

## Survival of Diploid Males in *Bracon* sp. near *hebetor* (Hymenoptera: Braconidae)

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**ABSTRACT** In the parasitoid wasp *Bracon* sp. near *hebetor*, unfertilized eggs develop as haploid males, but fertilized eggs are diploid and can develop into males or females. It is likely that the sex of diploid individuals is determined by allelic variation at a single sex locus within a system of sex determination known as single-locus complementary sex determination (CSD). Under single-locus CSD, individuals that are heterozygous at the sex locus are female and individuals that are homozygous or hemizygous are male. In *B. hebetor* (Say), a closely related species in which this mechanism of sex determination was 1st discovered, diploid males are sterile and usually do not survive. In this study, the survival of diploid males was confirmed in *B. sp.* near *hebetor* by the use of phenotypic and molecular markers. Females with a recessive light body color mutation (blonde) crossed with dark-bodied males produced families in which diploid males were identified through inheritance of the dominant dark-bodied allele from the father. Polymorphic RAPD-polymerase chain reaction (PCR) markers were used to confirm inheritance of paternal alleles in diploid males. In families that included diploid males, only 44% of diploids were male, indicating that diploid male survival is lower than female survival. Diploid males also are capable of mating, but mating by diploid males constrained females to producing only haploid male progeny. In the closely related species *B. hebetor*, diploid males rarely survive, but in other *Bracon* sp. (*B. serinopae* [Cherian] and *B. brevicornis* Wesmael) diploid males survive at high rates. The differential survival rates of diploid males between closely related species of *Bracon* may indicate that the CSD system is not as evolutionarily conserved as has been presumed.

**KEY WORDS** *Bracon*, complementary sex determination, diploid males, Hymenoptera

IN MOST HYMENOPTERA, sex is determined by haplodiploidy; unfertilized (haploid) eggs develop as males and fertilized (diploid) eggs develop as females. In some Hymenoptera, haplodiploidy is associated with a genetic mechanism known as complementary sex determination (CSD) (Cook 1993, Cook and Crozier 1995). Under single-locus CSD, diploid individuals that are heterozygous at the sex locus are female, whereas homozygous diploid or hemizygous (haploid) individuals are male. The individual sex alleles have no gender tendencies; females result from the complementary action of any 2 different alleles (Cook and Crozier 1995). The existence of CSD is identified by the production of diploid males and was 1st discovered in the parasitoid *Bracon hebetor* (Say) (Ichneumonoidea: Braconidae) (Whiting 1943). CSD is thought to be ancestral in the Hymenoptera and diploid males have been found in >30 species in the Symphyta (sawflies), Aculeata (ants, bees, and wasps), and Ichneumonoidea (parasitoids) (see re-

views by Stouthamer et al. 1992, Cook 1993, and Cook and Crozier 1995).

Although CSD in Hymenoptera has been studied since the 1940s (Whiting 1943), relatively little is known about the processes controlling female and male development in this group. Diploid male survival has been reported in a large number of species, and egg-to-adult survival of diploid males varies among distantly related species (see Cook 1993 for review). High viability of diploid males is found in sawflies (Smith and Wallace 1971, Naito and Suzuki 1991), bumble bees (Plowright and Pallett 1979, Duchateau et al. 1994), *Melipona* bees (Camargo 1982, Kerr 1987), Megachilid bees (McCorquodale and Owen 1994), and an ichneumonid parasitoid (Periquet et al. 1993). In these species, there is no difference in egg-to-adult survivorship between diploid males and females. In some social Hymenoptera, the viability of diploid males is determined mainly by whether they are eliminated by members of the worker caste (Ratnieks 1990). The best-studied examples of this phenomenon are the honey bees *Apis mellifera* (L.) and *A. cerana* F., where diploid males have high survivorship when reared artificially but are cannibalized as larvae by workers within the hive (Woyke 1986). Similarly, diploid males of the red imported fire ant, *Solenopsis invicta* Buren, and in some *Formica* species have via-

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bility equaling that of workers (Ross and Fletcher 1985), but they are eliminated along with other sexual larvae by workers under some conditions (Vargo and Fletcher 1987, Pamilo et al. 1994).

Perhaps the best-studied example of diploid male production under CSD is *B. hebetor*. In general, diploid male *B. hebetor* have <5% viability and die in the egg stage (Whiting 1943, Petters and Mettus 1980). However, there appear to be both genetic and environmental sources of variation in diploid male viability in *B. hebetor*. For instance, diploid male survival varies among strains, with 1 strain having diploid males that survive 44% of the time (Grosch 1945). Petters and Mettus (1980) increased the hatch rate of diploid male eggs by increasing humidity by rearing them under mineral oil. In addition, diploid male eggs had higher hatch rates when rearing temperatures were raised from 20°C (Whiting and Anderson 1932). Inter-specific variation in the viability of diploid males within the genus *Bracon* also has been found. Laboratory experiments indicate that diploid males develop normally in both *B. brevicornis* Wesmæl (Speicher and Speicher 1940) and *B. serinopae* (Cherian) (Clark et al. 1963).

Among species with CSD, diploid males that survive to adulthood are often sterile (Inaba 1939, MacBride 1946, Hung et al. 1974, Naito and Suzuki 1991, Stouthamer et al. 1992, El Agoze et al. 1994). During spermatogenesis in diploid male *B. hebetor*, the 1st maturation division is aborted and the 2nd equational division occurs normally, producing diploid sperm (Whiting 1927). MacBride (1946) showed that sterility results from the inability of large diploid sperm to penetrate the egg. In addition, diploid males in some sawfly species are larger and have difficulty in copulation and reduced fertility compared with haploid males (Smith and Wallace 1971, Naito and Suzuki 1991).

Although it is unknown why diploid male viability is so low in *B. hebetor*, CSD clearly has the potential to impose a significant genetic load on hymenopteran populations. Stouthamer et al. (1992) hypothesized that the high rate of biocontrol failure with Ichneumonidae and Braconidae may result from inbreeding, loss of genetic diversity during culturing, and the subsequent production of diploid males. The production of diploid males will negatively influence establishment and growth rates of wasp populations only under a certain set of circumstances. First, sex allele diversity must be low, causing diploid males to be produced in high numbers. Second, diploid males must survive and be sterile. Third, diploid males must have mating success relatively equal to that of haploid males. Finally, females must be monandrous. Under these conditions, females mated to diploid males would subsequently not remate and be constrained to produce only haploid sons, leading to male-biased sex ratios and decreased population growth rates.

CSD is likely to be restricted to outbreeding organisms, thus reducing the probability of shared-allele matings, and some evidence exists that species with CSD outbreed. Antolin and Strand (1992) discovered

that in natural populations of *B. hebetor*, females disperse from the site of emergence and only 14% are mated before dispersing. Additional studies have demonstrated that there is a premating refractory period where both males and females of *B. brevicornis* (Sudheendrakumar et al. 1978) and *B. hebetor* (Ode et al. 1995) will not mate for 1–4 h after emergence. Avoidance of inbreeding and diploid male production also are accomplished by females mating multiple times and by the formation of mating swarms (Thornhill and Alcock 1983). Regardless of the mating system, matings within populations with low sex allele diversity will produce high numbers of diploid males, which has important consequences for success of biocontrol efforts (Stouthamer et al. 1992). Future biocontrol efforts should examine the number of sex alleles and survival of diploid males in populations intended for release.

Recently, Heimpel et al. (1997) reported on a species of *Bracon* from the Caribbean island of Barbados that had been released for control of noctuid moths. This species was morphologically indistinguishable yet reproductively isolated from *B. hebetor* and has been referred to as *B. sp. near hebetor*. *Bracon sp. near hebetor* is a gregarious parasitoid of a number of moth species. This wasp appears to have a life history similar to that of *B. hebetor* in terms of fecundity and longevity, except that it attacks moths other than pyralids (Heimpel et al. 1997). Preliminary examination of *B. sp. near hebetor* during laboratory breeding indicated a highly male-biased sex ratio, suggesting low sex allele diversity and high diploid male survivorship. These observations, in addition to the discovery of a light-bodied mutation during laboratory rearing led us to characterize sex allele diversity, male survivorship, and consequences for females of mating with diploid males. We corroborated our detection of diploid males with molecular markers. The implications of our results for understanding the developmental and genetic aspects of CSD in Hymenoptera are discussed.

## Materials and Methods

**Purification of the Blonde Body Color Mutation.** A single light body colored male was observed during general colony maintenance of wild-type (dark body) *B. sp. near hebetor*. We subsequently demonstrated that this was a heritable recessive mutation for light body color that is referred to as blonde-bodied. *Bracon sp. near hebetor* are normally brown with dark markings on each sclerite. The blonde phenotype in *B. sp. near hebetor* has a yellow body color and tan coloring of the sclerites, much like the honey mutation of *B. hebetor* (Whiting 1934). The single blonde-bodied male was mated to several wild-type females. Unmated F<sub>1</sub> females from these matings were allowed to oviposit on 5th instars of *Plodia interpunctella* Hübner (≈20 mg) (Lepidoptera: Pyralidae) for several days and produced haploid wild-type and blonde-bodied males. The original wild-type females were allowed to lay eggs until death, continually producing heterozygous F<sub>1</sub> females that could be mated to blonde-bodied

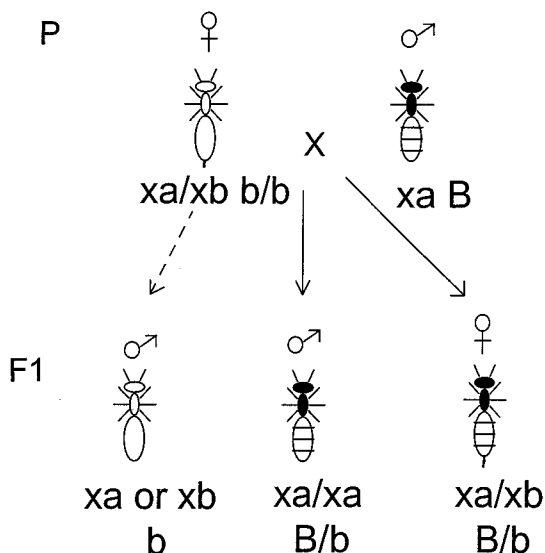


Fig. 1. Mating scheme to identify diploid males. xa, sex allele a; xb, sex allele b; B, dark body color allele; b, blonde body color allele. Dark-bodied males inherited the dark body color allele from the father and are therefore diploid.

$F_2$  males. In the next generation, blonde-bodied females were produced and mated to blonde-bodied males. These wasps were subsequently maintained as separate stocks.

All wasps throughout the study were housed in environmental chambers at 27°C, 16:8 (L:D) h, and 70% RH [see Strand and Godfray (1989) for laboratory rearing methods of *P. interpunctella* and *Bracon*].

**Matings to Identify Diploid Males.** To distinguish haploid from diploid males, blonde-bodied females were mated to haploid, wild-type (dark) males ( $n = 30$ ) (see Whiting 1943). Each female was housed separately in a plastic petri dish (6 cm diameter) and given 4 *P. interpunctella* 5th instars on which to lay eggs. Females were transferred to new petri dishes with 4 new hosts each day for 7 d. Any male progeny with the wild-type, dark body color from these matings could be identified as a diploid male by virtue of inheritance of the paternal dark body color allele (Fig. 1). Virgin females were treated similarly as a control ( $n = 15$ ). The numbers of eggs laid, egg-to-adult survival, and sex ratio (proportion male) of broods were recorded and compared between family types using the GLM procedure of SAS Institute (1988). Egg-to-adult survival and sex ratio values were arcsine square-root transformed for significance tests. The number of different sex alleles as well as 95% CL for the number of sex alleles were calculated for the laboratory population (Efron and Tibshirani 1993).

**Genotyping of Haploid and Diploid Wasps.** To verify the existence and viability of diploid males, we examined whether families that should have included haploid males, presumptive (dark-bodied) diploid males, and females had the predicted haploid or diploid genotypes, based upon molecular markers. We

used random amplified polymorphic DNA (RAPD) amplified by the PCR and single-strand conformational polymorphism analysis (SSCP). SSCP analysis detects large numbers of codominant molecular markers (Antolin et al. 1996). SSCPs result from different folding patterns of single strands of DNA and appear as multibanded alleles in heterozygotes. RAPD-PCR and DNA extraction protocols are described in Antolin et al. (1996) and Black and DuTeau (1996). Eleven 10-base pair oligonucleotide primers (A-05, A-09, A-20, AM-07, AM-10, B-18, B-20, C-01, C-03, C-04, and Q-16) from Operon Technologies, Alameda, CA, were initially screened. Two primers, A-05 and C-04, showed polymorphic codominant markers that make identification of homozygous (hemizygous) and heterozygous genotypes possible.

DNA analyses were conducted on 5 families that included dark-bodied males. DNA was extracted from the mother, father, 6 blonde-bodied males, 6 dark-bodied males, and 6 females of each family. Small amounts of DNA used for PCR reactions were stored at 4°C and the majority was stored in a -80°C freezer. PCR products were separated by electrophoresis on large-format (50 by 35 cm), thin (0.4 mm) glycerol (5%) native acrylamide (5%, 2% cross-linking) gels that were silver stained to visualize alleles. Complete details of SSCP analysis and silver staining are described in Antolin et al. (1996) and Black and DuTeau (1996).

**Fertilization by Diploid Males and Remating to Haploid Males.** To test the viability of matings between diploid males and females, 28 matings between dark-bodied diploid males and blonde females were established. Females were housed in plastic petri dishes (6 cm diameter) and given 4 *P. interpunctella* larvae per day. Eighteen of the females were allowed to lay eggs until death. Emerged progeny were counted and their sex determined to discover if any diploid offspring were produced. The remaining 10 females were given the opportunity to remate with haploid males after 3 d. If females mated to diploid males produced no diploid offspring, then production of females by remated individuals would indicate that remating had occurred.

To determine whether both diploid and haploid males transferred sperm during mating, reproductive tracts were dissected from females mated to haploid males, females mated to diploid males, and unmated females. The reproductive tracts were mounted on microscope slides with insect Ringer's solution and viewed at 1000× to examine spermathecae for the presence of sperm.

## Results

### *B. sp. near hebetor* Produced Viable Diploid Males.

Of the matings designed to detect diploid males, 15 produced only blonde-bodied males and dark-bodied females, whereas 15 produced blonde-bodied males, dark-bodied females, and dark-bodied (diploid) males. Analysis of variance (ANOVA) indicated no difference in survival among families with diploid

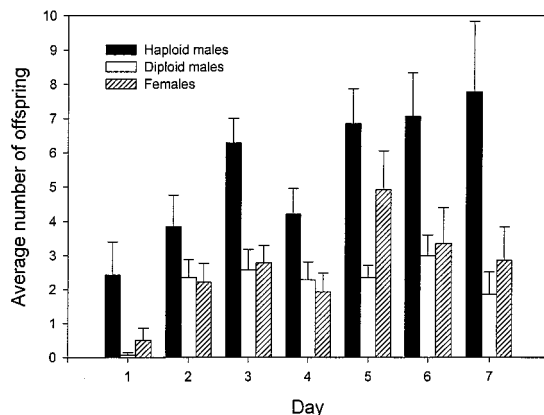
**Table 1.** Comparison of life history components among family types

Family type	<i>n</i>	Egg production over 7 d	Egg-to-adult survival	Sex ratio (proportion male)
Unmated females	15	123.7 ± 7.4	0.52 ± 0.01	1.00
No diploid males	15	137.3 ± 9.2	0.56 ± 0.01	0.54 ± 0.01*
Diploid males produced	14	139.9 ± 11.5	0.52 ± 0.01	0.77 ± 0.01*

Data are means ± SE. \*, Means significantly different according to *t*-test; *t* = 3.3801, *df* = 27, *P* = 0.0022.

males, families with females and haploid males only, and families of all haploid males from unmated females ( $F = 0.22$ ; *df* = 2, 41;  $P = 0.8082$ ; Table 1). Additionally, egg production did not differ significantly between these families ( $F = 0.86$ ; *df* = 2, 41;  $P = 0.4319$ ; Table 1). As expected, sex ratio was significantly more male-biased in families with diploid males than families without diploid males (Table 1).

The average number of haploid males, diploid males, and females varied among days for the 14 families that resulted from shared-allele matings (Fig. 2). Although 50% of fertilized eggs in shared-allele matings should result in diploid males, only 44% of diploids that survived to adulthood were male. Repeated measures ANOVA indicated that there were significant differences in the numbers of surviving diploid offspring between females and that overall, female survivorship was greater than that of diploid males (Table 2). However, the time × sex interaction also was significant, indicating that females were not more numerous than diploid males on all days (Table 2; Fig. 2). One female that produced diploid males had 86 female progeny, but only 7 diploid male progeny, was determined to be a statistical outlier. The inclusion of this female did not affect significance levels of ANOVA tests for survival and egg production. Although the lower survival of diploid males in this family followed the general trend in the data, inclusion of this female significantly increased the variance in diploid male survival. As a consequence, this female was excluded from analyses.



**Fig. 2.** Average daily broods for each offspring type ( $\pm$ SE) for each day of egg laying from females that produced diploid males.

The expected number of sex alleles in the experimental population, assuming monandry and random mating, is  $2/p$ , where  $p$  is the proportion of matings that produced diploid males (Stouthamer et al. 1992). Given that 15 of 30 matings produced diploid males, we estimate that our population harbors 4 different sex alleles. We calculated 95% CL for this estimate using standard bootstrapping techniques in which the original data set was resampled with replacement 1,000 times (Efron and Tibshirani 1993). This procedure led to lower and upper 95% CL for  $p$  of 0.33 and 0.68. Thus, for this experimental population the range of sex allele estimates within bootstrapped 95% confidence interval is between 2.9 and 6.1.

**Molecular Evidence.** Grosch (1945) found that diploid and haploid males in *B. hebetor* can be differentiated on the basis of bristle density on the wings. However, in *B. sp.* near *hebetor* we found no morphological differences between haploid and diploid males. To confirm identification of diploid males by body color, we examined 5 families for the presence of diploid genotypes in males. The results shown in Fig. 3 from 1 family are typical of our findings in all of the families. Two of the RAPD primers that we screened, A-05 and C-04, amplified markers with codominant SSCP alleles. For example, segregation of alleles of light-bodied (haploid) sons and banding pattern of their mother indicate that the mother was homozygous for the slow allele from the codominant marker A05.635 (Fig. 3), whereas the father had the fast allele. The heterozygous diploid genotype includes the slow allele from the mother and the fast allele from the father. Upper bands marked by arrows within the bracket in Fig. 3 are likely different conformations of the DNA from the A05.635 locus and also are found in all heterozygotes. The heterozygous genotype is seen in all daughters as well as in all dark-bodied males, thus

**Table 2.** Repeated measures ANOVA comparing the numbers of diploid males and females (sex) among females (family) over 7 d (time) of oviposition (data in Fig. 2)

Source	MS	<i>F</i>	<i>df</i>	<i>P</i>
Between females				
Family	30.829	9.24	13	0.0001
Sex	16.576	4.97	1	0.0440
Error	3.335	—	13	—
Within females				
Time	31.684	12.46	6	0.0001
Time × family	6.229	2.45	78	0.0001
Time × sex	6.707	2.64	6	0.0221
Error	2.543	—	78	—

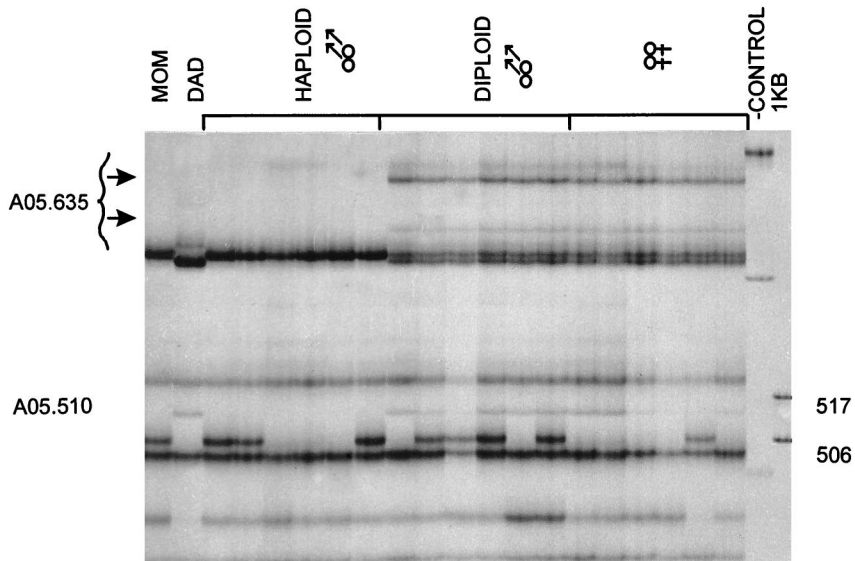


Fig. 3. Portion of silver-stained gel of primer A-05 indicating inheritance of paternal alleles in diploid progeny. All diploid progeny inherited both alleles of the codominant RAPD marker A05.635 as well as a band for the presence/absence marker A05.510 that is present in the father and not the mother. Arrows indicate SSCP banding patterns for heterozygous genotypes for the marker A05.635. All markers from these primers were named for the primer and the size in base pairs (bp) (e.g., A05.510 would be a fragment from primer A-05 that was 510 bp).

confirming that dark-bodied males are diploid. A similar pattern is seen in a presence/absence polymorphism, A05.510. The mother as well as all light-bodied sons had the band-absent allele. However, the father and all dark-bodied progeny had a band-present allele. Primer C-04 produced 2 polymorphic loci (1 codominant [C04.415], 1 presence/absence [C04.270]) that showed the same patterns.

**Fertilization by Diploid Males and Remating to Haploid Males.** The spermathecae of unmated females were empty and clear. In contrast, females mated to either diploid or haploid males had sperm in the spermathecae that was not motile and resembled a ball of twine. Although females mated to diploid males produced many haploid male offspring, none produced daughters ( $n = 18$ ). Additionally, none of the females mated to diploid males remated when subsequently provided with haploid males as mates ( $n = 10$ ).

### Discussion

Our results indicate that *B. sp. near hebetor* has single-locus CSD, like the closely related *B. hebetor*, but that diploid males survive at a high rate (78% that of females). In contrast, *B. hebetor* diploid males have survival rates between 2 and 44% that of females (Grosch 1945, Petters and Mettus 1980). We have evidence, however, that survival of diploid males is variable within *B. sp. near hebetor* (see Table 2). One female from a shared-allele mating in our experiments produced 86 female progeny, but only 7 diploid males. This indicates that some individual sex alleles within *B. sp. near hebetor* may have lethal effects in homozygous diploid individuals similar to those seen in *B.*

*hebetor*. Diploid males in *B. sp. near hebetor* were clearly identified through inheritance of body color and paternal alleles from RAPD markers. RAPD-SSCP provides a powerful tool for identifying diploid males in Hymenoptera because diploid males can be unambiguously identified by the banding patterns unique to diploid heterozygous individuals (see Fig. 3).

At the population level, the frequency and consequences of diploid male production will vary depending on the natural history of each species exhibiting single-locus CSD. Three aspects of this study demonstrate that the dynamics of natural populations of *B. sp. near hebetor* will be affected if diploid males have mating success equal to that of haploid males. First, when females were mated to haploid males that share a sex allele, they produced diploid male offspring at the cost of producing fewer daughters. Second, diploid males successfully mated females and transmitted sperm, but sired no daughters. Either the sperm from diploid males is not viable, or it is unable to successfully fertilize eggs (see Petters and Mettus 1980). Finally, we have found that mated female *B. sp. near hebetor*, even those mated to diploid males, rejected subsequent courtship and copulation attempts of haploid males. Single mating appears to be the norm in *Bracon*; *B. hebetor* is typically monandrous with remating occurring in <12% of females (Guertin et al. 1996, Ode et al. 1997). Because *B. sp. near hebetor* has been reported as a biocontrol agent (Heimpel et al. 1997), the production of viable diploid males will reduce the effectiveness of *B. sp. near hebetor* as a natural enemy of agricultural pests (Stouthamer et al. 1992).

Stouthamer et al. (1992) estimated how much productivity could be reduced in populations when diploid males survive, are sterile, and participate in mating as  $R(n) = SR(n) * (E)$ .  $R$  is the number of daughters produced per female,  $n$  is the number of sex alleles,  $SR$  is the sex ratio (proportion female), and  $E$  is the number of eggs laid. Our estimate for the number of different sex alleles in our laboratory population is 4 (95% confidence interval 3–6), as half of the matings resulted in families with diploid males. Given that our laboratory population of *B. sp.* near *hebetor* produced sex ratios of 0.32 and females laid 139 eggs (see Table 1), the presence of 4 sex alleles would result in each mated female producing on average 44 female progeny during a 7-d period. If 6 sex alleles were present in this population, only 1/5 of matings would result in diploid males and each female would produce 51 female progeny over 7 d. If egg-to-adult survival of females is 50%, and 75% of those mate and produce offspring, an increase from 4 to 6 sex alleles would result in a 10-fold difference in production of females over 10 generations. The number of females produced is important for the following 2 reasons (Stouthamer et al. 1992) (1) female wasps are responsible for host destruction in biological control and (2) female production is an important determinant of population growth rates.

Variation in the survival of diploid males also has implications for the genetic control and evolutionary stability of CSD. Many Hymenoptera exhibit CSD as a genetic mechanism of sex determination, but the developmental pathways for sexual differentiation may vary between species. Given the difference in survival of diploid males between *Bracon* species, we may speculate that the developmental pathways for sexual development under CSD are not evolutionarily conserved between even closely related species. Studies comparing sex determination systems in other groups have shown that the pathways for sexual development evolve rapidly (Bull 1983). For example, genetic mechanisms of sex determination and dosage compensation are well studied in *Drosophila* and *Caenorhabditis* and share many features in common, but the genes controlling the processes of sexual differentiation are not homologous between taxa (Hodgkin 1990). Further, conservation of sex-determining genes is low even within taxonomic groups (e.g., Diptera). A homologue of *sex-lethal*, a sex-determining gene in *Drosophila*, has been found to have similar structure in the blowfly, *Chrysomya rufifacies* (MacQuart) (Muller-Holtkamp 1995), but the *sex-lethal* homologue has no sex-specific function in the blowfly.

The relatively high survival rate of diploid males in *B. sp.* near *hebetor* makes this species an ideal subject for further study of CSD, including localization of sex-determining genes through linkage mapping of the *B. sp.* near *hebetor* genome. Further studies then can compare the different mechanisms controlling male and female development within organisms that exhibit CSD and determine the level of evolutionary conservation of CSD.

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## References Cited

- Antolin, M. F., C. F. Bosio, J. Cotton, W. Sweeney, M. R. Strand, and W. C. Black IV. 1996. Intensive linkage mapping in a wasp (*Bracon hebetor*) and a mosquito (*Aedes aegypti*) with single-strand conformation polymorphism analysis of random amplified polymorphic DNA markers. *Genetics* 143: 1727–1738.
- Antolin, M. F., and M. R. Strand. 1992. Mating system of *Bracon hebetor* (Hymenoptera: Braconidae). *Ecol. Entomol.* 17: 1–7.
- Black, W. C. IV and N. M. DuTeau. 1996. RAPD-PCR and SSCP analysis for insect population genetic studies, pp. 514–531. In J. Crampton [ed.], *The molecular biology of insect disease vectors: a methods manual*. Chapman & Hall, New York.
- Bull, J. J. 1983. *Evolution of sex determining mechanisms*. Benjamin/Cummings, Menlo Park, CA.
- Camargo, C. A. de. 1982. Longevity of diploid males, haploid males and workers of the social bee, *Melipona quadrifasciata* Lep. (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* 55: 8–12.
- Clark, A. M., H. A. Bertrand, and R. E. Smith. 1963. Life span differences between haploid and diploid males of *Habrobracon serinopae* after exposure as adults to x-rays. *Am. Nat.* 97: 203–208.
- Cook, J. M. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* 71: 421–435.
- Cook, J. M., and R. H. Crozier. 1995. Sex determination and population biology in the Hymenoptera. *Trends Ecol. Evol.* 10: 281–286.
- Duchateau, M. J., H. Hoshiba, and H.H.W. Velthuis. 1994. Diploid males in the bumble bee *Bombus terrestris*. *Entomol. Exp. Appl.* 71: 263–269.
- Efron, B., and R. J. Tibshirani. 1993. *An introduction to the bootstrap*. Chapman & Hall, New York.
- El Agoze, M., J. M. Drezon, S. Renault, and G. Periquet. 1994. Analysis of the reproductive potential of diploid males in the wasp *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Bull. Entomol. Res.* 84: 213–218.
- Grosch, D. S. 1945. The relation of cell size and organ size to mortality in *Habrobracon*. *Growth* 9: 1–17.
- Guertin, D. S., P. J. Ode, M. R. Strand, and M. F. Antolin. 1996. Host-searching and mating in an outbreeding parasitoid wasp. *Ecol. Entomol.* 21: 27–33.
- Heimpel, G. E., M. F. Antolin, R. A. Franqui, and M. R. Strand. 1997. Reproductive isolation and genetic divergence between 2 "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). *Biol. Control.* 9: 149–156.
- Hodgkin, J. 1990. Sex determination compared in *Drosophila* and *Caenorhabditis*. *Nature (Lond.)* 344: 721–728.
- Hung, A.C.F., S. B. Vinson, and J. W. Summerlin. 1974. Male sterility in the red imported fire ant, *Solenopsis invicta*. *Ann. Entomol. Soc. Am.* 67: 909–912.
- Inaba, F. 1939. Diploid males and triploid females of the parasitic wasp *Habrobracon pectinophorae* Watanabe. *Cytologia* 9: 517–534.
- Kerr, W. E. 1987. Sex determination in bees. XXI. Number of XO-heteroalleles in a natural population of *Melipona*

- compressipes fasciculata* (Apidae). Insectes Soc. 34: 274–279.
- MacBride, D. H. 1946. Failure of sperm of *Habrobracon* diploid males to penetrate the eggs. Genetics 31: 224.
- McCorquodale, D. B., and R. E. Owen. 1994. Laying sequence, diploid males, and nest usurpation in the leafcutter bee, *Megachile rotundata* (Hymenoptera: Megachilidae). J. Insect Behav. 7: 731–738.
- Muller-Holtkamp, F. 1995. The sex-lethal gene homologue in *Chrysomya rufifacies* is highly conserved in sequence and exon-intron organization. J. Mol. Evol. 41: 467–477.
- Naito, T., and H. Suzuki. 1991. Sex determination in the sawfly, *Athalia rosaeurificornis* (Hymenoptera): occurrence of triploid males. J. Hered. 82: 101–104.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1995. Brood-mate avoidance in the parasitic wasp *Bracon hebetor* Say. Anim. Behav. 49: 1239–1248.
1997. Constrained oviposition and female-biased sex allocation in a parasitic wasp. Oecologia 109: 547–555.
- Pamilo, P., L. Sundstrom, W. Fortelius, and R. Rosengren. 1994. Diploid males and colony-level selection in *Formica* ants. Ethol. Ecol. Evol. 6: 221–235.
- Periquet, G., M. P. Hedderwick, M. El Agoze, and M. Poirie. 1993. Sex determination in the hymenopteran *Diadromus pulchellus* (Ichneumonidae): validation of the one-locus multi-allele model. Heredity 70: 420–427.
- Petters, R. M., and R. V. Mettus. 1980. Decreased diploid male viability in the parasitic wasp, *Bracon hebetor*. J. Hered. 71: 353–356.
- Plowright, R. C., and M. J. Pallett. 1979. Worker-male conflict and inbreeding in bumble bees (Hymenoptera: Apidae). Can. Entomol. 111: 289–294.
- Ratnieks, F.L.W. 1990. The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. Behav. Ecol. Sociobiol. 26: 343–348.
- Ross, K. G., and D.J.C. Fletcher. 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. Evolution 39: 888–903.
- SAS Institute. 1988. SAS user's guide, version 6. SAS Institute, Cary, NC.
- Smith, S. G., and D. R. Wallace. 1971. Allelic sex determination in a lower hymenopteran, *Neodiprion digroscutum* Midd. Can. J. Genet. Cytol. 13: 617–621.
- Speicher, B. R., and K. G. Speicher. 1940. The occurrence of diploid males in *Habrobracon brevicornis*. Am. Nat. 74: 379–382.
- Stouthamer, R., R. F. Luck, and J. H. Werren. 1992. Genetics of sex determination and the improvement of biological control using parasitoids. Environ. Entomol. 21: 427–435.
- Strand, M. R., and H.C.J. Godfray. 1989. Superparasitism and ovicide in parasitic Hymenoptera: theory and a case study of the ectoparasitoid *Bracon hebetor*. Behav. Ecol. Sociobiol. 24: 421–432.
- Sudheendrakumar, V. V., U.V.K. Mohamed, U. C. Abdurahiman, and T. C. Narendran. 1978. Mating behavior of *Bracon brevicornis* Wesm. (Hymenoptera: Braconidae), a larval parasite of *Naphantis serinopa* Meyrick the black headed caterpillar of coconut. Agric. Res. J. Kerala. 16: 224–226.
- Thornhill, R., and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, MA.
- Vargo, E. L., and D.J.C. Fletcher. 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. Physiol. Entomol. 12: 109–116.
- Whiting, A. R. 1927. Genetic evidence for diploid males in *Habrobracon*. Biol. Bull. 53: 438.
1934. Mutants in *Habrobracon* II. Genetics 19: 268–291.
1943. Multiple alleles in complementary sex determination of *Habrobracon*. Genetics 28: 365–382.
- Whiting, P. W., and R. L. Anderson. 1932. Temperature and other factors concerned in male biparentalism in *Habrobracon*. Am. Nat. 66: 420–422.
- Woyke, J. 1986. Sex determination, pp. 91–119. In T. E. Rinderer [ed.], Bee genetics and breeding. Academic, Orlando, FL.

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