Hymenoptera.

Mating failures: females mating with haploid males

The most obvious form of mating failure caused by CSD results from a match-mated female's reduced ability to produce daughters. When a female mates with a male that does not share one of her sex alleles (i.e., unmatched mating), 100% of the diploid brood produced will develop into daughters, but only 50% of the diploid brood of a matched-mated female will develop into daughters assuming sl-CSD (Figure 3A–B). This reduction in productivity of match-mated females constitutes a partial-mating failure relative to unmatched-mated females. The consequences of matched matings are easiest to demonstrate using the 25 species with confirmed cases of sl- or ml-CSD (Table S1). The observed sex ratio of diploid brood produced by match-mated females from inbred crosses ranges from 1:1 (male to female) when diploid males are viable, to <1:1 when diploid males are partially viable and/or with ml-CSD (Figure 5). In most species, diploid males have equal viability relative to females (Smith & Wallace, 1971; Garofalo & Kerr, 1975; Butcher et al., 2000; Ayabe et al.,

2004). However, there is a hidden bias: the presence of diploid males in adult samples is often the first motivation towards characterizing CSD. As a result, species with inviable diploid males often go undetected. Nevertheless, the observation that diploid males often do not have reduced viability has consequences for the frequency at which females encounter diploid males in the field, as we discuss in the following section.

Ultimately, match-mated females produce fewer daughters, and this can have drastic effects when the fitness of the mother in question is contingent on the sex ratio of her brood. Consider that female haplodiploids are able to control the primary sex ratio by differentially inseminating eggs with semen stored in the spermatheca. In solitary species, controlling the primary sex ratio can be used adaptively to optimize a female's fitness in response to environmental conditions (Waage & Ming, 1984; Griffiths & Godfray, 1988; Ueno, 1998). The inability to control brood sex ratio can be more devastating in social hymenopterans because fewer daughters results in fewer workers, slower colony growth, and higher colony mortality (Ross & Fletcher, 1986; Buttermore et al., 1998).

Mating failures: females mating with diploid males

Diploid males appear to be viable or partially viable, and may thus contribute to additional forms of mating failures if they can successfully copulate with females. Six studies have compared the reproductive behaviours of diploid males relative to haploid males. Half of the studies noted no difference in the courtship or copulatory behaviours of diploid males relative to haploid males (Table 1). For example, in *C. vestalis*, diploid males performed wing-fanning behaviours, approached, and later mounted females in a manner that is similar to haploid males (de Boer et al., 2007). Similarly, the time spent on courtship did not differ based on male ploidy in *Euodynerus foraminatus* (Saussure) (Cowan & Stahlhut, 2004) and *Dia-dromus pulchellus* Wesmael (Agoze et al., 1994). In *Cotesia glomerata* (L.) and *Bombus terrestris* (L.), diploid males copulated faster or mated at a younger age in comparison to haploid males, respectively (Duchateau & Marien, 1995; Zhou et al., 2006). It is unclear if such differences offer an advantage to diploid males under field conditions, which likely depends on the timing of emergence of diploid males and females, and the pattern of post-emergence dispersal (Morbey & Ydenberg, 2001; van Wilgenburg et al., 2006). The sawfly *Neodiprion nigroscutum* Middleton is the only hymenopteran reported, to our knowledge, where diploid males have a drastically reduced ability to copulate with females;

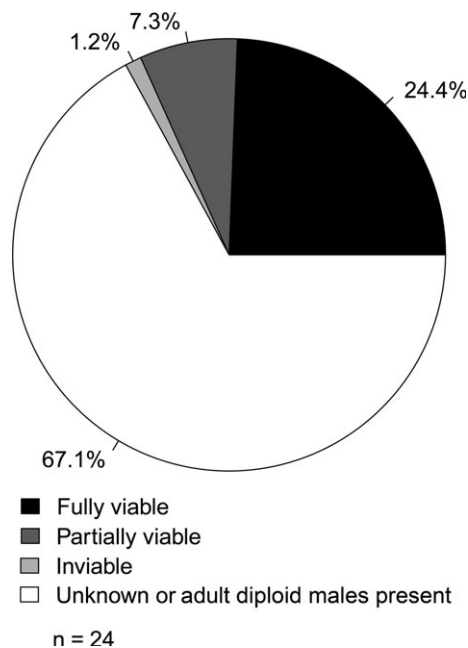


Figure 5 The ratio of adult diploid males to females in the brood of match-mated females indicates that diploid males are often equally viable as diploid females (1:1). Lower ratios indicate that diploid males are partially viable.

Table 1 Reproductive capacity of diploid (2n) males of Hymenoptera

Family	Species	Reproductive behaviour ¹	Insemination success ²	Sex ratio ³	Daughters sired ⁴	Daughters' ploidy	Reference
Tenthredinidae	<i>Athalia rosae</i>	?	82%	Male-biased	Few (32%)	3n	Naito & Suzuki (1991)
Diprionidae	<i>Neodiprion nigroscutum</i>	2n males cannot copulate	<1% (\ll 1n males)	Male-biased	Few (12%)	3n	Smith & Wallace (1971)
Braconidae	<i>Habrobracon</i> near <i>hebetor</i>	?	?	Male-biased	None	?	Holloway et al. (1999)
	<i>Habrobracon juglandis</i>	?	5%	?	Few	3n	Bostian (1934)
	<i>Cotesia glomerata</i>	2n males mate at a younger age	?(similar to 1n males)	Male-biased	Many (85%)	2n	Elias et al. (2009)
	<i>Cotesia vestalis</i>	Similar to 1n males	29% (\ll 1n males)	Male-biased	Few (29%)	3n	de Boer et al. (2007)
Ichneumonidae	<i>Diadromus pulchellus</i>	Similar to 1n males	?(similar to 1n males)	Male-biased	Rare (1%)	2n	Agoze et al. (1994)
Formicidae	<i>Lasius sakagami</i>	?	?	?	?	3n	Yamauchi et al. (2001)
	<i>Solenopsis invicta</i>	?	2.4% ⁵	?	?	3n	Krieger et al. (1999)
	<i>Tapinoma erraticum</i>	?	?	?	?	3n	Cournault & Aron (2009)
Vespidae	<i>Eudomynerus foraminatus</i>	Similar to 1n males	?(similar to 1n males)	Male-biased	Many (76%)	2n	Cowan & Stahlhut (2004)
	<i>Polistes dominulus</i>	?	?	?	?	3n	Liebert et al. (2005)
Apidae	<i>Bombus atratus</i>	?	17%	?	None ⁶	?	Garofalo & Kerr (1975)
	<i>Bombus floriclegus</i>	?	?	?	?	3n	Takahashi et al. (2008)
	<i>Bombus terrestris</i>	2n males mate at a younger age	9%	?	Few ⁵	?	Duchateau & Marien (1995)

¹We summarized findings from several studies that compared courtship and copulatory behaviour in diploid vs. haploid males.

²Defined by the proportion of diploid males that sired at least one daughter (diploid or triploid) in laboratory crosses. Where possible, we contrasted insemination success of diploid and haploid males and have indicated comparisons behind reported percentages.

³Secondary sex ratio of brood produced by females mated to diploid males.

⁴The proportion of daughters sired by diploid males relative to haploid males. We inferred this parameter for some studies based on qualitative statements made by the authors, or by shifts in sex ratio between laboratory crosses.

⁵Assumed based on analysis of sperm viability of diploid males.

⁶*Bombus atratus* diploid mated females produce intersex and aneuploid offspring.

diploid males are larger than haploid males and are unable to mount females successfully (Smith & Wallace, 1971). Although diploid males appear to have the necessary behavioural repertoire for courting and copulating with females, studies contrasting the reproductive behaviour of diploid vs. haploid males utilized no-choice experiments and/or restrained/anesthetized females (e.g., Whiting & Whiting, 1925; Speicher & Speicher, 1940). These experimental designs may overestimate mating success of diploid males if females prefer haploid males or if diploid males are unable to locate females in the field.

Although diploid males of most species can copulate with females, the insemination success of diploid males, defined by the proportion of mating attempts where diploid males sire at least one daughter, is inferior to haploid males in most species examined so far (Table 1). Only three species (*C. glomerata*, *D. pulchellus*, and *E. forminatus*) produce diploid males that are equally effective at inseminating females when contrasted to haploid males (Table 1). However, even when diploid males are effective at inseminating females, there is a clear difference in the productivity of females mated with diploid males relative to females mated with haploid males. Females mated to diploid males have a higher male-biased secondary sex ratio relative to females mated to haploid males in all species examined so far (Table 1). This pattern may be indicative of lower viability of diploid male sperm, lower viability of progeny arising from fertilized eggs, and/or a shift in investment towards producing haploids (i.e., higher unfertilized:fertilized ratio).

Finally, females mated to diploid males produced inviable or sterile triploid daughters in more than two-thirds of the species examined (Table 1), indicating that diploid males often produce unreduced, diploid sperm (Agoze et al., 1994; Holloway et al., 1999; Yamauchi et al., 2001). Therefore, in the majority of hymenopterans studied so far, females mated to diploid males experience a complete mating failure because they are unable to produce viable diploid daughters and are often constrained to producing haploid sons (Figure 3D). Fully fertile diploid males (i.e. diploid males that sire diploid daughters) have only been observed in *C. glomerata* and *E. forminatus*; diploid males sire diploid daughters (Cowan & Stahlhut, 2004; Elias et al., 2009). However, in both species, diploid males sire fewer daughters relative to haploid males, and females mated to diploid males produced a male-biased brood relative to females mated to haploid males (Cowan & Stahlhut, 2004; Elias et al., 2009). As such, females mated to fully fertile diploid males appear to experience partial-mating failures (Figure 3C).

Conclusion and summary

Our review clearly points at the need for more studies to better understand the prevalence of CSD in the Hymenoptera, and to better quantify the viability and fertility of diploid males. Particularly, more data are needed on the reproductive behaviour and success of diploid males across hymenopterans. Systematic studies of sex determination utilizing controlled crosses can be extremely effective at addressing these gaps of knowledge. We also need to understand how findings from laboratory experiments translate to natural populations. For example, are the negative effects of matched mating or mating with diploid males amplified under natural conditions relative to benign laboratory environments? Also, can female hymenopterans recognize matched-haploid males or diploid males, and avoid mating with them (van Wilgenburg et al., 2006)? The suggested studies will help us understand the relationship between life-history traits, the evolution of sex determination, and reproductive strategies of female hymenopterans.

Although more studies are clearly needed, we do find that CSD can increase the frequency of partial and complete mating failures in female hymenopterans. We highlight the following general patterns: (1) sl-CSD is far more common than ml-CSD in the Hymenoptera, (2) females mating with matched-haploid males often experience partial-mating failures because of their compromised ability to produce diploid daughters, (3) diploid males are often fully viable, and can usually mate, and (4) diploid males often produce inviable sperm, with only two species reported to produce fully fertile haploid sperm. As a consequence of this final point, (5) females mated to diploid males often experience complete mating failures; such females largely or exclusively produce haploid males and inviable or sterile triploid daughters. Complementary sex determination therefore contributes to both partial and complete mating failures in the Hymenoptera, and mating failures caused by CSD are expected to be largest in small populations that lack allelic diversity at the sex-determining locus or loci.

The increased frequency of mating failures in hymenopterans with CSD can have significant implications for economically important species. Several members of the Hymenoptera play important roles in crop pollination and biological control, but only females perform both of these crucial services. As such, mating failures can have negative consequences on the efficiency of pollination and biological control programs. Mating failures can also impact the persistence of natural hymenopteran populations, especially when such populations are small and

isolated (Goulson et al., 2005; Zayed & Packer, 2005; Hein et al., 2009).

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Supporting Information

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

Table S1. Diploid males in the Hymenoptera. Confirmed cases of single-locus complementary sex determination (sl-CSD) and multiple-locus (ml)-CSD are indicated

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