cardiaca along axons to their target organs. An electron micrograph by Meyer and Pflugfelder (*10*) showing axon terminals close to the nucleus of a cell in the corpus cardiacum of *Carausius,* and the presence of neurosecretory-type granules in the cytoplasm of intrinsic corpus cardiacum cells of *Carausius* (*10*), *Leucophaea* (*11*, *12*), and *Myzus persicae* (*13*) lend some support to this theory. The finding that materials are transported from the corpora cardiaca along nerve axons does not necessarily indicate that there is no release of neurohormones (*14*) from these organs into the blood; this may still occur, especially in view of evidence from a recent electron microscope study (*12*). But before accepting as fact such release into the blood in intact insects, it would be desirable to have more direct evidence than is at present available (*15*).

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15. This work was done while one of us (B.J.) was in receipt of a Rockefeller Foundation travel grant.
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Genetic Control of Differential Heat Tolerance in Two Strains of the Nematode Caenorhabditis elegans

Abstract. *Two strains (Bergerac and Bristol) of the nematode* Caenorhabditis elegans, *with different temperature tolerances, were reared axenically at 23° to 25°C. The Bergerac strain is heat sensitive (that is, it is sterile at maturity), whereas the Bristol strain is heat resistant (that is, it matures and reproduces normally). Hybrid hermaphrodites (F_1), produced by crossing Bristol males and Bergerac hermaphrodites, are heat tolerant. Heat sensitivity segregates as a simple Mendelian recessive in the F_2 and F_3 generations.*

Caenorhabditis elegans, (Maupas, 1900) Dougherty, 1953 (family Rhabditidae), has been maintained in continuous axenic cultivation (that is, in the absence of other living species) on various complex media since 1956 (*1*). This nematode can also be readily maintained monoxenically (that is, with one other species only—in this instance, *Escherichia coli*) on Difco nutrient agar slants. Populations of the organism, grown in the laboratory between 13° and 20°C, consist almost entirely of self-fertilizing protandrous hermaphrodites (*2*). Males are encountered occasionally and, under certain conditions, can be crossed with hermaphrodites. Typically the mated hermaphrodites produce progeny of mixed uniparental and biparental origin; consequently there is a considerable increase in the percentage of males. We have exploited this reproductive pattern in a study of the inheritance of heat tolerance as opposed to heat sensitivity in two strains of *C. elegans*, named according to their geographic origin—namely, Bergerac (France) (*3*) and Bristol (England) (*4*).

Reproduction of the Bergerac strain by self-fertilization under axenic conditions on a suitable medium is a function of temperature. When both it and the morphologically identical, but physiologically different, Bristol strain are grown in a number of organic media at temperatures between 10° and 25°C, the Bergerac strain matures and reproduces normally only up to about 18°C, whereas the Bristol strain matures and reproduces consistently. At 20°C reproduction of the Bergerac strain is variable; and at 23°C the worms grow to adult size but do not reproduce. This difference in temperature tolerance provides a basis for studying genetic control of reproduction in the two strains.

For the studies reported here, techniques of axenic cultivation have been largely those described by Dougherty *et al.* (*5*). A significant departure from work hitherto reported (*1*, *3–5*) was in the composition of the medium; ours is crudely organic, and contains a mixture of 50 percent, by volume, of Oxoid liver extract solution (*6*) at 45 mg per milliliter, 1 percent chick-embryo extract (*4*), and 49 percent glass-distilled water.

An initial difficulty encountered when attempts were made to obtain hybrids between the Bergerac and the Bristol strains was failure, despite a number of trials, to obtain successful matings under axenic conditions. This obstacle was overcome by crossing Bristol males with Bergerac hermaphrodites in monoxenic culture with *E. coli* on a petri plate containing Difco nutrient agar. (The reciprocal mating, Bergerac males with Bristol hermaphrodites, has so far been unsuccessful.) The males are left with the hermaphrodites for 24 hours at 20°C; then the mated hermaphrodites are rendered free of other organisms by being placed for 1 hour in an antibiotic solution, containing 1000 units of potassium penicillin G and 100 μg of streptomycin sulfate per milliliter. At the end of the hour the worms are washed through four changes of 0.067*M* potassium phosphate buffer (*p*H 7) to reduce the concentration of antibiotic to a negligible amount; they are then left in a fifth change of buffer (0.5 ml in a "Bureau of Plant Industry" watch glass) for 24 hours at 20°C to lay eggs. The larvae produced during this period are free of living bacteria. Cultures are then initiated in small test tubes (10 mm outside diameter), each containing 0.2 ml of medium; only one larva is placed in each tube and all of the tubes are incubated at 23°C.

Three types of F_1 progeny have been observed: (i) nonreproducing hermaphrodites (usually very few in successful matings), assumed to be the product of self-fertilization by Bergerac hermaphrodites; (ii) reproducing hermaphrodites, originating from cross-fertilization of Bristol males with Bergerac hermaphrodites; and (iii) males, also originating from cross-fertilization. The number of males has been approximately equal to the number of reproducing hermaphrodites. From the results of the study of the F_1 progeny we may assume that the presumptive gene (or genes) for heat tolerance, as ex-

pressed by the Bristol strain and the F_1 hybrid, is (or are) dominant over the presumptive gene (or genes) for heat sensitivity as expressed by the Bergerac strain. The genotypes are henceforth referred to as *Hs* for heat tolerance and *hs* for heat sensitivity. If we assume that *Hs* and *hs* segregate as simple Mendelian factors, we may expect the F_1 hybrid (with a presumptive genetic constitution *Hshs*) to produce equal numbers of *Hs* and *hs* gametes. By self-fertilization, the F_2 generation from individual hermaphrodites should segregate as three reproducing (1 *HsHs* : 2 *Hshs*) hermaphrodites to one nonreproducing (*hshs*) hermaphrodite.

The method for studying segregation of the *Hs* and *hs* factors in the F_2 and in succeeding generations was as follows. The tops of tubes containing reproductive adults with young larvae (1- to 2-days old after hatching) are flamed and allowed to cool for 1 minute. Then the tube contents are poured into small dishes (one tube per dish), each containing 0.5 ml of glass-distilled water. Next, 11 to 12 actively moving larvae are selected from each dish. Finally, inoculation and incubation are carried out, as with F_1 larvae. Figure 1 summarizes the results of four successive independent experiments. The total number of F_2 worms tested has been 401. Of these, 289 have reproduced and 103 have not, giving a ratio of 2.8:1.0 (which is close to the 3:1 ratio expected for a simple Mendelian segregation). In addition, nine "laggards"—larvae that grew little if at all (*5*)—were encountered in experiment IV.

In experiment IV the F_3 generation was studied by rearing, individually isolated, an average of 11 larvae from each of 38 tubes selected at random from the 163 F_2 larvae that were reproducing. The larvae from 15 out of the 38 all grew up to *Hs* adults and presumptively established their F_2 progenitors as homozygotes (*HsHs*). The other 23 tubes gave an approximate 3:1 ratio of reproductive as opposed to nonreproductive F_3's; thus the F_2 progenitors were established as heterozygotes (*Hshs*). The ratio of *HsHs* to *Hshs* F_2's was 1:1.5, not significantly different from the theoretically expected 1:2 in view of the relatively few F_2 progenitors tested.

Sex linkage was checked by backcrossing F_1 males (with presumptive genetic constitution *Hshs*, or, if the factor were sex linked, *hsO*) to the maternal Bergerac (*hshs*) hermaphrodite. If the presumptive *hs* gene were autosomal, reproducing *Hshs* and nonreproducing *hshs* individuals would have been expected; if the presumptive *hs* gene were sex linked, only nonreproducing *hshs* hermaphrodites would have appeared. Reproducing individuals were produced in two successive, independent experiments; and we may therefore assume that the *hs* character is autosomal.

To our knowledge the foregoing studies are the first in which the genetics of a physiological character in a member of the class Nematoda has been followed under axenic conditions. The difference between *Hs* and *hs* could be used as a convenient marker in the study of other naturally occurring or artificially induced mutations in *Caenorhabditis elegans* (*7*).

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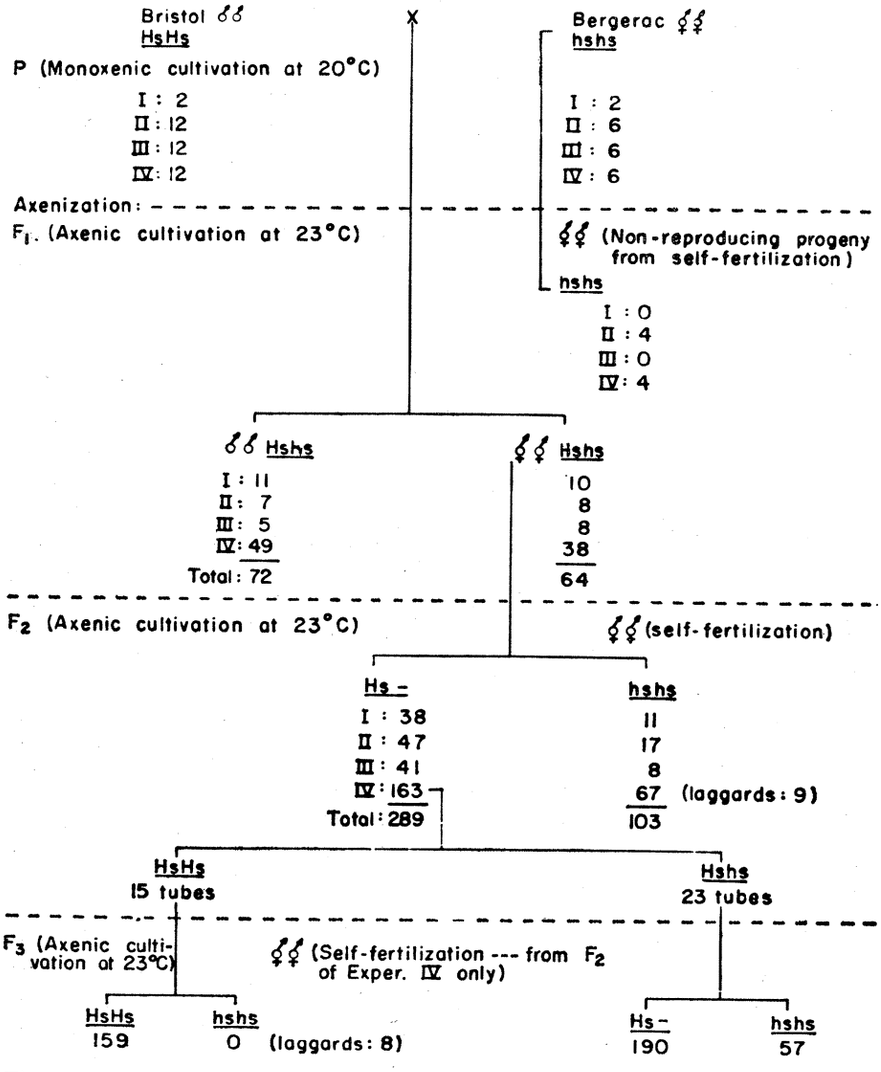

Fig. 1. F_1 and F_2 descendants of four crosses, and F_3 progeny of the fourth cross, between Bristol strain males and Bergerac strain hermaphrodites of *C. elegans*.

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6. Oxoid liver extract is obtainable from Oxoid Co., Ltd., London, E.C.4, England.
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