

Review article

Triploid females and diploid males: underreported phenomena in *Polistes* wasps?

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Summary. In hymenopteran species, males are usually haploid and females diploid. However, in species that have complementary sex determination (CSD), diploid males arise when a female produces offspring that are homozygous at the sex-determining locus. Although diploid males are often sterile, in some species they have been shown to produce diploid sperm, thus producing triploid daughters if they mate successfully. Diploid males have been observed in very few species of social wasps, and we know of no published reports of triploid females. In this paper, we review the existing literature on diploid males and triploid females in the Hymenoptera, and report the observation of triploid females in three species of *Polistes* paper wasps. Although polyploid offspring may be produced parthenogenetically, the more likely scenario is that *Polistes* wasps have CSD and produce diploid males via homozygosity at the sex-determining locus. Therefore, female triploidy indicates that diploid males do exist in *Polistes* species where they are presumed to be absent, and are likely to be even more frequent among species that have experienced a genetic bottleneck. We conclude by cautioning against the assumption of a selective advantage to the production of early males, and by discussing the implications of male diploidy and female triploidy for measurement of sex ratio investment and assumptions of reproductive skew theory.

Key words: *Polistes*, triploid females, diploid males, sterility, subfertility hypothesis, genetic bottleneck.

Introduction: sex determination in Hymenoptera

The genetic system of the Hymenoptera is arrhenotoky, in which haploid males develop from unfertilized eggs and diploid females result from fertilized eggs. The actual method of sex determination in these haplo-diploids is most commonly the single locus, multiple allele method of complementary sex determination (CSD) suggested by P.W. Whiting

(1933; 1943) and thought to be ancestral in Hymenoptera (Crozier, 1977; Crozier and Pamilo, 1996; Periquet et al., 1993). In this system, a heterozygote at the sex-determining locus is female, whereas a homozygote or hemizygote is male (Crozier, 1977). Evidence for single locus CSD has been found throughout the Hymenoptera (reviewed by Cook, 1993), including species ranging from the primitive sawflies (*Athalia rosae ruficornis*: Naito and Suzuki, 1991), to hornets (*Vespa crabro*: Foster et al., 2000), fire ants (*Solenopsis invicta*: Ross and Fletcher, 1985) and polistine paper wasps (*Polistes chinensis antennalis*; Tsuchida et al., 2002; Tsuchida et al., 2004). Recently, the sex-determining gene has been identified in the honey bee, *Apis mellifera*; heterozygotes at this locus were clearly shown to develop into females and homozygotes or hemizygotes into males (Beye et al., 2003).

Most hymenopteran males are haploid, but because of the single-locus CSD, diploid males are produced when a fertilized egg is homozygous at the sex-determining locus. Diploid males are often sterile, and therefore are generally considered part of a population's segregational genetic load, which is the reduction in a population's average fitness due to the segregation of alleles into homozygotes that have low fitness. The resources invested in the production of diploid males are effectively wasted because they are unlikely to produce fertile offspring. This waste of resources is even greater in social species where males do not contribute to colony productivity, and diploid males are produced at the expense of female workers. In the honey bee *Apis mellifera*, diploid males are removed as larvae and killed by workers (Drescher and Rothenbuhler, 1964). However, in other species, the diploid males develop into adults and some percentage of these males may mate successfully. For example, diploid male *Bombus terrestris* bumblebees are smaller and die earlier than haploid males, but they can mate and occasionally produce triploid female offspring (Duchateau and Marien, 1995). An introduced polygyne population of *Solenopsis invicta* in Georgia, USA produces a very high rate of diploid males (73–100%),

and these males have been observed to participate in normal summer mating flights (Ross and Fletcher, 1985). In addition, Krieger et al. (1999) found that 2.4% of diploid males in this population were able to produce sperm. They did not verify that this sperm was diploid, but other studies have shown that diploid males of varying species produce unreduced, diploid sperm (Crozier and Pamilo, 1996; Naito and Suzuki, 1991; Whiting, 1961; Yamauchi et al., 2001).

Diploid males have been found in at least 39 species of Hymenoptera (reviewed by Cook, 1993; Crozier and Pamilo, 1996; Periquet et al., 1993), but very few reports exist in social wasps. Under the category of social wasps, the most recent of the three reviews mentioned above lists only two species: the stenogastrine *Liostenogaster flavolineata*, and polistine *Mischocyttarus immarginatus* (both as pers. comm. from J.E. Strassmann; *L. flavolineata* observations published in Strassmann et al., 1994). Since the review by Crozier and Pamilo, diploid males have also been observed in the hornet *Vespa crabro* (Foster et al., 2000), the swarm-founding polis-

tine wasp *Polybioides tabidus* (Henshaw et al., 2002) and the independent founding *Polistes chinensis antennalis* (Tsuchida et al., 2002, 2004).

The apparent rarity of diploid males among polistine wasps may have several mutually compatible explanations. First, if there are many alleles at the sex-determining locus and a low rate of inbreeding, the chance of producing a homozygote at this locus is low. Second, if viability of diploid males is generally low and some develop abnormally (as found in *Bracon hebetor*; Whiting, 1924), nest residents may cannibalize diploid males rather than allow them to fully develop. Third, and perhaps most pertinent to *Polistes* research, many studies have genotyped brood without first identifying the sex of the individuals based on morphology (Table 1). Instead, these studies have identified sex based on genotype; individuals bearing only a single allele at all loci are considered males, and all others females. Clearly, this method would mistakenly categorize diploid male offspring as females.

Table 1. Methodology of *Polistes* wasp studies that genotyped colony offspring. Studies were chosen based on a literature search for studies (or included references) that genotyped *Polistes* wasps, then including only those where brood or emerging adult offspring were genotyped at variable allozyme or microsatellite loci. Sections A–C indicate whether diploid males could have been detected in the sampled offspring

<i>Polistes</i> sp.	Sample	Method of sex identification	Genetic markers	Reference
A. Studies that could not have found diploid males among offspring				
<i>annularis</i>	Eggs, larvae, pupae	All diploids, assumed female	Microsatellites	Queller et al., 1997
	Eggs, larvae, pupae	All assumed female	Microsatellites	Peters et al., 1995
<i>bellicosus</i>	Early and late season larvae	Homozygosity	Microsatellites	Field et al., 1998
<i>carolina</i>	Eggs, larvae, pupae	Homozygosity	Microsatellites	Seppä et al., 2002
	Larvae	Sex not identified	Microsatellites	Strassmann et al., 2000
<i>chinensis antennalis</i>	Emerged workers (all female)	Morphology	Allozymes	Miyano and Hasegawa, 1998
<i>fuscatus</i>	Late season larvae	Homozygosity	Microsatellites	Reeve et al., 2000
B. Studies that could have found diploid males in subset of sampled offspring				
<i>bellicosus, dorsalis</i>	Eggs, larvae, pupae	Homozygosity: eggs, larvae, young pupae; <i>Morphology: old pupae only</i>	Microsatellites	Arevalo et al., 1998
<i>gallicus</i>	Eggs, male pupae, female workers	Homozygosity: eggs <i>Morphology: male pupae, female workers</i>	Microsatellites	Strassmann et al., 2003
C. Studies that could have found diploid males in all sampled offspring.				
<i>apachus</i>	Emerged adults	Morphology ¹	Microsatellites	Liebert, unpublished data
<i>apachus, bellicosus, exclamans</i>	Pupae only (emerged as adults after collection)	Morphology ²	Allozymes	Lester and Selander, 1981
<i>aurifer</i>	Emerged adults	Morphology	Microsatellites	Liebert, 2003
<i>chinensis antennalis</i>	Emerged adults	Morphology	Allozymes	Tsuchida et al., 2002
	Emerged adults	Morphology	Microsatellites	Tsuchida et al., 2004

Bold type indicates studies that did observe diploid males.

¹ This study found only one diploid male.

² Eggs and larvae were specifically excluded from analysis because sex could not be determined.

Table 1 shows 14 studies of ten *Polistes* species in which offspring were genotyped either as immature brood or emerging adults. Of these 14, seven studies could not have found any diploid males among any sampled offspring because of their methodology of identifying sex by the presence of one allele at all loci, or simply assuming all offspring were females. The remaining seven studies identified at least a subset of sampled offspring by morphology, and thus had a chance of observing diploid males. Only three of these reported observing at least one diploid male, (two studies of *P. chinensis antennalis* and one of *P. apachus*). However, three of the four studies that did not report any diploid males identified only male pupae morphologically; eggs and larvae were either not examined or sexed based on genotype. One of these studies specifically cited the inability to sex larvae as the reason for excluding them from analysis (Lester and Selander, 1981). Despite the seemingly large body of research on *Polistes*, it appears that many genetic studies would not have detected diploid males. The fact that three of four studies that intensively genotyped adult male *Polistes* offspring found diploid males suggests that they may be more common than previously assumed.

Triploid females as indicators of diploid males

The presence of triploid females in a population indicates that diploid males are also being produced (Crozier and Pamilo, 1996). This is because diploid male Hymenoptera have been found to produce unreduced, diploid sperm (Crozier and Pamilo, 1996; Naito and Suzuki, 1991; Whiting, 1961; Yamauchi et al., 2001). Thus, if diploid males successfully mate, they will produce triploid daughters. Indeed, triploid females have been reported in a variety of Hymenoptera species (Table 2). Some of these have been produced through laboratory crosses of diploid males and diploid females (e. g., in the parasitoid wasp *Bracon hebetor* (= *Habrobracon juglandis*): Bostian, 1934; Torvik, 1931; Whiting, 1961; honeybee *Apis mellifera*: Chaud-Netto, 1975; and sawfly *Athalia rosae ruficornis*: Naito and Suzuki, 1991). A karyotype analysis of Australian ants by Imai et al. (1977) discovered one triploid *Crematogaster* sp. 2, and one tetraploid *Camponotus* sp. 5. Duchateau and Marien (1995) described the production of triploid bumblebee workers of the species *Bombus terrestris*. Krieger et al. (1999) studied the introduced population of the fire ant *Solenopsis invicta* with the 73–100% rate of male diploidy described above and found 12% triploidy among non-reproductive females. Finally, five triploid workers of the polistine wasp *Ropalidia revolutionalis* were found in one colony (out of 37 colonies and 270 workers genotyped; M. Henshaw, pers. comm.).

Evidence for triploidy in *Polistes*

To our knowledge, there are no published accounts of triploid female *Polistes* wasps, despite the large body of research in which many wasps have been genotyped with highly poly-

Table 2. Species of Hymenoptera in which female triploids have been reported

Taxon	Origin	References
Sawflies		
<i>Athalia rosae ruficornis</i>	Lab	Naito and Suzuki, 1991
Wasps		
Ichneumonidae		
<i>Bracon hebetor</i> (= <i>Habrobracon juglandis</i>)	Lab	Bostian, 1934; Torvik, 1931; Whiting, 1961
<i>Habrobracon</i> "pectinophorae"	Lab	Inaba, 1939, cited in Whiting, 1961
Vespidae		
<i>Ropalidia revolutionalis</i> ¹	Wild	M. Henshaw, personal communication
Ants		
Formicinae		
<i>Solenopsis invicta</i>	Wild	Krieger et al., 1999
<i>Camponotus</i> sp. 5 (tetraploid)	Wild	Imai et al., 1977
Myrmicinae		
<i>Crematogaster</i> sp. 2	Wild	Imai et al., 1977
Bees		
Apinae		
<i>Apis mellifera</i>	Lab	Chaud-Netto, 1975
<i>Bombus terrestris</i>	Lab	Duchateau and Marien, 1995

¹ Unpublished data: Of 270 workers in 37 colonies that were genotyped, five workers from one nest were found to be triploid at 2–3 loci. Genotypes were consistent with being full sisters produced by a diploid mother mated to a diploid male. Samples were not re-extracted for confirmation, but leakage between lanes was ruled out as a cause of a third DNA band.

morphic microsatellite loci. This may be because the phenomenon occurs with very low frequency, but also because researchers may have assumed the result to be due to contamination or genotyping error. We have observed a low rate of female triploidy (2.7% average) in three species of *Polistes* wasps, *P. fuscatus*, *P. dominulus*, and *P. aurifer* that were genotyped during the course of other studies (Table 3). Each of these studies genotyped many unrelated females using between six and 12 polymorphic microsatellite loci. Triploids were identified based on the presence of three alleles at a minimum of two independent loci (example shown in Fig. 1). When the genotypes of these individuals contained two alleles, they also tended to have abnormally asymmetric band intensities compared to heterozygotes from the same set of samples, as described for analysis of monomeric allozymes in triploid fire ants (Krieger et al., 1999). Given the number and variability of the microsatellite loci used in these studies, it is unlikely that we failed to detect triploids due to the presence of two or three copies of the same allele at all loci (i. e., no locus with three distinct alleles). Based on calculations by

Table 3. Populations of three *Polistes* species where triploid females were observed

Species	Year	Location	#3N females (% total)	#typed females	#loci typed	Genotyper
<i>P. fuscatus</i> ¹	1993	Ithaca, NY	3 (4.7)	64	6	Peters, J.M.
	1994	Ithaca, NY	3 (3.5)	86		
<i>P. dominulus</i>	2001	Andover, MA ²	2 (2.5)	79	12	Johnson, R.N.
	2003	Carlisle, MA	2 (3.9)	51	6	Liebert, A.E. and G.T. Switz
	2003	Rochester, MI	1 (2.8)	36	12	Liebert, A.E.
<i>P. aurifer</i>	2000	Calabasas, CA	1 (0.6) ³	164	6	Liebert, A.E.
	2001	Calabasas, CA	1 (0.6) ³	147	6	

¹ Three *P. fuscatus* individuals from 1993 and two from 1995 were not included because they were triploid at only one locus.

² One additional *P. dominulus* from this location was triploid at only one locus.

³ Triploidy could not be confirmed by re-extraction of genomic DNA from these samples due to lack of reserve tissue.

Locus:

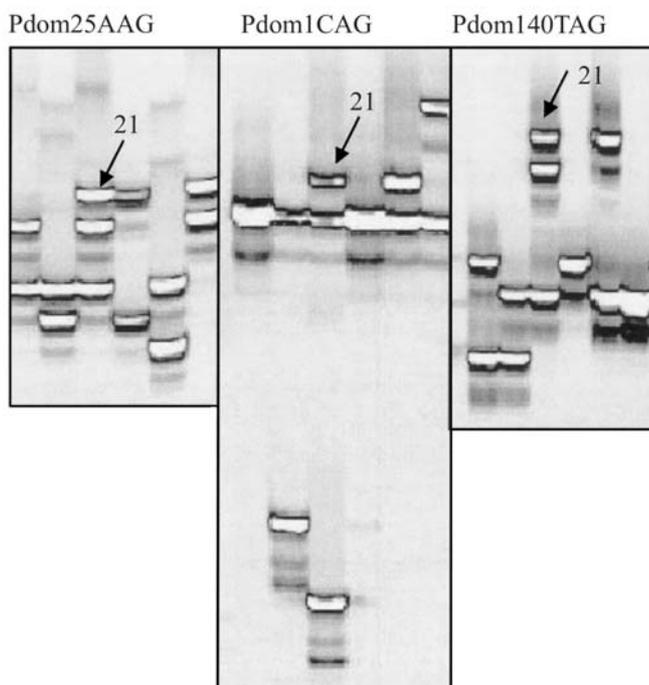


Figure 1. Example of genotypes for one *Polistes dominulus* female (sample #21: Andover, MA, 2001). In contrast with the adjacent samples, sample #21 has three clear bands indicating triploidy at each of the three microsatellite loci shown. Gel images were taken from SAGA^{GT} 2.1 software for microsatellite analysis

Krieger and Keller (1998), the loci used in these studies would have detected between 95–100% of triploids.

One may argue that the presence of three alleles indicates either contamination of extraction samples or an artifact of the methodology used to visualize alleles. Indeed, this assumption may help explain why there are no published accounts of triploid females. Mixing of DNA template from more than one individual or leakage between lanes during gel loading could both result in the presence of three alleles in

one sample. However, neither of these arguments explains the observations reported here.

First, to rule out contamination of DNA extraction solutions used in PCR, extractions were repeated using additional tissue from putative triploid individuals for *P. fuscatus* and *P. dominulus* (Table 3). In all cases, triploid status was confirmed for each locus upon re-extraction from new tissue. Second, the method used for visualizing alleles differed among studies: the *P. fuscatus* study used radioactively-labeled primers and the researchers visualized fragments using autoradiography (as described in Choudhary et al., 1993 and Reeve et al., 1998); the *P. aurifer* study used an automated capillary-method sequencer (CEQTM 2000 Fragment Analysis system, Beckman Coulter, Inc.; Liebert, 2003); and the *P. dominulus* study visualized alleles using a LI-COR automated sequencer with SagaTM GT analysis software. Extra alleles due to leakage between adjacent gel lanes could be ruled out by running the samples again after re-extraction in differing order than the original gel loading. In addition, leakage is not a problem for the capillary sequencer used for the *P. aurifer* study because PCR products are drawn from each sample directly into separate capillaries. The variety of methodology and multiple observers in these studies suggests that the presence of three alleles was not an artifact.

Significance of male diploidy/female triploidy for *Polistes* wasps

Diploid males and triploid females may be produced at generally low rates in *Polistes* wasps, and this may be the reason for the few reports in the literature. However, male diploidy (and thus female triploidy) is expected to increase with increased inbreeding, or a loss of alleles at the sex-determining locus due to a genetic bottleneck (Packer and Owen, 2001). The potential negative effects of this were raised by Stouthamer et al. (1992) concerning the use of inbred laboratory stocks of parasitoid wasps for biocontrol purposes. Loss of genetic diversity has been discussed in relation to male diploidy for populations of a variety of social Hymenoptera as well. In a study of the halictid bee *Lasioglossum*

(*dialictus*) *zephyrum*, Kukuk and May (1990) suggested that a genetic bottleneck may have led to a greater frequency of diploid males in one small, isolated population compared to a larger population. Ross et al. (1993) presented evidence that the high rate of male diploidy in the Georgia population of introduced fire ants is associated with a corresponding loss of alleles due to bottleneck effects. Zayed and Packer (2001) discussed the fragmented habitat of southern and central Florida as a primary cause of diploid male production in the bee *Halictus poeyi*. Similarly, Henshaw et al. (2002) suggested that the observed high rate of male diploidy in *Polybioides tabidus* may have been a result of the small size of the reserve in Africa where the wasps were found. Keller and Passera (1993) did not observe any adult diploid males produced via brother-sister matings of introduced Argentine ants (*Linepithema humile*, previously known as *Iridomyrmex humilis*) in the laboratory. However, it is unknown whether this result was due to a lack of diploid male production or selective elimination of diploid males at the egg or larval stage.

The recent interest in integrating invasion biology, population genetics, and social behavior (Cervo et al., 2000; Hänfling and Kollmann, 2002; Holway and Suarez, 1999; Starks, 2003) may lead to even more studies making this connection in the near future. For example, although we know that at least some of the triploid females discussed here were able to successfully overwinter (they were spring foundresses), it is currently unknown if they could mate or produce viable eggs. We know even less about diploid male success except that *P. chinensis antennalis* workers do not mate with diploid early males (Tsuchida et al., 2002). If diploid male production increases as a result of a genetic bottleneck, this might negatively affect colony productivity for introduced populations such as North American *Polistes dominulus*. Measurements of productivity that do not test for diploid males – such as counting cell number – may therefore lead to inflated results for introduced species. Studies that use colony productivities to predict the establishment and spread of introduced species would clearly need to identify diploid males to avoid overestimating the effect of the insect in question.

In addition to productivity impacts, there may arise a selective advantage to multiple mating for females in bottlenecked populations to reduce the probability of producing a high rate of diploid males. As demonstrated in previous studies of diploid male production in social insects (Page, 1980; Page and Marks, 1982; Pamilo et al., 1994; Tarpay and Page, 2001, 2002), increasing mate number decreases the variance in average brood variability so that the viability of brood is closer to the population average. However, whether selection would favor multiple mating also depends on the exact relationship between the percentage of viable brood and colony fitness. If the shape of this curve is concave, as is probably the case for the honey bee *Apis mellifera* (Tarpay and Page, 2002), selection will favor multiple mating; a convex shape will favor single mating (Page, 1980). *Polistes* colonies with low brood viability due to diploid males would produce a smaller workforce in addition to fewer female reproductives, and therefore fitness of the colony would probably be greatly reduced. However, the exact relationship between percentage

of diploid males and colony fitness is not known, and may vary among populations with varying life history characteristics. Although it is possible that high rates of diploid male production would select for multiple mating in *Polistes*, this would need to be confirmed with empirical measurement of the relationship between brood viability and fitness. CSD in social hymenopterans may therefore present two correlates indicating the degree of genetic bottleneck a species endures upon introduction: the population-level frequencies of diploid males and of multiple mating by females. The former factor is likely to impact conservation issues, and thus is especially important for studies of introduced species.

The presence of diploid males and triploid females may also have important effects on models of social behavior such as the adaptive significance of early male production, sex ratio investment, and reproductive skew theory. The production of early males has been discussed as an adaptation that allows early emerging females to mate and produce offspring if the foundress dies or the early female disperses to pursue independent reproduction (Kasuya, 1981; Page et al., 1989; Strassmann, 1981; Suzuki, 1985, 1997). However, as Tsuchida et al. (2002, 2004) found for *P. chinensis antennalis*, these early males may simply be an accidental result of a foundress that mated with a male sharing one of her alleles at the sex-determining locus. What has been assumed to serve an adaptive function may therefore be a drain on colony resources, as these diploid males are unlikely to produce many offspring and those they produce are likely to be sterile triploids. Researchers working with species that produce early males should genotype these males to determine whether they are haploid or diploid before assuming they act as a source of mates for early emerging females.

Tests of kin selection theory often involve measuring a colony's relative investment in males and females, but the presence of sterile diploid males in a population can complicate tests for worker or queen control over sex ratio investments. In a study of sex investment ratios in the polygyne fire ant *Solenopsis invicta*, Vargo (1996) pointed out that categorization of diploid males for such measurements depends on whether the workers that rear them perceive diploid males as male or female, or even as reproductives. Sex allocation studies of *Polistes* such as the recent work by Tsuchida et al. (2003) that compared male production by queens and workers of *Polistes chinensis antennalis* must take these factors into consideration when deciding how to account for diploid male production. The known occurrence of diploid early males in this species caused the authors to exclude such colonies from their analyses. This precaution would be impossible in other species where the possibility of male diploidy has not been examined.

Detection of diploid males and triploid females is also important for studies that test predictions of reproductive skew models. These models predict the degree of sharing of reproductive benefits among members of a cooperatively breeding group (details of different models reviewed by Johnstone, 2000), and they have been tested most frequently in species of social insects (reviewed by Reeve and Keller, 2001). The application of skew models to *Polistes* species has

generally assumed that all cooperatively nesting females (called foundresses) are physiologically capable of producing offspring. However, this may not be the case if a foundress is triploid and therefore would be unlikely to produce viable offspring. Because our data come from studies that were not designed to measure frequency of triploids, we cannot accurately calculate the frequency of nests with triploid individuals. However, to illustrate the extreme case, consider the 1993 population of *P. fuscatus* from Ithaca, NY with a triploidy rate of approximately 5% (Table 3). If this rate is representative of triploidy in the general population, then one of every ten multiple foundress nests (with a minimum of two foundresses per nest) is likely to contain one triploid female. Given that reproductive skew is calculated by first identifying individual offspring production, any foundress association with one triploid individual would be correctly classified as 'total skew' but misinterpreted within a social contract framework (cf. Reeve and Nonacs, 1992). In addition, if a foundress is capable of producing offspring, but produces diploid males or triploid females, these nonviable offspring clearly cannot carry the same weight as a female producing diploid females or haploid males. As reproductive skew models are our current best hypotheses for social evolution, it is essential that we are more careful to identify diploid males and triploid females in such studies.

Conclusions

The occurrence of triploid females in three different North American *Polistes* species suggests that diploid male production is more widespread in this genus than has been previously reported, and implies that some diploid males are indeed able to produce offspring. The fate of these rare triploids may be hard to track, but it would be very interesting to note whether these females attempt to nest alone, or seek subordinate roles in foundress associations. The "subfertility" hypothesis advanced by West-Eberhard (1969) to explain cooperative nest foundation has been generally dismissed based on evidence of subordinate reproductive abilities (Reeve, 1991); however, it might apply in this specific case where a female is unable to produce offspring successfully due to triploidy. Many *Polistes* nests are found abandoned in their early stages (pers. obs.), and this is usually assumed to be because the foundress has died. However, it is possible that in some of these cases the foundresses have abandoned their nests because they are triploid and unable to produce viable eggs, or they are producing only diploid males from fertilized eggs. These foundresses may improve their fitness by joining a sister rather than attempting independent reproduction.

We hope that this review will stimulate others to report findings of diploid males and triploid females in other *Polistes* species. In addition, we suggest that future studies should identify the sex of offspring morphologically when possible so that we can have a better idea of the frequency of male diploidy. The list of species in which this phenomenon has been observed has been steadily increasing since genetic

techniques have been developed and used to genotype individuals at many polymorphic microsatellite loci. We find it likely that more *Polistes* species will soon join this list.

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