ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

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

by

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Electronic appendices are refereed with the text; however, no attempt is made to impose a uniform editorial style on the electronic appendices.
Supplementary Materials and Methods:

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Microsatellite Genotyping:

All genetic analysis was conducted in the ISIRF genetics laboratory in Dana Hall at Tufts University. Genotyping protocols were the same as those in Johnson & Starks (2004), modified from Walsh et al., 1991, Strassmann et al., 1996, and Crozier 1999. We extracted genomic DNA from leg tissue for adults and pupae, and from head capsules for larvae and prepupae. We first froze samples on dry ice, and then used sterile minipestles to grind tissue into 0.5µl microcentrifuge tubes. We then immediately added 250µl of a 5% Chelex solution and placed the tubes into a water bath at 95ºC for 30-45 minutes, vortexing every 10 minutes. Finally, we centrifuged the samples at 13,000rpm for 5 minutes, diluting the supernatant 1:1 with sterile water for use directly in PCR.

We performed PCR on an Applied BioSystems GeneAmp PCR System 2700 thermocycler in 15μl reactions: 1x reaction buffer (Promega), 25mM MgCl₂, 200μM each dNTP, 300nM each primer, 0.75 units Taq polymerase (Promega). DNA was amplified using IRD800 labeled microsatellite primers developed for Polistes dominulus (Henshaw 2000), including Pdom1CAG, Pdom25AAG, Pdom117AAG, Pdom121AAG, Pdom122AAT, Pdom127bAAT, and Pdom140TAG. Visualization of microsatellite alleles was carried out on 6.5% denaturing polyacrylamide gels, using a LI-COR single channel 4200 NEN Global Edition IR² DNA Analyzer, scored with SAGA² GT 2.1 software.
**Probability of homozygosity vs. haploidy**

For males that had only one allele at four loci, an additional three loci were genotyped to distinguish haploidy from homozygosity. In all cases, these males also had only one allele at these loci. Although it is possible that these males could be diploid homozygotes, this is extremely unlikely. Using allele frequencies from females in the GB population in 2003, the probability of homozygosity at even four loci is very low (P < 0.00006; calculated with the formula $\prod(n_i^2)$, where $n_i$ represents the $i$th allele at locus $n$). Thus we considered these males to be haploid.

**References**


Walsh, S., Metzger, D. A. & Higuchi, R. 1991 Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10, 506-513.