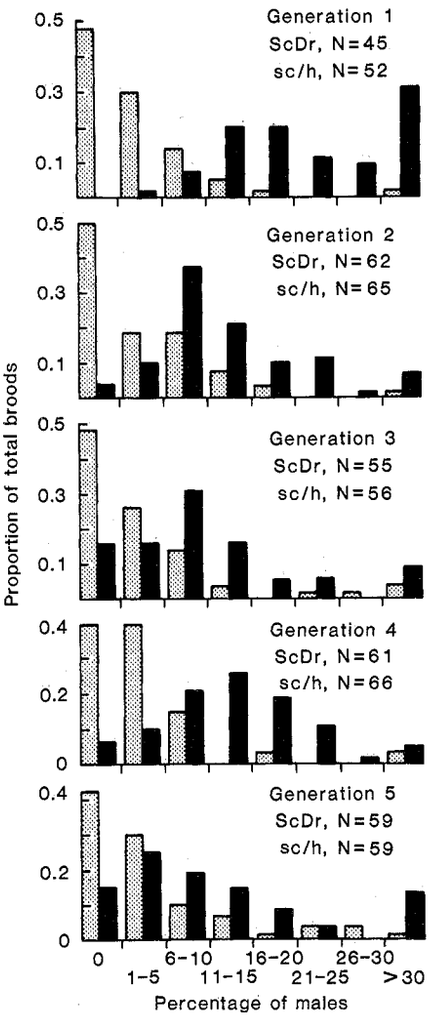

Fig. 2. Results of five generations of backcrossing females from two separate lines to sc/h males (see text for details). Stippled bars represent females with an sc/h maternal background (parental cross for generation 1 females was ScDr males × sc/h females). Solid bars represent females with an ScDr background (parental cross was sc/h males × ScDr females).

of ScDr and sc/h genes (50 percent each); they differed in maternal (cytoplasmic) background. Backcrossing to sc/h males increased the proportion of sc/h genes by roughly one-half with each generation, to ~ 97 percent sc/h in the fifth generation (*12*). In each generation, females were drawn at random from the ten broods in each line with the lowest sex ratios—thus biasing selection in favor of any genes for low sex ratio. If the trait is inherited through nuclear genes, this should result in both lines producing the low sex ratio within several generations, regardless of the genetic details. However, if the trait is maternally inherited, only line 1 should exhibit the trait, because only line 1 females have the sc/h maternal background.

Line 1 females exhibited the low sex ratio trait in all generations, whereas line 2 females never did (Fig. 2). Within each generation, the sex ratios of the two lines differed significantly ($P < .001$, Kruskal-Wallis one-way analysis of variance, d.f. = 1). Within each line, there were no significant shifts in sex ratio among generations, with the sole exception of generation 1 to generation 2 for line 2 (*13*). These data strongly suggest that the low sex ratio trait is maternally inherited and hence is extrachromosomal in nature.

Similar factors are known or suspected in a few other organisms (*14, 15*). Unlike autosomal genes, which are inherited through both sexes, extrachromosomal factors ("genes") are generally inherited through one sex only. Such extrachromosomal factors experience intrinsic selection to skew the sex ratio toward the sex through which they are inherited, whereas autosomal genes do not. This results in "conflict" over the sex ratio between extrachromosomal and autosomal factors (*14*). How the extrachromosomal factor in sc/h females causes the sex ratio skew is unknown. In some *Drosophila* species, a spiroplasma and an associated virus skew the sex ratio toward females through mortality of male offspring (*15*). Although the observed low number of sc/h males could be due to mortality, preliminary data suggest that this may not be the case. Virgin ScDr and sc/h females, which produce only males, were isolated with single hosts for 24 hours. After the females were removed, the hosts were opened, and all eggs were counted. Ten days later, the hosts were reexamined, and surviving offspring were counted. Twenty virgin ScDr females produced 487 eggs, 391 of which developed to pupae (80.3 percent); 28 virgin sc/h females produced 739 eggs, 577 of which developed (78.1 percent) (*16*). This indicates that the low sex ratios observed were not due to excess mortality of sc/h males.

Fertilization of eggs is required to produce female offspring in *Nasonia*; it occurs just before oviposition (*17*). It is possible that the sex ratio factor alters the reproductive morphology of sc/h females to prevent control over sperm access to eggs. Alternatively, the factor may actually mediate the behavior of sc/h females.

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6. The ScDr strain carries a mutant allele (*st*) with a scarlet eye-color phenotype. See G. Saul, S. W. Saul, S. Becker [*Genetics* **57**, 369 (1967)] for a review of *Nasonia* genetics including the *st* allele.
7. Stocks are maintained by allowing 15 inseminated females to parasitize 10 cm^3 of *Sarcophaga* pupae until the females die. Generation time at 27°C is 2 weeks. Hosts are obtained from Carolina Biological Supply Company.
8. Two replicates were performed; data are combined since there were no significant differences between replicates. *Sarcophaga bullata* were used as hosts in all experiments.
9. The mean number of offspring [± standard deviation (S.D.)] was 27.1 ± 11.1 per ScDr female ($N = 81$) and 24.4 ± 8.9 per sc/h female ($N = 83$) (Student's $t = 1.71$; $.1 > P > .05$).
10. The mean sex ratio of sc/h females was constant (~ 3 percent male) whereas that for ScDr females altered (~ 50 percent male in mass culture and ~ 10 percent male in isolation).
11. Mean sex ratios (± S.D.) of the reciprocal crosses were: for sc/h males × ScDr females, 11.9 ± 8.06 percent ($N = 29$) and for ScDr males × sc/h females, 3.1 ± 4.39 percent ($N = 38$).
12. This assumes perfectly random assortment.
13. The Kruskal-Wallis test for generation 1 versus generation 2 ($H = 27.60$, $P << .001$, d.f. = 1, $N = 107$). Females from the high end of the generation 1 distribution were used to start a new stock that has continued to produce unusually high sex ratios. Thus, the high sex ratios of generation 1 may represent nuclear genes subsequently selected out of the experimental line.
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16. The ~ 20 percent mortality in both groups is probably due to the experimental treatment, as survivorship is normally higher on *Sarcophaga* (*4*).
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Estrogen Receptors at Implantation Sites of Rat Endometrium

Martel and Psychoyos (*1*) have suggested that the increased concentrations of estradiol and progesterone nuclear receptors that we found at implantation sites in the endometrium of 6-day pregnant rats (*2*) were due to an artefact caused by binding of the hormone by trypan blue. We do not agree with this interpretation, and our reasons are as follows.

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1) Our assay involved measuring the saturable binding of estradiol (10 n*M* [^{3}H]estradiol displaced by 1 μ*M* diethylstilbestrol). To interfere in this assay trypan blue would have had to be saturable in this range of ligand concentrations and thus to bind both estrogens with a dissociation constant (K_D) equal to or lower than 10^{-7}*M*. This would mean an affinity for estrogen 10 to 100 times higher than that of albumin. We did, however, control for this possibility. One volume of a suspension of 0.5 percent charcoal and 0.05 percent dextran in tris buffer (0.01*M*), EDTA (1.5 m*M*), and HCl (*p*H 7.4) was used as an adsorbent, the time of adsorption being 60 minutes (*2*). We measured the concentration of radioactive estradiol not adsorbed by the charcoal in the absence and presence of various concentrations of trypan blue. Our results (see Table 1) show that negligible binding of [^{3}H]estradiol by trypan blue was observed (at most, at 0.46 percent of the total incubated hormone); moreover, this low level of binding was not significantly displaced by excess diethylstilbestrol and thus could not have interfered in the assay. Even the lowest concentrations of trypan blue used in these experiments were largely in excess of those present in the endometrium. Finally, we emphasize that we had compared binding characteristics of receptors at implantation and peri-implantation zones by Scatchard analysis and gradient ultracentrifugation and found no evidence of spurious nonreceptor binding (*2*).

2) Our main experiment was performed on intact pregnant rats (with one control experiment 1 day after castration but without hormonal treatment). It is thus difficult to compare our results with those of Martel and Psychoyos who reported on concentrations of estrogen receptors in castrated progesterone-estradiol–treated animals. However, these authors have published elsewhere (*3*) measurements performed on day 6 of normal pregnancy, that is, under conditions similar to those of our studies. The concentration of nuclear receptor which they observed was 287 ± 332 sites per cell (mean ± standard error, three determinations) outside implantation sites and 838 ± 573 in implantations sites. Hence, in normal pregnant rats they found practically null concentrations of receptors in the nuclei (cytosolic receptor concentration was ~ 11,000 sites per cell). Considering that circulating estradiol is present in these animals (even if one disputes any estrogen of embryonal origin) it is difficult to understand the absence of nuclear complexes. This raises questions about the method of measurement of nuclear receptor.

Table 1. Radioactivity nonadsorbed by treatment with dextran-charcoal suspension. The results show the mean of six determinations (± standard error) and are expressed as femtomoles per milliliter.

Trypan blue concentration	Incubation with 10 n*M* [^{3}H]estradiol	Incubation with 10 n*M* [^{3}H]estradiol plus 1 μ*M* diethylstilbestrol
0	18.02 ± 2.23	17.81 ± 1.91
1/50,000	24.17 ± 2.65	15.37 ± 1.80
1/10,000	28.20 ± 5.09	21.52 ± 3.18
1/5,000	28.94 ± 1.59	24.59 ± 4.77
1/1,000	46.32 ± 2.86	42.61 ± 4.77

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The trypan blue we used for our experiments (*1*) was purchased from Touzard & Matignon (Paris). Subsequently, we have obtained trypan blue from a number of other sources and have found that the ability to bind estradiol varies depending on the source: trypan blue R.A.L. from Kuhlmann (France) bound estradiol whereas that from Fluka (Buchs, Switzerland) did not. We suggested that the binding of steroids to trypan blue may explain the discrepancy between our data and those of Logeat *et al.* (*2*) as well as the observation by these authors of a tenfold difference in the estradiol binding capacity between the pseudopregnant horns of control animals (not injected with trypan blue) and the interimplantation areas of animals injected with the dye. If, in their original study, Logeat *et al.* used a "nonbinding estrogen" batch of trypan blue for revealing the implantation sites, these differences remain to be explained.

We think that it is not difficult to understand the low concentration of nuclear estrogen receptor on day 6 of pregnancy (*3*) considering (i) the low plasma estrogen level on this day in this species (rat) (*4*), (ii) the increased uterine estrogen metabolism on this day (*5*), and (iii) the selective reduction of nuclear estradiol receptor concentration, by progesterone (*6*).

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