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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

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Table 1. Sex ratio of early emerging brood and male genotyping data for nests collected in June–July 2004. (Includes only those nests that produced five or more early offspring. Bold type indicates nests from which males were genotyped.)

nest	sex ratio (M : F)	no. males genotyped	no. (%) of diploid males
site 1: Derby Farm (DF), Stow, MA			
DF1	**4 : 4**	**4**	**4 (100%)**
DF2	2 : 3	0	n/a
DF3	0 : 7	n/a	n/a
DF4	**7 : 2**	**5**	**5 (100%)**
site 2: Tufts University (TU), Medford, MA			
TU1	**14 : 14**	**8**	**4 (50%)**
site 3: Great Brook Farm State Park (GB), Carlisle, MA			
GB1	**6 : 4**	**4**	**0 (0%)**
GB2	0 : 18	n/a	n/a
GB3	15 : 6	0	n/a
GB4	6 : 9	0	n/a
GB5	**7 : 18**	**7**	**7 (100%)**
totals		**28**	**20 (71.4%)**

offspring (larval instars 2–5 and pupae from 13 nests) at a minimum of six loci (see Electronic Appendix). Triploidy was determined by the presence of three bands at two or more loci (Liebert *et al.* 2004; figure 1). We dissected foundresses' reproductive tracts to assess ovarian development and mating status (Chandrashekara & Gadagkar 1991).

3. RESULTS

(a) *Diploid males*

Four of five nests—and all three populations—represented in the sample contained diploid males. Of 28 males genotyped, 20 (71.4%) were diploid (table 1). The remaining eight males, from two different nests, had only one allele at seven loci and thus were probably haploid (see Electronic Appendix). Three nests had 100% male diploidy, one contained only haploid males and one had 50% haploid males (table 1).

(b) *Triploids*

We found triploids on three nests (table 2): 1 (7.7%) out of 13 nests with genotyped brood contained triploid offspring (figure 1) and triploid females were among the cooperating foundresses on 2 (11.1%) out of 18 nests with genotyped adults. These triploid foundresses were unrelated and thus originated from two different matrilines.

Triploid offspring genotypes were consistent with being produced by one collected foundress mated to a diploid male (figure 1). At each locus, all offspring genotypes contained: (i) one allele that was also present in the foundress, and (ii) the same one or two additional alleles presumably contributed by the male's diploid sperm. The two foundresses on this nest were unrelated, allowing unambiguous maternity assignment.

Both triploid foundresses were mated and their ovaries contained yellow bodies, indicating previously laid eggs. However, neither foundress possessed more than one mature oocyte and one foundress had only four instead of six ovarioles. Both triploid foundresses' genotypes were incompatible with having produced offspring in their respective colonies or with being related to their cofoundresses.

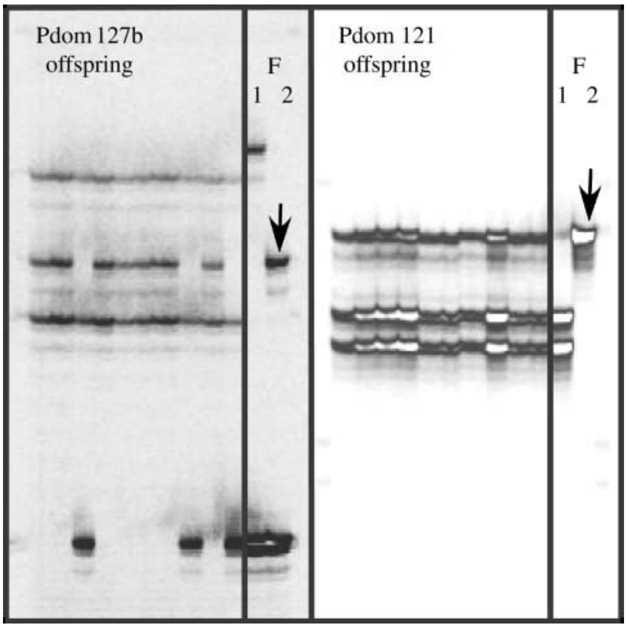

Figure 1. Microsatellite genotypes of triploid offspring and diploid foundresses from *P. dominulus* nest Z. The panels show two loci (Pdom127b and Pdom121), each with offspring genotypes on the left and the two foundress (F) genotypes on the right. An arrow points to the foundress whose genotype at all loci was consistent with having produced the triploid offspring.

4. DISCUSSION

We have presented the first direct evidence, to our knowledge, for diploid males in *P. dominulus* and the first evidence that these males are capable of mating and producing triploid offspring via unreduced diploid sperm. Our data also show that a female's failure to recognize a diploid mate leads to a cascade of costly recognition errors. For example, a triploid-producing foundress was able to monopolize reproduction on a nest with an unrelated cofoundress, and the presence of triploid pupae shows that neither foundress recognized and removed triploid brood. Both triploid foundresses had mated; thus their mates also may have been unable to recognize the females' triploid status. Although helping kin would have provided these foundresses with their only possible fitness benefit, they joined non-relatives. At this point, the 'dead-end' originating with the diploid male is finally reached 2 years later.

These results have special meaning because *P. dominulus* is a very successful species in both its native and introduced ranges and has been actively studied in Europe since the 1940s (e.g. Pardi 1948). Despite its recent introduction (1978), *P. dominulus* has been the subject of two decades of behavioural research in North America. However, our data may prove problematic for current methodology, in which behaviour studies are conducted without regard to population genetics. Such studies are common in *Polistes* wasps, which are often used to develop and

Table 2. Morphological and offspring production data for adult females on three nests collected from Carlisle, MA, USA in 2003. (Bold type indicates triploid status. All others are diploid.)

nest	foundress no.	head width (mm)	wet weight (g)	total no. of oocytes	no. of mature oocytes	no. own offspring	total no. of colony offspring genotyped
X	1	3.4	0.11	—	—	0	**28**
	2	3.4	0.13	—	—	**28**	**28**
Y	1	3.3	0.14	66	5	26	26
	2	3.6	0.14	7	1	0	26
	3	3.4	0.13	60	8	0	26
Z	**1**	3.6	0.14	16	0	0	26
	2	3.4	0.09	36	0	0	26
	3[a]	—	—	—	—	26	26

[a] This female was not collected, but we were able to reconstruct the maternal genotype based on the offspring genotypes.

test theories of social behaviour such as sex investment ratios and reproductive skew models (e.g. Nonacs 2002). Without genetic data to identify factors such as diploid male and triploid female production, we risk interpreting these studies erroneously.

Investigation of sex investment ratios is complicated by the consideration of diploid males (Vargo 1996). For example, previous studies have suggested that early males served an adaptive function as potential mates for early emerging female offspring (Kasuya 1981; Strassmann 1981; Suzuki 1985, 1997; Page *et al.* 1989). If early males are diploid, they may not be able to mate or will produce sterile offspring. Previous authors found 95% diploidy among early males in one population of *P. chinensis antennalis* (Tsuchida *et al.* 2002, 2004) and concluded that the reproductive success of early males could be ignored in that species. Our results demonstrate that some diploid male *P. dominulus* may also be an unintended consequence of the sex-determining mechanism; however, two out of the five nests in this study contained haploid males. Consequently, we cannot make assumptions about the ploidy of early males in this species without collecting relevant genetic data.

Another example demonstrating the necessity of testing assumptions relates to reproductive skew models, which make predictions about the division of reproduction within a group (reviewed in Johnstone 2000). These models assume that all group members are capable of independent reproduction, but triploid females clearly do not have the same options for solitary nesting as their diploid cofoundresses and, therefore, cannot be equal partners in a 'social contract' (Reeve & Nonacs 1992). Our finding of triploid foundresses among 11.1% of cooperative nesting associations suggests that this possibility cannot be ignored.

Genetic data are particularly important for studies of introduced or fragmented populations, which are likely to have fewer alleles at the sex-determining locus and thus relatively high rates of diploid male production. Indeed, this phenomenon has been suggested as an indicator of pollinator decline (Zayed *et al.* 2003). High diploid male frequency may, in turn, result in strong selective pressure for increased fertility of diploid males as recently described for a solitary vespid wasp (Cowan & Stahlhut 2004). Introduced species provide a tremendous opportunity to study such evolutionary processes in action (Lee 2002) and this can best be accomplished by integrating population genetics with behavioural and life-history data.

Although high rates of diploid male production are expected in bottlenecked populations, our evidence suggests that this phenomenon also occurs when population genetic diversity is not severely reduced. Our data were collected near the site of the oldest known *P. dominulus* introduction in Cambridge, MA in the late 1970s (Eickwort 1978; Hathaway 1981). Recent evidence suggests that this population has a high level of genetic diversity and has not experienced a genetic bottleneck (Johnson & Starks 2004). It is possible that allelic diversity has been reduced at the sex locus but not at the tested microsatellite loci, but the successful establishment of this species across the USA suggests that a high level of inbreeding is unlikely. In combination with the previous report of 4.7% triploidy among foundresses in a native population of *Polistes fuscatus* (Liebert *et al.* 2004), our results demonstrate that this phenomenon is not limited to introduced or bottlenecked populations. Integration of genetic data with behavioural research on *Polistes* wasps will help us to examine the impact of diploid males and triploid females in this model genus.

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