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Effective population size in Hymenoptera with complementary sex determination

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Complementary sex determination in the haplodiploid Hymenoptera leads to the production of inviable or effectively sterile diploid males when diploid progeny are homozygous at the sex-determining locus. The production of diploid males reduces the number of females in a population and biases the effective breeding sex ratio in favor of haploid males. This in turn will reduce the effective population size (N_e) of hymenopteran populations with complementary sex determination relative to the expected reductions due to haplodiploidy alone. The effects of diploid male production on N_e in hymenopterans with complementary sex determination when diploid males are either inviable or effectively sterile are assessed theoretically. In both models, low allelic diversity at the sex locus reduces the N_e of hymenopteran populations, and this effect is largest when diploid males are effectively sterile.

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Introduction

Wright (1933) demonstrated that the effective population size, the size of a genetically ideal population that has the same rate of heterozygosity loss as an actual population under consideration, for diploids, $N_{e.d}$, is

$$N_{e.d} = \frac{4N_f N_m}{N_f + N_m} \qquad (1)$$

and for haplodiploids, $N_{e.hd}$, is

$$N_{e.hd} = \frac{9N_f N_m}{2N_f + 4N_m} \qquad (2)$$

where N_f and N_m are the number of breeding females and males, respectively. The difference between equations (1) and (2) arises due to the lower number of gene copies in haplodiploids, as males are hemizygous at all loci (Hedrick and Parker, 1997). Given equal numbers of breeding males and females, $N_{e.hd} = 0.75N_{e.d}$ (Crozier, 1976; Hedrick and Parker, 1997).

In the haplodiploid Hymenoptera (ants, bees, wasps, and sawflies), sex is usually determined through the complementary actions of alleles at an autosomal locus (Cook, 1993; Cook and Crozier, 1995; Evans *et al*, 2004). Complementary sex determination (CSD) is the ancestral sex-determining mechanism in the Hymenoptera (Cook, 1993; Cook and Crozier, 1995), and the CSD locus has recently been characterized in the honeybee *Apis mellifera* (Beye *et al*, 2003). Hemizygotes at the sex-determining locus develop from unfertilized eggs into haploid males, while for diploid individuals, heterozygotes and homozygotes develop from fertilized eggs into females and males, respectively (Cook and Crozier, 1995; Beye *et al*,

2003). Diploid males are either inviable or effectively sterile (Agoze *et al*, 1994; Cook and Crozier, 1995; Holloway *et al*, 1999) and, therefore, do not contribute to reproduction in hymenopteran populations. The production of diploid males is essentially equivalent to differential female mortality (Cook and Crozier, 1995). Differential mortality at the end of the parental investment period does not select for changes in the primary sex ratio (Charnov, 1982; Trivers, 1985), and thus the production of diploid males will ultimately bias the effective breeding sex ratio in favor of haploid males (Stouthamer *et al*, 1992; Cook and Crozier, 1995) in parasitic and mass provisioning species (the majority of bees and solitary wasps mass provision; Bohart and Menke, 1976; Hunt, 1999; Michener, 2000; O'Neil, 2000). This in turn will result in a decrease in N_e of hymenopterans with CSD relative to the expectations of equation (2) for haplodiploids. The effects of diploid male production on N_e in hymenopterans have never been examined.

The effects of diploid male production in hymenopteran populations are assessed theoretically by modifying equation (2) under the following conditions: (1) diploid males are inviable (ie diploid males never emerge as adults) and (2) diploid males are viable but effectively sterile (ie diploid males emerge as adults that are capable of mating, but females mating with diploid males produce triploid progeny). Both scenarios have been documented in the Hymenoptera in both natural and laboratory populations (eg Plowright and Pallett, 1979; Agoze *et al*, 1994; Duchateau *et al*, 1994; Cook and Crozier, 1995; Holloway *et al*, 1999; Krieger *et al*, 1999; Ayabe *et al*, 2004; Liebert *et al*, 2004).

Model

Consider a mass provisioning hymenopteran population producing a proportion of h haploid and $1-h$ diploid

progeny per generation. In haplodiploid populations without CSD, all diploid progeny are expected to be female. However, with CSD, a proportion of diploid progeny will develop into inviable or effectively sterile diploid males that will not contribute to reproduction. Given K effective alleles at the sex-determining locus, the frequency of matched matings, where a mating male and female share a sex allele in common, in a random mating population is (Adams *et al*, 1977)

$$\theta = \frac{2}{K} \tag{3}$$

Half of the diploid progeny in a matched mating will be homozygous at the sex-determining locus, and thus will develop into diploid males. Assuming that females mate singly, the norm for hymenopterans (eg Eickwort and Ginsberg, 1980; Strassmann, 2001), the proportion of diploids that are male is (Adams *et al*, 1977; Owen and Packer, 1994)

$$\Phi = \frac{1}{K} \tag{4}$$

Given CSD and inviable diploid males, the expected proportion of breeding females is reduced by a factor of $1-\Phi$. The effective population size for haplodiploids exhibiting CSD with inviable diploid males can then be represented as

$$N_{e.csd1} = \frac{(9(1-h)(1-\Phi)N)hN}{2(1-h)(1-\Phi)N + 4hN} \tag{5}$$

where N is the total number of eggs produced per generation. The predictions of this model will also hold if diploid males are viable but never achieve matings, or if they mate but females mating with diploids remate with haploids and utilize the latter's sperm.

When diploid males are effectively sterile (diploid males mate but father only triploid progeny), the proportion of females mating with a diploid male is equivalent to the frequency of males that are diploid, ϕ:

$$\phi = \frac{(1-h)\Phi}{h + (1-h)\Phi} \tag{6}$$

Only females mating with haploid males will contribute to diploid reproduction, and thus the expected proportion of breeding females is further reduced by a factor of $1-\phi$ when diploid males are effectively sterile and achieve matings. The effective population size for haplodiploids exhibiting CSD with effectively sterile diploid males can then be represented as

$$N_{e.csd2} = \frac{(9(1-h)(1-\Phi)(1-\phi)N)hN}{2(1-h)(1-\Phi)(1-\phi)N + 4hN} \tag{7}$$

Results

As the effective number of sex alleles approaches infinity, diploid males are seldom produced, and thus equations (6) and (7) converge to equation (2). However, K is finite, and thus the actual proportion of breeding females with CSD, in both models, will always be smaller than the expected proportion of breeding females without CSD, reducing the number of females breeding and biasing the realized sex ratio in favor of haploids. For example, given a primary even sex ratio, where $h = (1-h) = 0.5$ and $K = 4$,

the actual proportion of breeding females is reduced by 25% when diploid males are inviable, resulting in an effective breeding sex ratio of 4:3 (haploid males:diploid females). When diploid males are effectively sterile, the same starting parameters result in a 40% reduction in the number of females breeding, and a breeding sex ratio of 5:3.

The effective population size for haplodiploids, and haplodiploids with CSD for different primary sex ratios and numbers of sex alleles are presented in Figure 1. In all cases, the effective population size of hymenopterans with CSD is lower than that expected due to just haplodiploidy. The difference between N_e of populations without CSD (equation (2)) and with CSD (equations (6) and (7)) increases when the effective number of sex alleles is reduced, as expected. The effect of CSD on reducing N_e is stronger when diploid males are effectively sterile *versus* when they are inviable (Figure 1b *versus* Figure 1a). When diploid males are inviable (Figure 1a), the magnitude of reduction in N_e increases with male biased primary sex ratios, but the opposite pattern occurs when diploid males are effec-
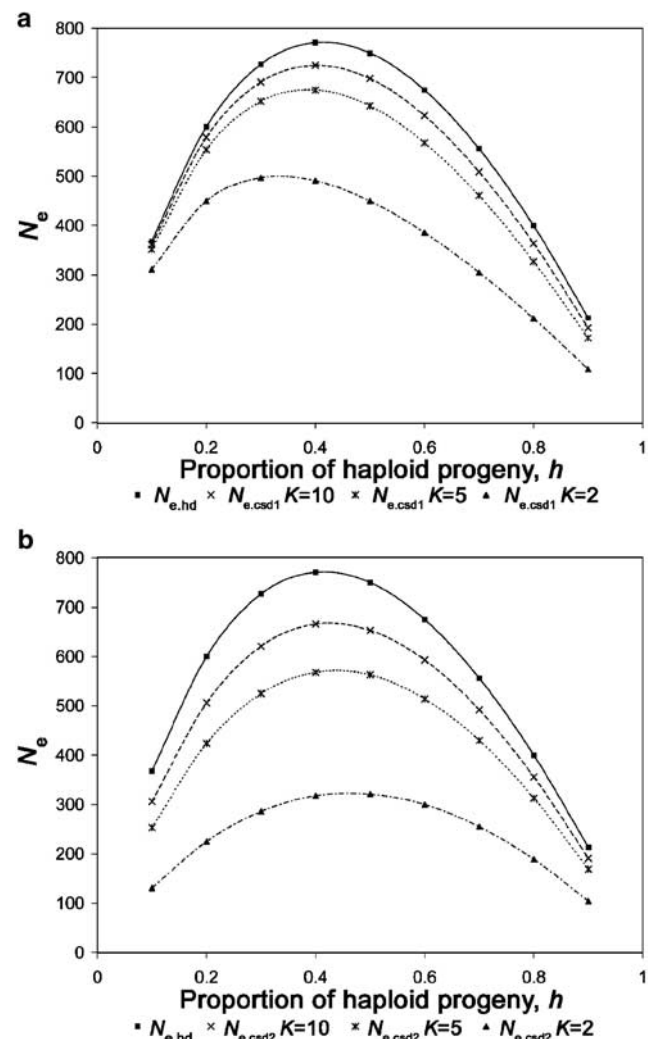

Figure 1 The effective population size of haplodiploids with CSD given (**a**) inviable or (**b**) effectively sterile diploid males, based on $N = 1000$.

tively sterile (Figure 1b). The explanation for the former lies in the fact that diploid male production acts to reduce female numbers, the effect of which is most extreme when females are already rare. The latter pattern occurs due to the increased probability of a female mating with a diploid male with female biased primary sex ratios. For example, starting with $h = 0.1$ and $K = 4$, the proportion of females mating with a diploid male is 69% *versus* only 3% when $h = 0.9$.

Discussion

The effective population size is a parameter of great importance in evolutionary, population, and conservation genetics. For example, N_e controls equilibrium levels of neutral genetic variation, rates of heterozygosity loss, and the relative importance of selection *versus* drift (Crow and Kimura, 1970; Frankham, 1995; Hedrick, 2000). As many hymenopterans exhibit CSD (Cook and Crozier, 1995), they are expected to have even lower effective population sizes than previously predicted due to haplodiploidy alone. Hymenopteran species usually exhibit lower levels of genetic variation, when compared to diploid insects (reviewed by Packer and Owen, 2001) even when the confounding effects of haplodiploidy and social behavior are removed (Hedrick and Parker, 1997; Packer and Owen, 2001), consistent with the present analysis. Surprisingly low empirical estimates of N_e ($\ll 100$) for natural hymenopteran populations (Zayed and Packer, 2001; Antolin *et al*, 2003; Zayed *et al*, 2004) also lend support to the view that hymenopterans with CSD have lower N_e than previously expected.

The number of sex alleles in large natural hymenopteran populations usually ranges from 9 to 20 (Cook and Crozier, 1995); however, several studies have documented low allelic diversity at the sex locus ($K \leqslant 5$) in natural populations (Heimpel *et al*, 1999; Holloway *et al*, 1999; Butcher *et al*, 2000a,b; Carvalho, 2001; Zayed and Packer, 2001; Zayed *et al*, 2004). Large reductions in N_e relative to equation (2) are thus very likely to occur in small, fragmented, and bottlenecked/introduced populations where allelic diversity at the sex locus is expected to be low (eg Krieger *et al*, 1999; Zayed and Packer, 2001; Zayed *et al*, 2004). Similarly, large reductions in N_e are likely to occur in populations of diet specialists expected to have lower allelic diversity at the sex-determining locus than generalist species (Packer *et al*, 2004). Further, it is theoretically expected that diploid males show higher survival than haploid males, as deleterious recessive alleles are always expressed in the latter. Effective sterility of diploid males is thus likely to be more common than inviability, further increasing the effects of diploid male production on reducing N_e. This is supported by recent reports of triploid females, the product of matings between females and diploid males, in several hymenopteran species (Krieger *et al*, 1999; Ayabe *et al*, 2004; reviewed by Liebert *et al*, 2004). Failure to incorporate diploid male production into population and conservation genetic models may lead to overestimates of N_e, a parameter of great significance to the evolutionary genetics of the Hymenoptera, especially in populations expected to have low allelic diversity at the sex locus.

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