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Paternal inheritance of a daughterless sex ratio factor

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Here we report an unusual case of extrachromosomal inheritance in the parasitic wasp, Nasonia vitripennis. It was accidentally discovered during attempts to select for genetic variability in the sex ratio produced by females of this wasp. The trait, henceforth termed `Daughterless' (DI), is transferred paternally and causes the mates of carrier males to produce only sons. The effect is not due to mortality of female offspring but rather to an increase in the number of male offspring. DI can be shown to be extrachromosomally inherited by experimental use of the haplodiploid sex determination of this wasp. After introduction at low frequency, the trait increases to predominance in an experimental population within a few generations. The DI trait is of theoretical interest because of its paternal inheritance, and may have practical applications as a biological control agent in pest organisms with haplodiploid sex determination.

N. vitripennis is a small chalcidoid wasp which parasitizes the pupae of various fly species. Typically, 20-40 eggs are laid on to a fleshfly *(Sarcophaga bullata)* host and only 5-15% of these are males. Development from egg to adult takes 14 days at 24'C. Males and females can be easily sexed at the pupal stage and then isolated for controlled matings. As in other hymenoptera, *N. vitripennis* has haplodiploid sex determination; unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop into females. This mode of sex determination gives N. *vitripennis* control over the sex ratio among offspring'".

Originally, an experiment was designed to select for genetic variability in the sex ratio produced by females of the wasp. Ten virgin males and females were collected from each of 10 different wild-type stocks of *N. vitripennis* and mated randomly. Five of the stocks had been collected from nature within the previous 2 months. The following regime was used for each generation. Females were isolated singly on hosts for 24 h, then after -12 days the hosts were opened, sex ratios recorded and wasp pupae removed. For each generation, 400 female and 200 male pupae were selected and placed in a quart container where eclosion and mating occurred. Females (100-150) were then removed from this container and isolated singly on hosts for 24 h to begin the next generation.

For the first two generations, pupae were selected randomly from all the broods for the next generation. This allowed for repeated interbreeding between the original cultures. On the third generation, `high' and `low' sex ratio selection lines were begun. For the high (high proportion of males) line, half of the broods with the highest sex ratios was used to select the 400 female and 200 male pupae for the next generation. For the low line, we used half of the broods with the lowest sex ratios. For the first selection generation, progeny for high and low lines were taken from the mixing population. In subsequent generations, they were genetically isolated from one another (see Fig. 1), with selection occurring in each generation.

It was expected that if there was heritable variability in the sex ratio, then the high line would evolve a high proportion of sons. As the normal sex ratio of the wasp was very low (5-15 % sons)[2], little change in the low line was expected.

Results are shown in Fig. 1. There was a dramatic increase in the sex ratio of the high line, due to the production of all-male broods. By the fourth selection generation there were so few females in the high line that selection had to be relaxed. The

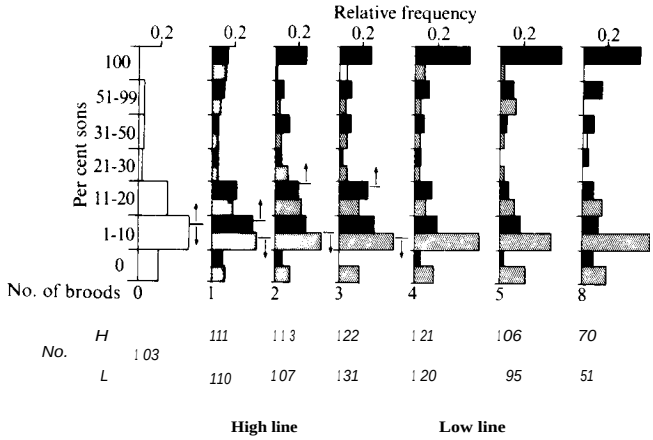

Fig. 1 Selection experiment for high and low brood sex ratios in *N. vitripennis.* The cut-off sex ratios for progeny selected for the next generation are indicated by arrows. Within two generations there was a dramatic increase in the frequency of all-male broods in the high line. The all-male trait persisted in the population after selection was relaxed in the fourth generation.

production of all-male broods persisted even in the absence of selection, remaining at a frequency of 0.40-0.65 until the ninth generation, when the line was terminated. The occurrence of all-male broods was not due to differential mortality of female offspring. For example, in generation 5 the mean number of progeny' in high line all-male broods (25.9 ± 7.8(s.d.), $n = 54$) was not significantly different from high line mixed-sex ratio (26.9 ± 6.0, n = 67; t test, P > 0.4) or low line (27.4 ± 8.2, n = 120; P>0.35) broods. The sex ratio bias is caused by the production of male offspring instead of female offspring.

A series of crosses were done to determine whether the all-male trait was a characteristic of high line males or high line females. High line males and females were crossed to either low line or scarlet-eye (ScDr) stocks'. The all-male brood trait was expressed in crosses involving high line males, indicating that they were the carriers of the trait (see Table 1). This result was rather surprising because haploid males transfer no chromosomal genes to their all-male broods (due to haplodiploidy). Thus, the males are effectively sterile, and it is difficult to select for chromosomal genes for male sterility in a population experiment. Furthermore, such genes should not have persisted once selection was relaxed.

The Dl trait was shown to be inherited extrachromosomally by testing all-male brood progeny from the high line male x ScDr female crosses. As expected, male progeny from the crosses were ScDr in genotype, indicating that an unusual mechanism such as the replacement of the female (ScDr) pronucleus by the high line male pronucleus was not occurring. Therefore, the males did not receive chromosomal genes from

Table 1 Crosses between the high line, low line and ScDr demonstrate that the all-male (Dl) trait is a characteristic of high line males

Cross *d x 4*	*No.* of broods All-male	Mixed
High x low	33	10
High x high	22	13
High x ScDr	52	23.
Total	107	46
Low *x low*	0	31
Low x high	3	20
Low x ScDr	3	63
Total	6	114
ScDr x low	0	29
ScDr x high	2	53
ScDr x ScDr	2	28
Total	4	110

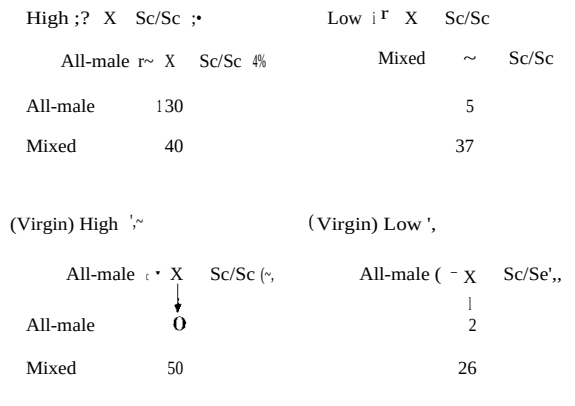

Fig. 2 A test for the inheritance of the high line DI trait demonstrates that it is transmitted from high line males to the male progeny of their mates. High line females do not transmit the trait to their male progeny. Sc/Sc indicates ScDr genotype females.

the high line. However, 76% of the males received the high line DI trait, producing all-male broods when mated with ScDr females (Fig. 2). By definition, therefore, the DI trait is extrachromosomally inherited through the paternal line. Figure 2 also shows a simultaneous test of virgin high line females to determine whether DI is transmitted to their male offspring. This test revealed no maternal transmission, although it is possible that there was low-level maternal transmission. DI is only the second known example of extrachromosomal inheritance primarily through the male line[5].

Crosses to three different wild-type stocks have substantiated the paternal extrachromosomal inheritance of DI. It is transferred to each successive generation, with ~75% of the males in any all-male brood inheriting the trait. DI males readily mate; they have (normal) haploid spermatagonia and produce motile sperm which are transferred to the female spermatheca during copulation.

To determine whether the trait would increase in the absence of selection, DI carrier males were introduced into an experimental population at a frequency of 0.05. The same design was used as in the previous population experiment, but without selection for brood sex ratio. The DI trait increased to a frequency of 0.63 in two generations and remained at about that level for two more generations, until the line was terminated. Thus the trait spread rapidly through a randomly mating population in the absence of any experimentally induced selection.

The causative agent of the DI trait is unknown. DI could be a cytoplasmic factor located within the spermatozoa, which interferes with incorporation of the male pronucleus after fertilization has occurred. However, transmission electron microscopy has so far revealed no unusual cytoplasmic particles associated with spermiogenesis in carrier males. Alternatively, Dl may be an extracellular infectious agent which is transferred to the female reproductive tract during copulation. There it could interfere with the fertilization mechanism and be transmitted to ova passing down the reproductive tract. These possibilities are being investigated.

In many organisms, virus-, rickettsia- and spirochaete-like factors are known to be cytoplasmically transmitted through the maternal line[4-1]. Some of these cause a female-biased sex ratio, either by mortality of male offspring[11] or by affecting sex determination[12]. Population genetic theory predicts that maternally transmitted factors which bias sex ratio towards female offspring will increase in frequency in many circumstances[5,6]. Similarly, paternally transmitted factors should bias the sex ratio towards male offspring. Dl is the first paternally transmitted extrachromosomal factor discovered which causes a sex ratio bias, and it is consistent with theory. By producing all-male broods, a DI factor increases its frequency of paternal transmittance and will spread rapidly through an outbreeding population, as shown experimentally. The demic population structure of N. *ritripennis* in nature probably limits the increase of DI in this species[2]. For example, some host pupae are highly dispersed such that the (flightless) males can only mate with sisters. A Dl factor producing only males is eliminated in these broods because the males have no mates.

Haplodiploid sex determination presents unique opportunities for paternal extrachromosomal inheritance. In diploid organisms, a paternal extrachromosomal factor which causes effective male sterility will not be transmitted to the progeny because there will be few, if any, viable progeny to inherit the trait. In contrast, females of many haplodiploid organisms who mate with effectively sterile males can still produce (haploid) male progeny. This presents an 'evolutionary opportunity' for paternal transmission. We therefore predict that DI factors will be found in many haplodiploid species. Evidence supporting this is the occurrence of colony extinctions due to the production of all-male broods in laboratory rearing of parasitic wasps[13]. Such occurrences may be due to the form of genetic sex determination[14], but DI factors may also be responsible. Whether DI occurs in a particular species will depend on the population structure of that species, its normal sex ratio and the nature of transmission of the factor.

A DI factor introduced into a pest haplodiploid species may persist for many generations and could drive the population to extinction or maintain it at low levels. Thus DI factors may be of value in controlling haplodiploid pests such as sawflies, seed chalcids and certain phytophagous mites.

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