Old Guilford, and it is possible that the reduction in susceptibility is partially explained by this change. However, individual variations in enzyme activity led us to suspect an additional genetic factor. Specifically, we hypothesized a mutation which suppressed seizures, possibly by modifying the enzyme inhibitory properties of *d*. Susceptibility of DBA/1 mice (*dd*) from lines (in the Russell-Wolfe colony) which had never been crossed to *D*-carrying animals was only 18 percent (33 subjects). Heterozygotes from the colony showed an incidence of 21 percent and their dilute siblings, 17 percent. Therefore, the change of seizure incidence was not mediated by the presence of the *D* allele in the pedigree. If a genetic change had taken place, it must have occurred originally in a dilute mouse.

The data in Table 1 show that the ranking of the three genotypes for seizure susceptibility was actually the reverse of that originally predicted. Further analysis demonstrated that *Dd* mice from *DD* × *dd* matings showed a higher incidence of seizures (25 percent of 81) than *Dd* offspring from *Dd* × *dd* matings (14 percent of 168) ($\chi^2 = 4.608$, $p < .05$). The finding is compatible with the existence of a seizure suppressor *Sz*, originally occurring in the dilute stock in the same linkage group as *d*. Dense mice from *D sz/D sz* × *d Sz/d Sz* would all be *D sz/d Sz*. Dense mice from *Dd* × *dd* matings could be of two classes, *D sz/d Sz* (noncrossover) and *D Sz/d Sz* (crossover). The latter type, homozygous for the suppressor, would be less susceptible to seizures. For verification of the original data, crosses were made as follows, *DD* × *dd*, *dd* × *DD*, *Dd* × *dd*, and *dd* × *Dd*. No seizures occurred in 27 *Dd* offspring from *Dd* × *dd* matings; 19 seizures in the first trial at 30 days were obtained in 138 offspring from *DD* × *dd* matings. The lowering of seizures incidence was found in other groups from different experiments conducted during the summer months and appears to be an environmental effect. Comparisons between seizure frequencies made at different periods must be made with caution.

A confirmation of the genetic origin of seizure suppression found in our DBA/1 colony was sought through selection. Four groups of matings were made: *DD* susceptible; *DD* nonsusceptible; *dd* susceptible; and *dd* nonsusceptible. Families were classified as having high or low susceptibility to seizures, based on the testing of at least two litters. All individuals selected as parents had been tested and were phenotypically characteristic of their family category. From five to eight matings were made in each category and the results are given in Table 4. The change in seizure incidence among offspring of *DD*-susceptible matings compared with nonselected mice is significant ($\chi^2 = 7.05$; $p < .01$), but no change was observed in the other groups. Whatever effects nutritional and other environmental factors are exerting, appropriate selection restored the expected high level of susceptibility characteristic of the DBA/1 strain. It seems reasonable to interpret these results as follows: *dd* mice chosen for the selection experiment are not segregating at the suppressor locus, and variability in susceptibility is therefore largely environmental. The asymmetry of selection in the *DD* groups suggests that the heterozygote *Sz sz* is phenotypically closer to the resistant *Sz Sz* than to the susceptible *sz sz*. If the nonsusceptible *DD* mice are chiefly heterozygous with respect to the suppressor locus, a likely condition according to the data presented here, one would expect little change in one generation of selection.

The high susceptibility of appropriately selected *DD* mice indicates that the *d* gene has little effect on proneness to seizures within this particular genetic and environmental background. Further biochemical studies are necessary for conclusions regarding the original hypothesis of a positive relationship between seizure susceptibility and deficient phenylalanine hydroxylase activity. The coincidence of the decreased susceptibility in our DBA/1 colony with an increase of enzyme activity is highly suggestive of such relationship. The possible role of nutritional changes in modifying both seizure susceptibility and enzyme activity is acknowledged, but was not tested in this experiment. Evidence was found that variation in susceptibility within the colony was associated with a genetic change distinct from the dilute locus, but linked with it.

SALLY D. HUFF*
JOHN L. FULLER

Jackson Laboratory, Bar Harbor, Maine

References and Notes

1. D. L. Coleman, *Arch. Biochem. Biophys.* **91**, 300 (1960).
2. R. A. Friedland, I. M. Wadzinski, H. A. Waisman, *Biochem. Biophys. Res. Commun.* **5**, 94 (1961).
3. H. L. Wang, V. H. Harwalkar, H. A. Waisman, *Arch. Biochem. Biophys.* **97**, 181 (1962).
4. R. G. Busnel and A. Lehmann, *J. Physiol., Paris* **52**, 37 (1960).
5. J. L. Fuller, C. Easler, M. E. Smith, *Genetics* **35**, 622 (1950).
6. S. D. Huff and R. L. Huff, *Science* **136**, 318 (1962).
7. The original stocks were provided by H. G. Wolfe and E. S. Russell.
8. Liver phenylalanine hydroxylase activities were analyzed by D. L. Coleman.
9. We thank Jane Harris, Frank Clark, Roy Hostetter, and Gayle Hostetter for their aid in testing mice. This work was supported in part by USPHS grant MY-1775 from the National Institute of Mental Health.
* Present address: Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City.

10 February 1964

Inherited Male-Producing Factor in an Insect That Produces Its Males from Unfertilized Eggs

Abstract. *An inherited factor causing the normal sex ratio of 92 percent females to drop to about 5 percent has been produced by selective breeding in a laboratory strain of the arrhenotokous parasitic wasp* Dahlbominus fuliginosus *(Nees) (Hymenoptera, Eulophidae). The factor is known to be of genetic origin and is sex-limited, being transmitted by females to their sons. Its effect on the sex ratio is constant and not influenced by the female parent, the host, or the environment.*

In the hymenopteran *Dahlbominus fuliginosus*, as with most other arrhenotokous insects, maleness has previously been considered to be determined solely by the lack of fertilization of the egg. Haploid males arise from unfertilized eggs and diploid females from fertilized eggs. The underlying genetic mechanism of sex determination is as yet unknown. In *D. fuliginosus* there are no known series of sex alleles, no diploid males resulting from close inbreeding, such as in *Habrobracon juglandis* (*1*), and no apparent sex chromosomes. Unlike what Flanders (*2*) believes to be the situation in arrhenotokous species, females of *D. fuliginosus* cannot control fertilization of their eggs, and the sex ratio of progeny from females producing offspring of both sexes is usually rather uniform, except when environmental conditions

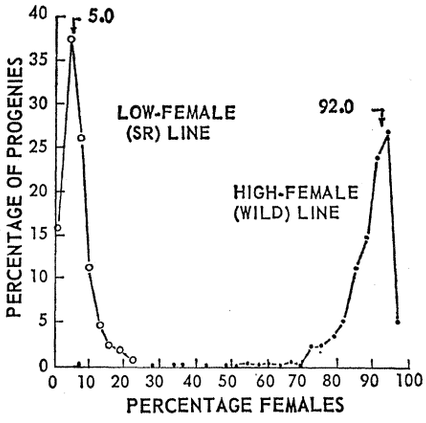

Fig. 1. Distribution of the sex ratios (percentage of females) of progenies from single-pair matings of selected low-female (SR) and high-female (wild) lines; 13,765 progeny from 273 pairs of F_{17} to F_{27} of the SR line A and 41,228 progeny from 824 pairs of F_2 to F_{27} of the wild line. The mean sex ratios were 6.11 ± 0.020 and 88.56 ± 0.091, respectively.

are extremely unfavorable (*3*) or when the male-producing factor reported here is present in a population.

The sex ratio of laboratory populations reared at or near 23°C and at a relative humidity of 70 percent has a somewhat skewed frequency distribution, with a mode at 92 percent females and a mean of 89 percent (Fig. 1, right). There is a noticeable negative skewness in the distribution and very occasionally progenies appear with less than 20 percent females—about one in every 15,000 pairs.

In an attempt to determine the causes of this variability in the sex ratio, the progeny of a stock laboratory female having a 6.8 percent female sex ratio was used to establish two, single pair, inbred (sibling-mating) lines selected for high and low percentages of females. One was selected for a mean ratio of 89 percent females or higher (wild line) and another (SR line) for 20 percent females or lower (SR referring to the sex-ratio factor described in this report). A second SR line (B) was started from the SR line at the F_4 generation but discontinued at the F_{10} generation.

Special efforts were made to prevent the sex-ratio from being influenced by the environment or mortality during development of the immature stages. All the insects were reared simultaneously and in the same manner for each generation of all the lines, and uniform incubation conditions were maintained at 23°C and 70 percent relative humidity. Sex ratios were determined for the most part by examination of mature pupae, but checks were made later on samples of newly emerged adults.

For the first five selected generations, the sex ratios of the high and low lines did not differ significantly but remained almost alike and relatively constant at just over 90 percent females (Fig. 2). In the low lines, progeny with noticeably lower percentages of females began to appear in the F_6 generation of SR line B and in the F_7 generation of line A. The sex ratio of the high-female line, although fluctuating slightly, remained at a high level during subsequent generations but that of the two SR lines continued to fall. From the F_{16} generation on, the line A became more stabilized, remaining at a uniform low level of about 5 percent females, as shown by the frequency distribution of the sex ratios in generations 16 to 26 (Fig. 1).

Crosses between the high- and low-female lines have shown that the low percentage of females in the low line is due to a factor transmitted by females to their sons, here designated as the sex-ratio (SR) factor. From crosses of males of the low-female line with females of both the high-female line and the standard, laboratory stock (mated at random), the percentage of females of the progenies was always low. Reciprocal crosses in which both heterozygous and homozygous (SR/SR) females were used produced progenies that had a high or "normal" percentage of females. The number of progeny per female parent was the same in all crosses.

Five hundred and eighty-seven males produced by virgin heterozygous (SR/+) females, half from crosses of SR/SR ♀♀ × + ♂♂ and half from crosses of +/+ ♀♀ × SR ♂♂, were tested by mating them individually to wild females; 43.6 percent were found to be normal (wild type), 32.0 percent were SR and 24.4 percent produced no female offspring and were classed as "sterile." Further tests are being made to determine the exact ratio of the three classes from these crosses. Although many "sterile" males occurred in the SR lines during the first 13 generations of selection, fewer have occurred since. Approximately 19 percent of 1427 males tested in generations 14 to 26 were "sterile." It has been shown in dissections of these wasps that the testes, and the numbers and motility of the sperm, of the +, SR, and so-called sterile males are alike.

To prove that the abnormal sex ratio was not due to cytoplasmic agents transmitted only through the egg, progenies of females individually mated successively to both normal and SR males were reared. Wild, red-eyed (*rr*) females were first mated to *r*/SR males and immediately after to +/+ males.

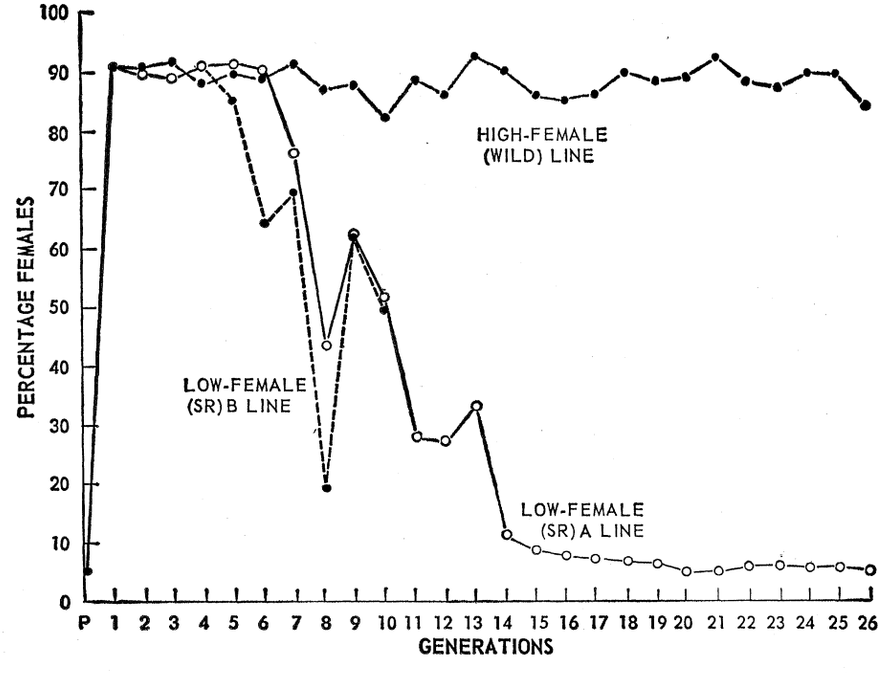

Fig. 2. Sex ratios (percentage of females) of progenies from pair matings of inbred low-female (SR) and high-female (wild) selected lines; 20,973 progeny from 466 pairs and 41,537 from 830 pairs, respectively.

In a comparable series, the order of mating was reversed. The progenies produced by the 257 females that received marked sperm from both wild and SR males showed that their eggs were fertilized by both types of sperm and in the same ratio as the eggs produced by females that received either kind of sperm alone. The sex ratio of the 19,010 offspring from females inseminated by both kinds of males was, for normal sperm, 89.1 percent females, and for SR sperm, 6.3 percent females. The male-producing factor was not therefore transmitted by the egg alone. From the records of adult emergence, the sperm taking part in fertilization appears to have been random, and not selective as has been suggested for this as well as other arrhenotokous species (*2*).

Although the mode of action of the male-producing factor is unknown, its presence, as in the case of nonreciprocal cross incompatibility in the pteromalid wasp *Nasonia (Mormoniella) vitripennis* (Walker) reported by Saul (*4*), appears to hinder successful fertilization, either by preventing the sperm from successfully entering the egg, or by preventing the sperm nucleus from uniting with the female pronucleus after it has entered the egg. In *D. fuliginosus* the action of the factor is clearly independent of the agents considered to be responsible for altering the sex ratio of arrhenotokous animals. The factor can be increased and presumably decreased by selection and is transmitted by females to their sons. Its effects are not due to selective mortality but may be associated with male sterility. Although the sex ratio of certain selected crosses are constant, the factor is, no doubt, widely scattered through natural populations and might account for the variability of the sex ratio of this species and quite possibly the remarkable variability of the sex ratio that characterizes so many species of Hymenoptera both in the laboratory and in the field.

A. WILKES

Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario

References

1. P. W. Whiting, *Advan. Genet.* **10**, 295 (1961).
2. S. E. Flanders, *Quart. Rev. Biol.* **21**, 135 (1946).
3. A. Wilkes, *Can. Entomologist* **95**, 183 (1963).
4. G. B. Saul, *Z. Vererbungslehre* **92**, 28 (1961).

13 January 1964

Dispensable and Indispensable Genes in Neurospora

Abstract. *Twenty-four recessive lethal mutations were detected in a heterokaryon by the method of Atwood and Mukai. All controlled indispensable functions. Genetic analysis revealed that six of these failed to transmit the defect to their progeny. These mutants are believed to be semilethal genetic changes which express a lethal phenotype only in certain genetic backgrounds.*

The hereditary determinants of a microorganism can be divided into two classes: dispensable genes, whose loss or inactivation can be overcome by complete medium (that is, auxotrophs), and indispensable genes, whose loss results in lethality even in the presence of a complete medium. In *Neurospora* the relative proportions of the two classes have been estimated by two methods which gave quite different results. Horowitz (*1*) found that 14 out of 26 *Neurospora* mutants whose phenotype was temperature dependent grew on complete medium at the temperature at which they were unable to grow on minimal medium; these mutants represent dispensable genes. On the other hand, Atwood and Mukai (*2*) employed a heterokaryon method by which they demonstrated that only two out of 26 mutants examined were dispensable.

In the present study, a genetic analysis of mutants isolated by the Atwood-Mukai heterokaryon method suggests a partial explanation for this discrepancy.

The heterokaryon used in these experiments consists of two components, both of which are identifiable by their morphological and biochemical characteristics. One nucleus contains the genes *al-2* (colorless conida), *me-2* (methionine requirement) and *igloo* (a new morphological marker, linked to *al-2*). The other nucleus contains the genes *tr-1* (tryptophan requirement), and *flat* (another morphological marker). Both nuclei contain the temperature colonial gene, *cot*, but the heterokaryon is nutritionally like the wild type.

Conidia from the heterokaryon were filtered through glass wool and plated on minimal medium (*3*) at 33°C. Colonies were picked up and grown on minimal slants until conidiation, at which time they were tested for recessive lethals by plating on methionine-containing medium, also at 33°C. If no *igloo* colony types arose, this was presumed to be evidence of a recessive lethal in the *igloo* nucleus and the strain was set aside for further testing. With this method six spontaneous and 26 ultraviolet-induced mutants were isolated. The frequency was 0.95 percent for the spontaneous mutation and 8.6 and 12.5 percent for the ultraviolet induced mutations at 70 percent and 20 percent survival, respectively. All the mutants isolated were found to be indispensable, that is, none gave *igloo* colonies on Horowitz (*4*) complete medium.

Table 1. Summary of results of the tetrad analysis of spontaneous and induced mutants. Unless more than four spores germinated on the methionine-containing dissection agar, the spores were not transferred to small slants of complete agar. The last column indicates whether or not the mutants behaved according to the assumptions made by Atwood and Mukai.

Origin of mutants	No. examined	No. giving aberrant pattern
Spontaneous	6	1
Ultraviolet	18	5

All of the spontaneous mutants and a sample of the "ultraviolet" mutants were tested for allelism by the method of Atwood and Mukai (*2*). None gave identical complementation patterns and therefore all were different by this criterion.

Eighteen of the "ultraviolet" mutants and all six of the spontaneous mutants were crossed to *cot a* and asci were dissected on methionine medium and germinated at 33°C. Control crosses of *Igloo* A × +a were also dissected, and no lethal genetic factors were found to be segregating.

Crosses were judged as either normal or aberrant. A heterokaryon mutant was said to be normal if no tetrads were dissected in which more than four spores out of eight grew. If more than four spores grew, and if the ascus in question was segregating for the *igloo* gene, then the spores were transferred to slants and allowed to grow. Strains with this behavior were aberrant. Table 1 gives the data for the dissections. Since crosses were always dissected on methionine medium, the other nucleus