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Science **315**, 206 (2007);
DOI: 10.1126/science.1133388

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Haploid Females in the Parasitic Wasp *Nasonia vitripennis*

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Haplodiploidy, a sex determination mechanism, occurs in rotifers, nematodes, mites, and insects. The order Hymenoptera consists almost entirely of haplodiploid species, in which haploid males develop from unfertilized eggs and diploid females develop from fertilized eggs. In complementary sex determination (CSD), sex is determined by the allelic state of one locus with multiple alleles (1). Females are heterozygous, and males receive a single chromosome set from their mother. Diploid males can result under CSD by inbreeding, but they are mostly infertile (2). The parasitic wasp *Nasonia vitripennis* Walker does not have CSD and inbreeds readily. No cases of haploid females among Hymenoptera have been found previously. We report that haploid females can develop in *N. vitripennis*.

We found a Canadian strain in which ~10% of unfertilized eggs developed into gynandromorphs, that is, individuals with both male and female features. Their frequency was increased by both directional selection (~20%) and high culturing temperature (~40%). Females differ

externally from males in the dark color of antennae and legs, large wings, and presence of an ovipositor. Gynandromorphs show an anterior-posterior gradient of feminization ranging from individuals with only female antennae to individuals with complete female morphology (parthenogenetic females). Tetracycline used to remove *Wolbachia* (and other bacterial symbionts) did not affect gynandromorph frequency.

Ploidy level of gynandromorphs and parthenogenetic females was determined in 459 eggs at 4, 8, 12, and 26 hours of development and in brain tissue of 99 4-day-old larvae. No diploids were found, although 40% were expected on the basis of emerging control wasps. Rare diploid cells (<1%) were observed at frequencies similar to those of normal haploid males. Flow cytometric analysis showed that all gynandromorphic individuals and parthenogenetic females contained the haploid amount of DNA (Fig. 1). The diploid *Nasonia* genome was estimated at 312 Mb, consistent with previous reports (3) and confirming that parthenogenetic

females are haploid and do not arise by chromosome duplication in unfertilized eggs.

Parthenogenetic females have low fertility; only 2 of 281 females produced offspring (one son and two diapause larvae, respectively). Normal diploid females ($n = 24$) have four ovarioles on either abdominal side. Ovariole number in parthenogenetic females ($n = 17$) varies, averaging 3.6 (range from 1 to 5) on either side. Diploid females carried on average 28.8 ± 1.8 SE eggs upon emergence (Fig. 1B), whereas haploid females had only 4.2 ± 0.7 SE eggs (Fig. 1D). Oogenesis proceeds normally in haploid females. However, instead of arresting in first prophase, as in diploid females, mature eggs in haploid females consistently showed five condensed chromosomes that appeared to have proceeded further into the meiotic cycle (Fig. 1, B and D), which may account for the sterility of haploid females. Gynandromorphs with a male abdomen had normal testes (Fig. 1, A and C) and live sperm.

Previously only a mite species had been reported to produce haploid females among the higher metazoans (4). Parthenogenetic *Nasonia* females have severely reduced fecundity and fertility. In contrast, diploid *Nasonia* males are fully fertile and produce diploid sperm mitotically, indicating that ploidy is more important for female than male germline development. These findings argue against female development depending on paternally inherited chromosomes, but are consistent (along with other data) with a maternal imprinting model for *Nasonia* sex determination (5).

References and Notes

1. M. Beye, M. Hasselmann, M. K. Fondrk, R. E. Page, S. W. Omholt, *Cell* **114**, 419 (2003).
2. J. M. Cook, R. H. Crozier, *Trends Ecol. Evol.* **10**, 281 (1995).
3. *Nasonia* Genome Project, www.rochester.edu/College/BIO/labs/WerrenLab/nasonia/genomeprojectindex.html (2004).
4. A. R. Weeks, F. Marec, J. A. J. Breeuwer, *Science* **292**, 2479 (2001).
5. L. W. Beukeboom, A. Kamping, *Genetics* **172**, 981 (2006).
6. Supported by a Pioneer grant of the Netherlands Science Organization. We thank J. P. M. Camacho, I. Mateo Leach, and G. Mesander for help with flow cytometry; G. Pomp for the figure; and L. van de Zande for comments on the manuscript. J.H.W. thanks NSF and NIH for funding support (grant nos. DEB-9981634 and 5 R01 GM070026-02).

Supporting Online Material

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Materials and Methods
Fig. S1

2 August 2006; accepted 27 October 2006
10.1126/science.1133388

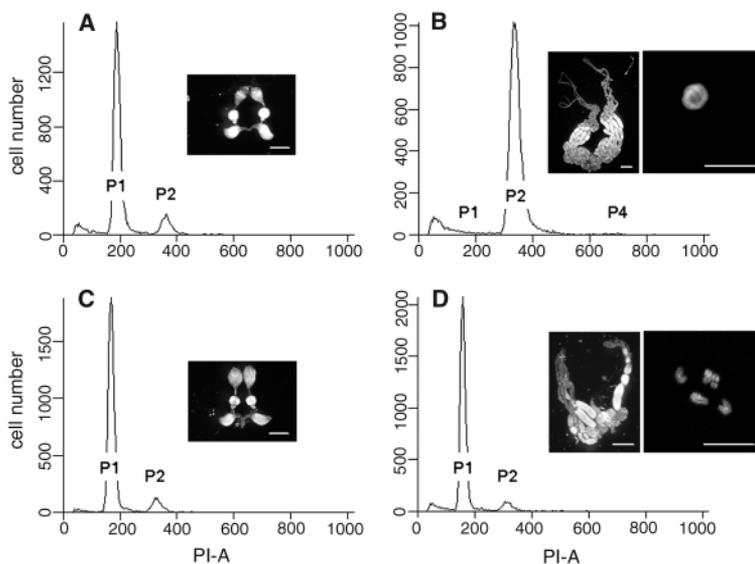


Fig. 1. Flow cytometry and photographs of ovaries and testes of *Nasonia*. (A) Normal haploid male. (B) Normal diploid female. (C) Gynandromorph with female antennae and wings but male abdomen. (D) Parthenogenetic female. Peak P1 corresponds to haploid, P2 to diploid, and P4 to tetraploid cells. Some endopolyploidization is evident in males (P2) and females (P4). PI-A indicates propidium iodide area as a measure for DNA content. Photo inserts in (A) and (C) are testes; for (B) and (D), ovaries and meiosis in eggs. Scale bars indicate 150 μ m in testes and ovaries and 40 μ m in eggs.

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