

Diploid males and their triploid offspring in the paper wasp *Polistes dominulus*

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Although the hymenopteran sex-determining mechanism generally results in haploid males and diploid females, diploid males can be produced via homozygosity at the sex-determining locus. Diploid males have low fitness because they are effectively sterile or produce presumably sterile triploid offspring. Previously, triploid females were observed in three species of North American *Polistes* paper wasps, and this was interpreted as indirect evidence of diploid males. Here we report what is, to our knowledge, the first direct evidence: four of five early male-producing *Polistes dominulus* nests from three populations contained diploid males. Because haploid males were also found, however, the adaptive value of early males cannot be ignored. Using genetic and morphological data from triploid females, we also present evidence that both diploid males and triploid females remain undetected throughout the colony cycle. Consequently, diploid male production may result in a delayed fitness cost for two generations. This phenomenon is particularly relevant for introduced populations with few alleles at the sex-determining locus, but cannot be ignored in native populations without supporting genetic data. Future research using paper wasp populations to test theories of social evolution should explicitly consider the potential impacts of diploid males.

Keywords: hymenoptera; sex determination; sex investment ratio; reproductive skew

1. INTRODUCTION

In the insect order Hymenoptera, fertilized eggs usually develop into diploid females and unfertilized eggs develop into haploid males. However, because of the single-locus complementary sex determination mechanism ancestral in this order (Crozier 1977; Periquet *et al.* 1993; Crozier & Pamilo 1996), diploid males are occasionally produced via homozygosity at the sex-determining locus. If a female's mate shares one of her alleles at this locus, their diploid offspring will be 50% female and 50% male.

Diploid males are considered 'reproductive dead-ends' for at least two reasons. First, they are a drain on colony resources because they perform no labour

and are produced at the expense of female workers. Second, diploid males are often functionally sterile, producing unreduced diploid sperm that result in sterile triploid offspring if fertilization occurs (Whiting 1961; Naito & Suzuki 1991; Ayabe *et al.* 2004; but see Cowan & Stahlhut 2004).

Diploid males have been reported in over 39 hymenopteran species (Crozier & Pamilo 1996), but rarely in social wasps. This is surprising given that social wasps, especially *Polistes* species, serve as model systems for social evolution research (Reeve 1991; Gamboa 1996; Queller *et al.* 2000). Diploid males have been observed directly in only one population of a single *Polistes* species, *P. chinensis antennalis* (Tsuchida *et al.* 2002, 2004). The presence of triploid females recently reported in three North American *Polistes* species suggests only indirectly that diploid males are more common than previously thought and are capable of successful mating (Liebert *et al.* 2004). Two critical pieces of evidence would confirm this speculation: direct observation of diploid males and diploid male paternity of triploid offspring.

Additionally, we lack information regarding the behaviour and reproductive success of diploid males and triploid females. In temperate climates, the *Polistes* annual colony cycle consists of four phases (Reeve 1991). In the spring, mated females ('foundresses') build nests alone or in small groups and perform all colony tasks until female workers emerge in early summer. Workers maintain the nest and care for reproductive-destined male and female offspring that emerge in late summer/early autumn. These offspring eventually leave the nest to mate and mated females enter winter diapause until spring, when the cycle repeats. Triploid females have been collected among spring foundresses (Liebert *et al.* 2004), which demonstrates that they are able to survive winter diapause. However, we do not know whether triploids mate or lay eggs. If triploids are sterile, their best nesting option would be to aid relatives.

Here we report the first direct observation, to our knowledge, of diploid males in *Polistes dominulus*. Using a combination of genetic and morphological data, we also present evidence that diploid males mate and produce triploid female offspring, and that despite their probable sterility, these triploid females mate, overwinter and join spring nesting associations.

2. MATERIALS AND METHODS

(a) Diploid males

We collected 21 pre-emergence nests in 2004 from three distinct populations (separated by at least 20 km), in MA, USA (table 1). Nests were kept in the laboratory at Tufts University where we monitored the emergence of early offspring. Because a matched mating at the sex locus will produce diploid offspring in a 1 : 1 sex ratio, we focused on nests that produced males and females in similar numbers. Using five such nests, with at least one nest representing each of the three collection sites, we genotyped 28 males at four microsatellite loci. Genotyping protocols were the same as in previous studies (Johnson & Starks 2004; see Electronic Appendix). A male was considered diploid if at least one locus had two distinct alleles. Males with only one allele at four loci were genotyped at three additional loci, to help distinguish haploidy from homozygosity.

(b) Triploid foundresses and offspring

In 2003, we collected 18 pre-emergence multiple-foundress nests from Carlisle, MA. We genotyped all 51 foundresses and 291