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Supporting Online Material for

Haploid Females in the Parasitic Wasp *Nasonia vitripennis*

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Materials and Methods
Fig. S1

Supporting Online Material

Material and Methods

Model

Material and Methods

Nasonia are parasitoid wasps of cycloraphous flies and are cultured on *Sarcophaga* pupae. Females typically lay 20-30 eggs per host and generation time is 14 days at 25C (eggs hatch after 1 day, larval stages last from day 2-5 and pupal stages from day 6-14). Virgin females are obtained by opening parasitized hosts and separating male and female pupae. Due to haplodiploidy unmated females normally produce all-male offspring.

Virgin females were individually given two hosts for oviposition.

Development of embryos at 31C greatly increased the probability to obtain gynandromorphs. Four categories of gynandromorphs were distinguished with an increasing degree of feminization, (i) only female antennae, (ii) female antennae and wings, (iii) female antennae, wings and legs, and (iv) complete female external morphology.

For cytology of embryos, individual females were allowed to oviposit at 31C during a 4h time window to restrict developmental variation between embryos. Eggs and embryos were dissected from the fly hosts and fixed in a 3 parts ethanol and 1 part glacial acetic acid mixture. After removal of superfluous fixative they were covered with a drop of 4,6-diamino-2-phenylindole (DAPI, Molecular Probes, Carlsbad, California, USA) on an microscope slide, squashed after 24h under an object glass and examined under a Zeiss (Oberkochen, Germany) Axiophot fluorescent microscope. DNA in ovaries was stained with OliGreen from Molecular

Probes (Carlsbad, California, USA). Haploid and diploid cells were scored by counting the number of chromosomes ($n=5$). Larval brain ganglia were dissected and treated similarly. A subset of broods was allowed to develop into adults for obtaining an estimate of the expected proportion of gynandromorphs among each batch of embryos examined.

For flow cytometry, adult wasps were homogenized in Galbraith buffer (21mM MgCl₂, 30 mM tri-Na citrate dihydrate, 20mM MOPS, 0.1% Triton X-100, 1mg/l RNase, pH 7.2), filtered (50µm), stained overnight in propidium iodide (Sigma, St. Louis, Missouri, USA) and loaded on a LFR II flow cytometer (BD BioSciences, Franklin Lakes, New Jersey, USA). Ovaries and testes from freshly killed wasps were dissected in PBS buffer (1.1g/l Na₂HPO₄, 0.3g/l NaH₂PO₄, 9g/l NaCl, pH 7.2) by gently pulling the abdomen tip with a pair of forceps.

Figure S1. A model for sex determination in *Nasonia*. A maternal sex determining gene (*msd*) imprints a zygotic sex determining gene (*zsd*) into a masculinizing state. Unfertilized haploid eggs with only a maternal *zsd* develop into males. A paternally inherited copy of *zsd* is not (or differently) imprinted. Fertilized haploid eggs with both a maternal and paternal *zsd* become females. Uniparental haploid females are considered resulting from incomplete imprinting, either due to a mutation in the *msd* or *zsd* gene.

msd = masculinizing *maternal* sex *determiner*

zsd = feminizing *zygotic* sex *determiner*

* = gynandromorphic mutant