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Population structure, mating system, and sex-determining allele diversity of the parasitoid wasp *Habrobracon hebetor*

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Besides haplo-diploid sex determination, where females develop from fertilized diploid eggs and males from unfertilized haploid eggs, some Hymenoptera have a secondary system called complementary sex determination (CSD). This depends on genotypes of a 'sex locus' with numerous sex-determining alleles. Diploid heterozygotes develop as females, but diploid homozygotes become sterile or nonviable diploid males. Thus, when females share sex-determining alleles with their mates and produce low fitness diploid males, CSD creates a genetic load. The parasitoid wasp *Habrobracon hebetor* has CSD and displays mating behaviours that lessen CSD load, including mating at aggregations of males and inbreeding avoidance by females. To examine the influence of population structure and the mating system on CSD load, we conducted genetic analyses of an *H. hebetor* population in Wisconsin. Given the frequency of diploid males, we estimated that the population harboured 10–16 sex-determining alleles. Overall, marker allele frequencies did not differ between subpopulations, but frequencies changed dramatically between years. This reduced estimates of effective size of subpopulations to only $N_e \sim 20$–50, which probably reflected annual fluctuations of abundance of *H. hebetor*. We also determined that the mating system is effectively monogamous. Models relating sex-determining allele diversity and the mating system to female productivity showed that inbreeding avoidance always decreased CSD loads, but multiple mating only reduced loads in populations with fewer than five sex-determining alleles. Populations with N_e less than 100 should have fewer sex-determining alleles than we found, but high diversity could be maintained by a combination of frequency-dependent selection and gene flow between populations.
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Introduction

The haplo-diploid sex determination of most parasitoid Hymenoptera makes them ideal subjects for studying the joint evolution of sex determination and mating systems (Godfray, 1994; Antolin, 1999). Under haplo-diploidy, females develop from fertilized eggs (diploid), males develop parthenogenetically from unfertilized eggs (haploid), and the primary sex ratio is determined by mothers' control of fertilization at the time of oviposition. In some Hymenoptera, sex determination is also influenced by a genetic process called complementary sex determination (CSD), which is usually based on a single sex-determining locus and a high diversity of complementary sex-determining alleles (Whiting, 1943; Cook and Crozier, 1995). Under CSD, diploid individuals heterozygous at the sex-determining locus are female, while haploid individuals are hemizygous and are male. However, diploid individuals homozygous at the sex-determining locus develop into diploid males, which are usually not viable or sterile (Stouthamer *et al*, 1992; Cook and Crozier, 1995; Krieger *et al*, 1999). High sex-determining allele diversity is expected within populations because of frequency-dependent selection; with K sex-determining alleles the equilibrium frequency of each allele will be $1/K$. Gene dynamics of CSD are similar to those in genetic incompatibility systems of plants (eg, Yokoyama and Nei, 1979; Richman and Kohn, 1999) and major histocompatibility loci of vertebrates (Penn and Potts, 1999).

Diploid male production constitutes a genetic load within populations, especially when inbreeding is high and/or sex-determining allele diversity is low (Stouthamer *et al*, 1992; Pamilo *et al*, 1994; Cook and Crozier, 1995). For instance, one generation of brother–sister mating under single-locus CSD leads to severe inbreeding depression, with half of the fertilized eggs yielding low-fitness diploid males (Stouthamer *et al*, 1992; Cook and Crozier, 1995). Generally, the number of sex-

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determining alleles in a population influences the probability of 'matched' matings, where females share sex-determining alleles with their mates. With K sex-determining alleles at equilibrium, the probability of matched mating is $2/K$ under random mating (Adams *et al*, 1979; Yokoyama and Nei, 1979; Owen and Packer, 1994; Cook and Crozier, 1995), and CSD load increases dramatically when the number of sex-determining alleles in a population drops below six (Stouthamer *et al*, 1992). Diploid males and CSD have been identified in 30 species within four superfamilies of Hymenoptera including the primitive sawflies, which points to CSD being an ancestral means of sex determination in this group. Given the detrimental aspects of CSD, the question of how CSD load is avoided in Hymenoptera naturally arises.

Four aspects of population structure and mating systems influence the genetic load arising from CSD (Pamilo *et al*, 1994; Cook and Crozier, 1995). First, large populations with random mating should harbour many sex-determining alleles because frequency-dependent selection favours alleles when they become rare. Hymenoptera commonly have six or more sex-determining alleles (Whiting, 1943; Adams *et al*, 1977; Periquet *et al*, 1993; Ross *et al*, 1993; Kerr, 1997; Heimpel *et al*, 1999; Butcher *et al*, 2000). Second, multiple mating by females decreases the variance in diploid male production among females. Singly mated females produce either no diploid males if their sex-determining alleles do not match their mates, or produce diploid males in 50% of their fertilized eggs if they have a matched mate. When females mate more than once, the total number of diploid males produced in the population remains the same, but each female is expected to produce fewer of them (Cook and Crozier, 1995). Multiple mating is found in some but not all Hymenoptera that have CSD (Ridley, 1993; Boomsma and Ratneiks, 1996). Third, both females and males can directly avoid matched matings and diploid male production by avoiding inbreeding (Ode *et al*, 1995; Paxton *et al*, 2000). Fourth, a sex ratio skew towards female offspring can reduce the genetic load in Hymenoptera that have population-wide random mating (Cook and Crozier, 1995). In species where diploid males are fully functional and able to mate, females mated to these sterile diploid males usually will be constrained to produce haploid males only. Selection for 1:1 population-wide sex ratios will favour a higher fertilization rate in mated individuals, to compensate for the overproduction of males by the constrained females (Godfray, 1994).

The biology and life histories of most Hymenoptera are too poorly known to explore how population genetic structure and mating systems influence CSD load. One exception is *Habrobracon* (*Bracon*) *hebetor* (Say) (Hymenoptera: Braconidae), the first species in which single-locus CSD was described (Whiting, 1943). The mating system, sex allocation, and genetics of sex determination are better known in *Habrobracon* than in most other parasitoid wasps (see below). Here we examined the roles of both genetic structure and mating system on CSD in a population from Wisconsin, addressing these questions: (1) What is the genetic structure and effective size of *H. hebetor* populations, which are found in spatially patchy habitats in grain storages where they undergo annual fluctuations in abundance. (2) How common are diploid males and how many sex-determining alleles segregate in populations? (3) Do *H. hebetor* females have multiple mates? We use a model of CSD to examine how these aspects of the population biology of *H. hebetor* affect the expected CSD load.

Natural history of *Habrobracon hebetor*

Habrobracon hebetor is an ectoparasitoid of larvae of pyralid moths that infest stored grains, nuts, and fruits. Currently, *H. hebetor* has a worldwide distribution, is present throughout the USA, and is sold commercially as a biological control agent (Brower *et al*, 1996). A primary host for *H. hebetor* in stored grain is the Indian meal moth, *Plodia interpunctella*. Female wasps inject a paralysing venom into fourth or fifth instar *P. interpunctella* larvae, and then deposit between three and 20 eggs on the outside of the host. Larval/pupal development is completed in 10–11 days at 27°C. Egg hatch is greater than 90% for females and haploid males, but 10% or lower for diploid males (Whiting, 1943; Ode *et al*, 1997). Diploid males that reach adulthood readily mate, but almost all are sterile. Females typically fertilize two-thirds of their eggs, leading to female-biased sex ratios when the parents are not inbred (Antolin *et al*, 1995; Ode *et al*, 1997; Heimpel *et al*, 1999).

Mating behaviour and life history attributes of *H. hebetor* facilitate outbreeding (Antolin and Strand, 1992; Ode *et al*, 1995, 1998; Guertin *et al*, 1996). Wasp larvae pupate in close proximity to the consumed host, but after adult emergence both sexes disperse before mating (Ode *et al*, 1995). Males subsequently form aggregations on the surface of grain storages where females come to mate (Antolin and Strand, 1992). Laboratory experiments demonstrate that adult females actively avoid mating with males that develop in the same brood as themselves (Ode *et al*, 1995), and that 5–15% of females will mate more than once (Guertin *et al*, 1996; Ode *et al*, 1997).

Populations of *H. hebetor* greatly fluctuate in abundance on an annual basis (Antolin and Strand, 1992; Ode *et al*, 1997). Wasps are largely undetectable during spring and early summer, but become abundant in late summer (August) until severe cold weather settles in for winter. During autumn, wasp populations within each grain storage likely number in the thousands of individuals. It is during these periods of high abundance that male mating aggregations are commonly seen (Antolin and Strand, 1992).

Materials and methods

Field collection

Wasps were collected from two corn storage barns at Theis Farms (TH I, TH II), located 3 km from each other and 15 km southwest of the University of Wisconsin-Madison campus in Dane County, Wisconsin. The TH I facility was a small 5 m wide, 10 m long, and 5 m high wooden shed with a cement floor. The grain had remained in place for at least 5 years prior to the first sampling in 1991, with more grain added in the summer of 1992. TH II was a larger structure (40 m long, 20 m wide, 5 m high) that had grain moved into and out of it each year. Both populations were sampled during autumn of 1991 and 1992 (Ode *et al*, 1997). From each sample, 19–25 females were frozen at −80°C for genetic analysis. A similar number of males (20–23) were

sampled at the same time, except at TH I in 1992 when no males were collected.

Laboratory rearing

In 1993, field-captured females were returned from TH I to the laboratory for measurement of sex ratios and other life history characteristics (Ode *et al*, 1997). A total of 34 females ovipostied on hosts individually by being provided with four hosts per day for their lifetimes. Progeny were reared and sexed before being stored at −80°C for genetic analysis. In all, 34 females were allowed to oviposit individually. The progeny from each female were genotyped to determine the number of times each female had mated and to look for diploid male progeny. We isolated DNA individually from 10 male and 10 female progeny from each of the 34 females.

DNA isolation and molecular markers

Our population genetic studies used Randomly Amplified Polymorphic DNA (RAPD) markers analysed by polyacrylamide gel electrophoresis on native gels to detect single-strand conformation polymorphisms (SSCP) (Antolin *et al*, 1996; Black and DuTeau, 1996). Using RAPD-SSCP analysis, we can resolve a high proportion of markers that segregate as codominant polymorphisms and are dispersed throughout the genome (Antolin *et al*, 1996; Vaughn and Antolin, 1998).

DNA was isolated by salt extraction (Black and DuTeau, 1996). DNA was resuspended in 100 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at −80°C until use. RAPD-PCR protocols followed Black and DuTeau (1996) using 1 µl of DNA template in each 50 µl reaction, and included a negative control (all reagents but no template DNA). Field samples were analysed by amplifying DNA using four random primers (Operon Technologies, Alameda, CA, USA: AM10, B18, C1, Q16), which yielded 27 polymorphic markers. Laboratory-reared broods from 1993 were examined for genetic variation amplified by two primers (AM10 and Q16, see below) which yielded 11 polymorphic markers. RAPD markers were named using the primer name followed by the estimated size of the fragment. Only repeatable bands that segregated in Mendelian fashion were scored for analysis, including 15 markers that had been mapped previously (Antolin *et al*, 1996). SSCP and silver staining protocols were as described in Antolin *et al* (1996) and Black and DuTeau (1996). Sizes of amplified DNA fragments were estimated using an inverse function that relates fragment size to mobility in the gel (Antolin *et al*, 1996).

Population genetic analysis

Three pairs of markers were linked to each other (Antolin *et al*, 1996) and were in linkage disequilibrium in field samples. One of each pair was excluded from analyses (B18.1600, C01.3640, Q16.1240), leaving a total of 24 markers: 15 dominant presence/absence markers, and nine codominant markers. Excluding the linked markers resulted in fewer markers in linkage disequilibrium than expected by chance, corrected for the number of pairwise tests.

Genetic structure of the populations was determined by analysis of variance (Weir and Cockerham, 1984) using the program Genetic Data Analysis (Lewis and Zaykin, 2001). Potential biases in allele frequency estimates of dominant RAPD markers (band presence/absence) were corrected (Lynch and Milligan, 1994). The data were analysed for females separately and for males and females combined. Allele frequency estimates from males and females did not differ within samples (correlation coefficients: TH I 1991: $r=0.85$; TH II 1991: $r=0.78$; TH II 1992: $r=0.59$; TH I 1993: $r=0.89$). Thus, estimates from males and females for each marker in each sample were combined by a weighted average: $p(a_i)=[N_m p(a_{im})+2N_f p(a_{if})]/[N_m+2N_f]$, where $p(a_{im})$ and $p(a_{if})$ are frequencies of marker allele a_i in males and females, and N_m and N_f are sample sizes of males and females. The data were analysed hierarchically, with sampling dates nested within each of the populations to estimate differences between localities (Θ_{LOC}) and between sampling times nested within localities ($\Theta_{YEAR(LOC)}$). Within-population inbreeding was estimated using the nine codominant markers in females. An analysis that included data from Th I in 1993 used genotypes of males and females deduced from offspring of field-captured females. Confidence limits were estimated by bootstrapping across markers from 10 000 permutations.

Effective population sizes, N_e, of TH I and TH II were estimated from temporal changes in allele frequencies between 1991 and 1992, following Waples (1989). Estimates depend upon the standardized variance of allele frequency change, F_k,

$$F_k = \frac{1}{A-1}\sum_{i=1}^{A}\frac{[p_0(a_i)-p_t(a_i)]^2}{[p_0(a_i)+p_t(a_i)]/2} \quad (1)$$

with A being the number of alleles for each marker, and $p_0(a_i)$ and $p_t(a_i)$ the frequencies of marker allele a_i in the first and second samples. When actual population size is unknown and sampling is after reproduction, F_k relates to effective population size as

$$N_e = \frac{tg-2}{2g[F_k-1/(2S_0)-1/(2S_t)]} \quad (2)$$

where t denotes the number of generations between samples, S_0 and S_t indicate sample size at the two intervals, and g is the ratio of population size to effective size, N/N_e (equation (14), Waples (1989)). Estimates of N_e that used values of g between two and 10 differed by less than 10%. We report values assuming $g=2.5$, which is a common value known from a large number of studies of effective population size (Nunney, 1993; Frankham, 1995). To combine data from all markers, harmonic means of S_0, and S_t for each marker were used and average F_k was weighted by the number of alleles from each marker. Confidence intervals around N_e were estimated from the χ^2 distribution (Waples, 1989).

Mating frequency and sex-determining allele diversity

We examined genotypes within broods to determine the number of times females mated and the number of matched matings. We genotyped 10 male and 10 female offspring from each of 34 females collected from the TH I population in 1993, and thus categorized each field-caught female as having mated once or more than once. Progeny were scored for RAPD markers amplified by primers AM10 (five markers: four dominant, one codominant) and Q16 (six markers: two dominant, four

codominant). Most male offspring were haploid and reflected their mother's genotypes. Given the mother's genotype, multiple mating was indicated when genotypes among female progeny exceeded the number expected from a single mate.

Using genotypes of broods to measure the frequency of mating requires some correction because simple counts of the number of mates fail to account for biases in detection, leading to two nondetection errors (Pedersen and Boomsma, 1999). The first error arises from missing offspring of a second mate because of inadequate sample size, the second error depends on the number of molecular markers examined and the possibility of failing to detect offspring of a second mate because of poor genetic resolution. When females mate with two males, both errors are influenced by unequal sperm use (paternity skew), including sperm precedence of the first male. We used methods from Pedersen and Boomsma (1999) to estimate paternity skew ($\hat{C}$) and the probability of nondetection (f_i). Given the observed number of double-mated females, D_{obs}, the effective number of mates per female is $m_{ep} = 1/[1-2D\hat{C}\ (1-\hat{C})]$. The frequency of double matings is corrected for nondetection bias by estimating the number of double matings: $D_{est} = D_{obs}/(1-f_i)$; D_{est} is used in conjunction with $\hat{C}$ to estimate the effective number of mates, m_{ep}.

Two broods included diploid males, which were heterozygous for at least three RAPD-SSCP markers. Using this, we calculated the probability of matched matings (Ψ) and the numbers of sex-determining alleles (K) following the procedures of Adams *et al* (1977) and Owen and Packer (1994). The symbol 'Θ' was used previously to indicate the probability of matched mating, but we used the symbol 'Ψ' to avoid confusion with population genetic parameters. Under single-locus CSD, Ψ is estimated from rates of fertilization by females, r, and survival of diploid males relative to haploid males, s. The proportion of diploid male eggs from a matched mating is $0.5r/[0.5r+(1-r)] = r/(2-r)$. The survival of diploid males relative to haploid males determines the probability of detecting diploid males among the offspring of matched matings: $p = sr/(2-r)$. The distribution of diploid males in a sample of n males from each mating is binomial and the probability of sampling m diploid males in a brood is

$$P(M=m) = \begin{cases} (1-\psi) + \psi(1-p)^n, & m=0 \\ \binom{n}{m}(p)^m(1-p)^{n-m}\psi, & m>0 \end{cases} \quad (3)$$

The sample size of each brood, n, was the same. Thus, $(1-p)^n$ was treated as a parameter, b, a function of relative diploid male survival and the fertilization rate. As *H. hebetor* diploid males have low survivorship and were rare, we approximated their occurrence with a simplified binomial, with broods having either no diploid males or at least one diploid male:

$$\begin{aligned} P(M=0) &= [(1-\psi) + \psi b] = 1 - \psi(1-b) \\ P(M>0) &= 1 - [(1-\psi) + \psi b] = \psi(1-b) \end{aligned} \quad (4)$$

We estimated Ψ from the number of broods that contained diploid males, which relates to the probability $P(M>0)$. With a binomial distribution of matched matings, the variance of Ψ for m matings is $V(\Psi) = [\Psi(1-b)][1-\Psi(1-b)]/m$.

The number of sex-determining alleles in a population at equilibrium is $K = 2/\Psi$ (Adams *et al*, 1977) when each sex-determining allele is at frequency $1/K$ and mating is random. When sex-determining allele frequencies are unequal, the number of alleles detected in a population will be lower than the actual number (Adams *et al*, 1977; Owen and Packer, 1994). Thus, our sex-determining allele diversity estimates may have been biased downwards.

Results

Population genetic analysis

Allele frequencies of markers varied temporally, but the populations were not significantly differentiated (Table 1). All estimates of Θ_{LOC}, which reflected overall divergence between the TH I and TH II, had 95% confidence intervals (CIs) that included zero. In contrast, all estimates of $\Theta_{YEAR(LOC)}$, which measured differences between years within localities, were significantly greater than zero. Analysis of 3 years of samples from the TH I population (1991, 1992, 1993), based on 11 markers, also showed differences between years ($\Theta_{YEAR} = 0.054$, 95% CI 0.028, 0.086). The within-population inbreeding

Table 1 Description of population structure based upon the Weir and Cockerham (1984) hierarchical analysis of variance

	No. of markers	$\Theta_{YEAR(LOC)}$	Θ_{LOC}	*Inbreeding coefficient*, f
Females				
Dominant markers	15	0.111	−0.056	—
(95% CI)		(0.046, 0.20)	(−0.16, 0.00)	
Females				
Codominant markers	9	0.084	0.021	0.12
(95% CI)		(0.028, 0.16)	(−0.008, 0.054)	(−0.007, 0.25)
Males and females				
All markers	24	0.079	−0.019	—
(95% CI)		(0.042, 0.12)	(−0.052, 0.010)	

Θ_{LOC} measures differentiation between the two field localities, $\Theta_{YEAR(LOC)}$ measures differentiation between sampling years (1991, 1992) within localities. Analyses were conducted on samples of females for both dominant (band present/band absent markers) and codominant markers. Similar results were obtained when allele frequencies from male and female samples were combined. Bootstrapped 95% CIs based on 10 000 permutations are in parentheses below each estimate.

coefficient estimated from genotypes of females for nine codominant markers was 0.12, with a lower confidence limit that slightly overlapped zero.

Upper 95% confidence limits of effective population size of both TH I and TH II were less than 87 individuals (Table 2). As sampling occurred during autumn when populations were at peak abundance (Ode *et al*, 1997), N_e of this magnitude reflects population bottlenecks that must occur between times of high abundance. It is likely that 10–15 generations pass each year in Wisconsin, as development from egg to adult in the laboratory at 27°C is 11 days, and females can live for up to 6 weeks. Thus, we estimate that N_e of these populations was on the order of 20–50 individuals. This may overestimate the actual value because we assume that the effects of each generation are additive rather than multiplicative (Luikart *et al*, 1999). On the other hand, if as few as five generations passed between sampling times, effective size could have been less than 20.

Mating frequency and sex-determining allele diversity

Of the 34 broods sampled in 1993, seven resulted from multiple mating. Determination of multiple mates in each brood was on the basis of two or more markers. We detected double matings only; we did not find genotypes suggesting more than two mates. The 11 RAPD markers provided high resolution; the average probability of nondetection of a second mate by genetic markers was less than 0.012, with the range of values spanning 0.002–0.04. The observed frequency of double mating was $D_{obs} = 0.21$, and from this we may conclude that *H. hebetor* females mated with 1.21 males. However, contributions by the two males in double-mated broods were heavily skewed, with 7, 8, or 9 of the 10 offspring sired by one male. Following Pedersen and Boomsma (1999), this translated into a paternity skew of $\hat{C} = 0.87$, which increased the overall probability of nondetection of a second male to $f_i = 0.28$. The corrected frequency of double mating was $D_{est} = 0.31$, slightly greater than the observed value. Regardless, the severity of the paternity skew reduced the effective number of mates per female to $m_{ep} = 1.08$.

Two single-mated females produced diploid males, and in both cases the diploid males were heterozygous for three markers. One brood had one diploid male, and the other had two. We calculated the probability that we missed diploid males in our samples because they were homozygous for all markers, and found it unlikely that we failed to detect diploid males because they were homozygous for all three codominant markers. For samples of 10 males per brood in 34 broods, the overall probability of misclassifying homozygotes is

$$1 - \left[1 - \left(\left(\frac{sr}{2-r}\right)\prod_{i=1}^{3}\left(p(a_i)^2 + p(b_i)^2\right)\right)^{10}\right]^{34} \qquad (5)$$

where $p(a_i)$ and $p(b_i)$ are allele frequencies of the three codominant markers that indicated diploid males. Using the typical rates of survival of diploid males relative to haploid males, $s = 0.1$, and fertilization, $r = 0.7$, the probability of misclassifying diploid males in our samples was estimated to be less than 1×10^{-18}. Even if all diploid males survived ($s = 1.0$), the probability was less than 3×10^{-9}.

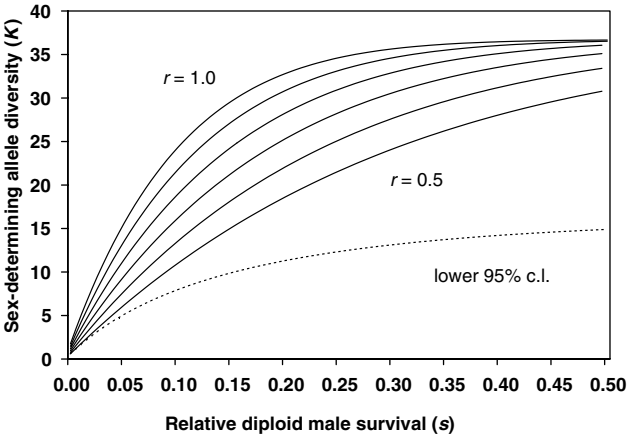

Figure 1 Estimated number of sex-determining alleles, *K*, as a function of survival of diploid males relative to haploid males, *s*, when the fertilization rate, *r*, varies between 0.5 and 1.0. The broken line is the lower 95% confidence limit for $r = 0.5$.

We used the frequency of diploid males to estimate the probability of matched mating, Ψ, and the numbers of sex-determining alleles, *K* (equation (4)). With 34 broods tested and an effective number of mates $m_{ep} = 1.08$, the effective number of males was 36.7. Two of these matings resulted in diploid males, making the probability of finding one or more diploid males in each brood $P(M>0) = \Psi(1-b) = 2/36.7$, where $(1-b)$ is the probability of observing diploid males in our sample. After rearranging, the estimate of matched mating was $\Psi = (2/36.7)[1/(1-b)]$. The estimate of sex-determining allele diversity is $K = 2/\Psi$ (Adams *et al*, 1977; Owen and Packer, 1994).

Again using typical values of diploid male survival ($s = 0.1$) and fertilization rates ($r = 0.7$), our best estimate for this population was 15.6 sex-determining alleles, with a lower 95% confidence limit of 10.1 alleles (Figure 1). Female *H. hebetor* almost always produce female-biased sex ratios in the laboratory, so assuming a fertilization proportion of $r = 0.5$ likely provided a low estimate of diversity. With this low fertilization rate and rates of diploid male survival of $s = 0.05$ and 0.1, estimates dropped to 5.7 and 10.6 sex-determining alleles, with lower 95% confidence limits of 4.7 and 7.7 alleles. Estimates asymptote at an unlikely maximum of 36.7 alleles (with a lower confidence limit of 16.0), which would be expected when diploid and haploid males survive at the same rate, all alleles are in equal frequency (one unique allele from each male mate), and no matched matings occur.

Discussion

The Theis population in Wisconsin showed relatively large genetic differences between years, but no overall genetic differentiation between subpopulations (Table 1). The lack of differentiation between subpopulations could result from dispersal between the sites, as wasps disperse at high rates from stored grains (Ode *et al*, 1998), and female longevity is sufficient (as high as 42 days in the laboratory) to facilitate successful dispersal. It is likely that between-year differences arose because of

Table 2 Estimates of effective population size (N_e) for the TH I and TH II populations based on changes in marker allele frequencies between samples, and for t=5, 10, or 15 generations between samples

	TH I		*TH II*	
t	N_e	*(95% CI)*	N_e	*(95% CI)*
5	13.8	(6.8, 25.7)	11.8	(5.9, 21.0)
10	30.3	(14.9, 56.2)	25.8	(13.0, 45.9)
15	46.8	(23.0, 86.8)	39.7	(20.1, 70.8)

Estimates are from a model that assumes sampling of individuals after reproduction (Waples, 1989), and for a ratio of census number to effective size (N/N_e=g) of 2.5. Estimates of CIs (in parentheses) followed Waples (1989).

the dramatic population fluctuations that occur annually. Wasp populations are small during the spring and early summer, increase in late summer, and then decline to nearly undetectable levels when temperatures drop below freezing in the fall (Antolin and Strand, 1992; Ode *et al*, 1997). Our estimates of effective population size of only 20–50 individuals reflect these fluctuations (Table 2). These annual reductions in population size could also account for the observation that the Theis populations appear to be slightly inbred (inbreeding coefficient = 0.12).

Our best estimate of sex-determining allele diversity for this population was 10–16 alleles, which is higher than previously reported for *H. hebetor* but not as high as that in other species (Yokoyama and Nei, 1979; Stouthamer *et al*, 1992; Cook and Crozier, 1995). Whiting (1943) found nine sex-determining alleles in crosses between six populations from California, Iowa, New York, and Pennsylvania. Recently, Heimpel *et al* (1999) identified sex-determining alleles from crosses among isofemale lines originating in Wisconsin (five alleles), California (nine alleles), Kansas (six alleles), and Texas (five alleles). Crosses between populations showed a combined total of 12 alleles in these populations, with alleles shared between Wisconsin, Kansas, and Texas, but not California. Heimpel *et al* (1999) estimated four sex-determining alleles in the Theis population, based on counting alleles in crosses between five isofemale lines derived from colonies that had been maintained in the laboratory for several years. This value is probably low. It is likely that the five isofemale lines incompletely represented total diversity and that some alleles were lost by genetic drift during the approximately 75 generations of laboratory rearing.

On the other hand, finding 10–16 sex-determining alleles in the Theis population may be construed as high. Only five sex-determining alleles are expected in populations with $N_e < 100$ (Yokoyama and Nei, 1979) and our estimate of N_e fell into this range. However, our data also suggest that the TH I and TH II were not genetically differentiated (Table 1) and that regular gene flow between the populations occurred. Yokoyama and Nei (1979) noted that even small amounts of gene flow could dramatically increase sex-determining allele diversity. Gene flow in conjunction with frequency-dependent selection would slow the potential loss of sex-determining alleles because of genetic drift.

Mating system and reducing CSD load

The mating system of *H. hebetor* is based on males forming aggregations on the surface of grain storages. Females rarely remate, even if they deplete the sperm in their spermathecae, and females discriminate against mating with brood-mates. In the laboratory studies, few females produce progeny from more than one mate (Antolin and Strand, 1992; Guertin *et al*, 1996; Ode *et al*, 1997, 1998). Collectively, these studies suggest that multiple mating in field populations also should be rare. This was corroborated, as we found that 21% of females collected in the field mated more than once, but that unequal sperm use (paternity skew) in double-mated broods reduced the effective mate number to only 1.08. Our sample size of each brood allows for nondetection error as high as 0.28, and we cannot completely exclude the possibility that sperm is used sequentially by each female (eg sperm precedence). Thus, we have likely underestimated the number of mates per female. Regardless, because of unequal sperm use, our data collectively point to much lower effective rates of multiple mating than seen in other species with CSD, for instance honeybees (*Apis mellifera*; Adams *et al*, 1977).

We evaluated the consequences of the mating system on diploid male production and CSD load using a model that relates genetic load to sex-determining allele diversity. The model followed Adams *et al* (1977), Page and Marks (1982), and Stouthamer *et al* (1992), and assumed population-wide mating, lifetime female fecundity of 100 eggs, and each sex-determining allele at frequency $1/K$. The model calculated the number of female and diploid male offspring as a function of the number of sex-determining alleles (K), the effective number of males that mate with each female (m), and the number of mates that share sex-determining alleles with the females (y). Among fertilized diploid eggs, the proportion that is female is binomial with mean $1-1/K$ and variance $(1/2m)(1/K)(1-2/K)$. The distribution of females mated with y males having alleles matching theirs is binomial:

$$P(Y=y)=\binom{m}{y}\left(\frac{2}{K}\right)^{y}\left(1-\frac{2}{K}\right)^{m-y} \qquad (6)$$

in which $y = 0, 1, 2, \ldots, m$. When females lay E eggs and fertilize them at rate r, the productivity (female offspring) of each female mated m times with y matched mates is $R(m, y) = r(1-y/2m)E$ and the average productivity of the population is $R(K) = r(1-1/K)E$ (Page and Marks, 1982; Stouthamer *et al*, 1992).

Our analysis of the effects of multiple mating on CSD load showed that multiple mating is favoured only when there are five or fewer sex-determining alleles in populations. We begin by noting that multiple mating does not change the total number of diploid males or average productivity within populations, but changes the distribution of diploid male production between females and reduces the between-female variance in productivity (Figure 2). With single mating, females will either produce no diploid males or produce diploid males in 50% of the eggs they fertilize. When females mate many times, more females produce diploid males but each produces fewer of them (Page and Marks, 1982).

As a consequence, single mating will usually be favoured over multiple mating because more females

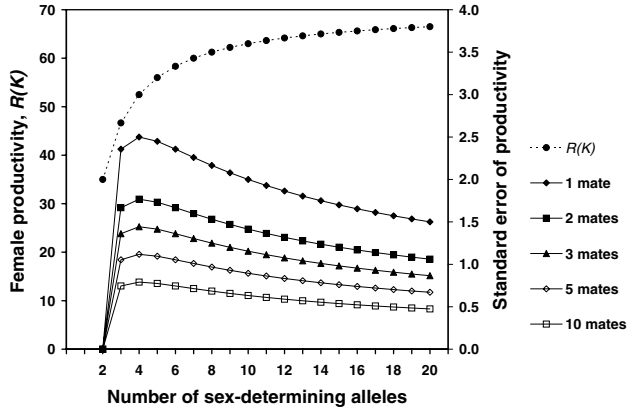

Figure 2 Expected relationship between the number of sex-determining alleles, relative female productivity ($R(K)$, broken line), and variance in female productivity (solid lines) in a large randomly mating population with egg fertilization rate $r = 0.7$. Female productivity is the average number of female offspring per female and is a function of sex-determining allele diversity only; the number of times females mate does not change overall diploid male production or average productivity in a population (Page and Marks, 1982). Variance in productivity is plotted for $n = 1$, 2, 3, 5, and 10 mates.

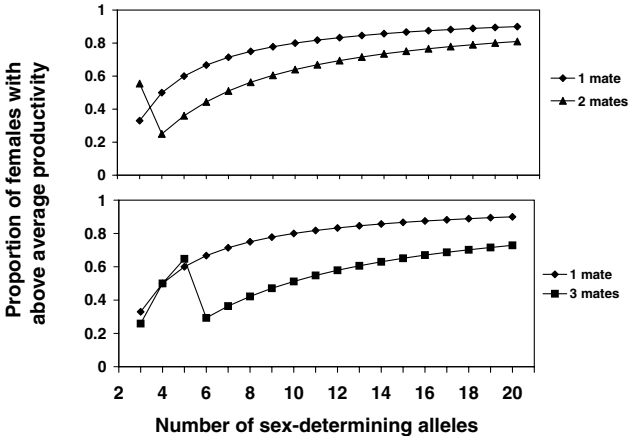

Figure 3 Proportion of females in a large randomly mating population with above-average productivity, as a function of sex-determining allele diversity when females mate with 1, 2, and 3 males. When the number of sex-determining alleles is low, females who mate more than once can have much higher than average fitness and the distribution of female productivity becomes greatly skewed. A small proportion of females have matched matings only, resulting in many diploid male progeny and extremely low productivity for those females.

will have greater than average productivity (Figure 3). The exception is when five or fewer sex-determining alleles are found in a population. This result depends on the skewness in the distribution of female productivity. In monogamous populations with many sex-determining alleles, few females have matched matings and most escape CSD load. In populations with multiple mating, more females will suffer at least some effects of CSD load, but the distribution of female productivity will be skewed. Some females will suffer severely, but many more will have greater than average productivity, especially when there are few sex alleles in the population. With few sex-determining alleles in the population, multiple mating is favoured because it reduces the probability that females will be involved only in matched matings.

Multiple mating occurs in other parasitoid species (Ridley, 1993), including ichneumonids and braconids where CSD is expected. In parasitoid wasps, however, multiple mating is associated with oviposition behaviour rather than with CSD; females that produce gregarious broods (many offspring develop from each host) are much more likely to multimate than females that are solitary (one offspring per host) (Ridley, 1993). As multiple mating correlates with oviposition strategy rather than CSD, it is unlikely that multiple mating evolved to reduce the potential costs of CSD.

This contrasts with the situation in social Hymenoptera, where the relative advantages of single and multiple mating depend on details of colony growth and timing of production of sexual offspring (Pamilo *et al*, 1994; Boomsma and Ratneiks, 1996). The cost of diploid males can be high during initial colony growth because diploid males develop in the place of worker females. With single mating, queens either produce no diploid males or suffer a 50% reduction in the number of workers if the queen has a matched mating. On average, single mating results in higher fitness (colony growth) than multiple mating, because with multiple mating more queens suffer the fitness reduction of losing workers to diploid male production. The advantage of single mating is especially strong if sexual reproduction occurs early in the growth of colonies or if rapid colony growth is critical to their survival. Multiple mating is favoured only if initial colony growth does not affect colony survival and if sexual reproduction is delayed until after colonies reach their maximum size. Multiple mating is favoured even more in species like the honeybee, *A. mellifera*, whose diploid males are consumed by workers early in development so that the cost of diploid male production is low (Boomsma and Ratneiks, 1996).

How great is the advantage of avoiding inbreeding in species with CSD? The effect of inbreeding avoidance can be analysed by considering the penalty for failing to avoid matched matings. This penalty is expressed as the reduction in the number of daughters produced by females with one or more matched mating, compared to females that completely avoid matched matings. The fitness advantage of inbreeding avoidance is a function of both sex-determining allele diversity and multiple mating (Figure 4). With a fertilization rate of 0.7 and each female producing 100 eggs, females that avoid matched matings have 70 daughters, regardless of the number of mates. With two sex-determining alleles, all matings are matched and females can only produce 35 daughters. When females mate once, the penalty of matched mating is 35, regardless of the number of sex-determining alleles in the population. Avoiding matched mating is always advantageous, but the penalty for matched mating is lower when sex-determining allele diversity is high and females mate multiple times. Prior studies indicate that *H. hebetor* females avoid mating with brood mates, but in the laboratory mating readily occurs between siblings reared on different hosts and between mothers and sons (Ode *et al*, 1995; Heimpel *et al*, 1999). This suggests that sex-determining alleles *per se* are not used as cues to

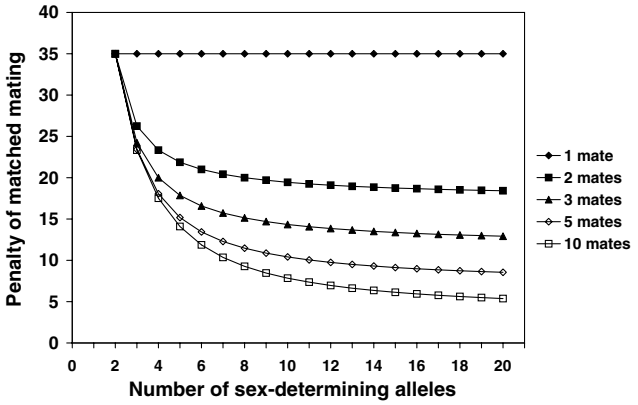

Figure 4 Penalty of matched matings as a function of the number of sex-determining alleles, when females mate with $m = 1$, 2, 3, 5, and 10 males. Penalty is the difference in productivity between female that avoid matched mating ($y = 0$) and the average productivity of females with one or more matched mates ($y > 0$).

avoid matched matings in *H. hebetor*, nor do they appear to be used by other hymenopterans in which CSD operates (Ratneiks, 1991; Cook and Crozier, 1995). On the other hand, *H. hebetor* females can avoid some matched matings if they discriminate against mating with males from their natal brood (Ode *et al*, 1995).

Although it has been suggested that biased sex ratios in parasitoids can arise as a consequence of diploid male production, it is doubtful that CSD influences sex ratios in *H. hebetor*. For outbreeding Hymenoptera like *H. hebetor*, primary sex ratios can evolve towards female bias if some females are constrained to producing only haploid male progeny (Godfray, 1994; Ode *et al*, 1997). In *H. hebetor*, females that mate with diploid males cannot fertilize eggs and are constrained to producing haploid males only (Heimpel *et al*, 1999). If diploid males participate in mating and thus constrain their mates to produce only haploid males, sex ratio selection favours a higher fertilization rate in mated individuals (Cook and Crozier, 1995; Godfray and Cook, 1997). Low diploid male survival in *H. hebetor* means that diploid males seldom participate in mating, making it unlikely that skewed sex ratios arise as a consequence of CSD. On the other hand, many *H. hebetor* females are constrained to produce all-male broods because they never mate or are depleted of sperm (Guertin *et al*, 1996; Ode *et al*, 1997). Thus, we think it more likely that oviposition by these constrained females is responsible for the female-biased sex ratios produced by mated *H. hebetor* females (Ode *et al*, 1997).

In conclusion, our results suggest that sex-determining allele diversity was high enough in the Theis *H. hebetor* population to avoid a severe genetic load from CSD. On the other hand, it appears that these populations may have been slightly inbred because of population fluctuations and reductions in effective population size. Thus, selection for outcrossing should remain high in *H. hebetor*. Females have only 1.08 effective mates, which makes the *H. hebetor* mating system effectively monogamous. Our model suggests that multiple mating could be favoured during population bottlenecks when population size is relatively small and local sex-determining allele diversity within subpopulations is low. However, other aspects of the mating system (male mating aggregations, female inbreeding avoidance) combined with occasional gene flow and frequency-dependent selection could work to maintain high sex-determining allele diversity in this species.

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