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7 September 1965

Genetic Adaptation of Caenorhabditis elegans (Nematoda) to High Temperatures

Abstract. *When taken directly from a strain kept for several years at 18°C in the laboratory,* Caenorhabditis elegans *cannot reproduce indefinitely at temperatures higher than 22°C. By progressive and very slow increments of the breeding temperature, a strain fecund at 24.5°C was obtained.*

Caenorhabditis elegans is a species consisting almost entirely of hermaphroditic, self-fertilizing individuals (about one male out of 1000 hermaphrodites). Its fecundity is easy to measure when the nematodes are raised individually on a special agar medium (*1*). At 18°C, the strain Bergerac (*2*) studied shows an average fecundity of 141 offspring per hermaphrodite.

When an embryo grown at 18°C is transferred to growing conditions at 24.5°C, the embryo develops into a sterile adult whose morphology is apparently normal. When the growth conditions are changed from 18° to 23°C, the resulting adult has a very low fecundity. If the succeeding generations are kept at 23°C, fecundity decreases, and complete sterility is reached with the fifth or sixth generation. Thus there exists between normal (around 20°C) and immediately sterilizing (above 24°C) temperatures a range that leads to the extinction of the strain within a few generations.

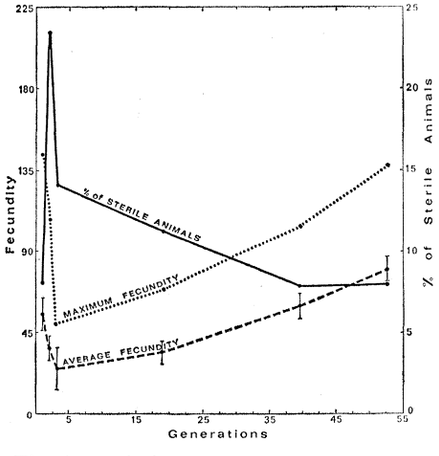

Fig. 1. Evolution of fecundity and sterility for successive generations of *C. elegans* after transfer from 18° to 22°C. Each point is the average of three experiments.

When the nematodes were transferred from 18° to 22°C, fecundity dropped during the first few generations to a minimum and then gradually increased (Fig. 1). Hence it appears that a strain perfectly adapted to 22°C has been obtained.

Attempts to obtain a new strain at 23°C depend upon the time of transfer of the nematodes from 22° to 23°C. Transfers made up to approximately the 90th generation yielded results already noted: fecundity decreased to zero over several generations. However, transfers made starting with the 95th generation showed fecundity that decreased to a minimum and rose afterward. Thus a strain, stable at 23°C, was obtained.

If animals that were stable at 23°C were transferred to 23.5°C, similar results were obtained: adaptation to the new temperature occurred if the transfer was made within or after the 252nd generation.

Further, by successive increments of 0.5°C, a permanently fertile strain was finally obtained at 24.5°C (Fig. 2). This strain is radically different from the initial strain grown at 18°C, which, when directly transferred to 24.5°C, becomes immediately and irreversibly sterile.

Cytological study of gametogenesis showed that sterility of the nematodes transferred to high temperature came from an abnormal oogenesis similar to that produced by thermal shocks (*3*). Hence, the adapted animals had undergone a change of ovarian physiology that permitted normal gametogenesis.

The gradual changes in fecundity and the apparently repetitive process at each stage of adaptation suggest that the corresponding genetic modifications occur in successive steps of small degree over the generations studied. Since *C. elegans* is self-fertilizing, selection in a highly heterozygous state presumably cannot be exploited to achieve a progressive adaptation. Comprehensive observations

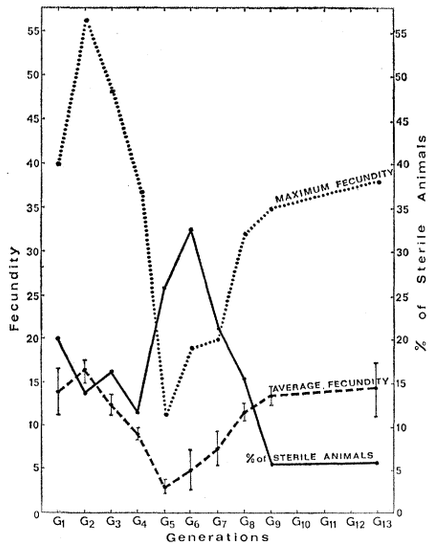

Fig. 2. Evolution of fecundity and sterility for successive generations of *C. elegans* after transfer from 24° to 24.5°C.

(*4*) support the assumption that adaptive transmissible cytoplasmic states are produced gradually and are responsible for the production of fertile high-temperature strains.

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5. I thank Prof. V. Nigon for guidance during this investigation and Dr. E. C. Dougherty for help with the manuscript.

29 September 1965

Antibodies in Gastric Juice

Abstract. *The presence in gastric juice of specific antibody has been demonstrated. It is mainly an IgG antibody reacting with the cytoplasm of gastric cells; it has been detected in patients with atrophic gastritis, with or without pernicious anemia, whose serums contain antibodies to parietal-cell cytoplasm. Evidence is presented that associated circulating antibody to cytoplasm of thyroid acinar cell does not appear in the gastric juice.*

There is a growing body of evidence that, in man, immunoglobulins are normal components of various secretions, such as tears, saliva, colostrum

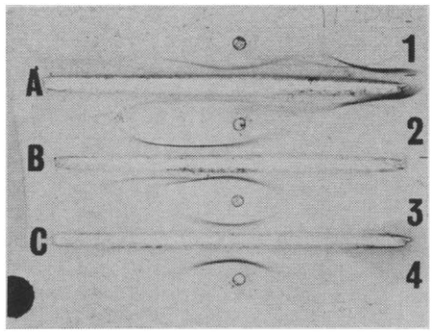

Fig. 1. Immunoelectrophoresis of gastric juice. Gastric juice of normal subjects in wells 1 and 3 and of patient with atrophic gastritis in wells 2 and 4. Troughs: (*A*) Rabbit antiserum to human serum. (*B*) Rabbit antiserum to Cohn's fraction II. (*C*) Goat antiserum to IgA.

and the succus entericus (*1, 2*). Complete definition of the protein components of gastric juice, however, has been hampered by the rapid proteolysis which occurs in acid gastric juice, both in vivo and in vitro. When neutralization in vivo of gastric juice is achieved in normal subjects, several proteins, including IgG and IgA globulins, having immunological identity with serum proteins, are present in the juice (*3*). These immunoglobulins are also found in the gastric juice of patients with atrophic gastritis with or without achlorhydria.

There is a significantly higher incidence of circulating antibodies to a cytoplasmic, microsomal component of the gastric parietal cell and the thyroid acinar cells in subjects with pernicious anemia or atrophic gastritis without pernicious anemia than in normal subjects (*4*). Samples of the gastric juice of four patients with pernicious anemia and three patients with atrophic gastritis and "histamine-fast" achlorhydria, all of whom had circulating antibodies to the gastric parietal cell, were studied by immunoelectrophoresis and by immunofluorescence. Five gastric juices, from normal (control) subjects, which had been neutralized in vivo with 0.07*M* phosphate buffer (*p*H 7.8) were studied similarly. The dialyzed, lyophilized gastric juices were reconstituted in saline, and immunoelectrophoresis of these preparations was performed in agar gel (barbital buffer, *p*H 8.2) with rabbit antiserums prepared against human serum and Cohn's fraction II and with goat antiserum against IgA from human serum. The gastric juices were similarly reconstituted in Coons' buffer and were tested by the indirect Coons' immunofluorescent technique against quick-frozen sections of human and hog gastric mucosa, with fluorescein-conjugated rabbit antiserum to human globulin and goat antiserum to human IgG and IgA. The gastric juices were tested similarly against human thyroid tissue with a fluorescein-conjugated rabbit antiserum to human globulin.

All the gastric juices tested by immunoelectrophoresis showed several lines of reaction with rabbit antiserum to whole human serum. Lines of reaction that corresponded with IgG and IgA were also noted in all samples when the specific antiserums were used (Fig. 1).

When tested by immunofluorescence against human gastric mucosa with fluorescein-conjugated rabbit antiserum to human globulin, four out of seven gastric juices from patients with circulating parietal-cell autoantibodies gave positive parietal-cell staining, two gastric juices were equivocal, and one showed no evidence of antibody activity (Table 1). All the control gastric juices gave negative reactions. The serums of five patients contained antibodies to the cytoplasm of thyroid acinar cells, but none of the gastric juices of these patients contained antibody to thyroid tissue, as judged by immunofluorescence (Table 1).

In order to determine whether the antibody was present in the IgG or IgA components of the test samples, we tested serums and gastric juices on hog gastric mucosa by the indirect Coons' technique, using fluorescein-conjugated goat antiserum to human IgA and IgG, respectively. All gave

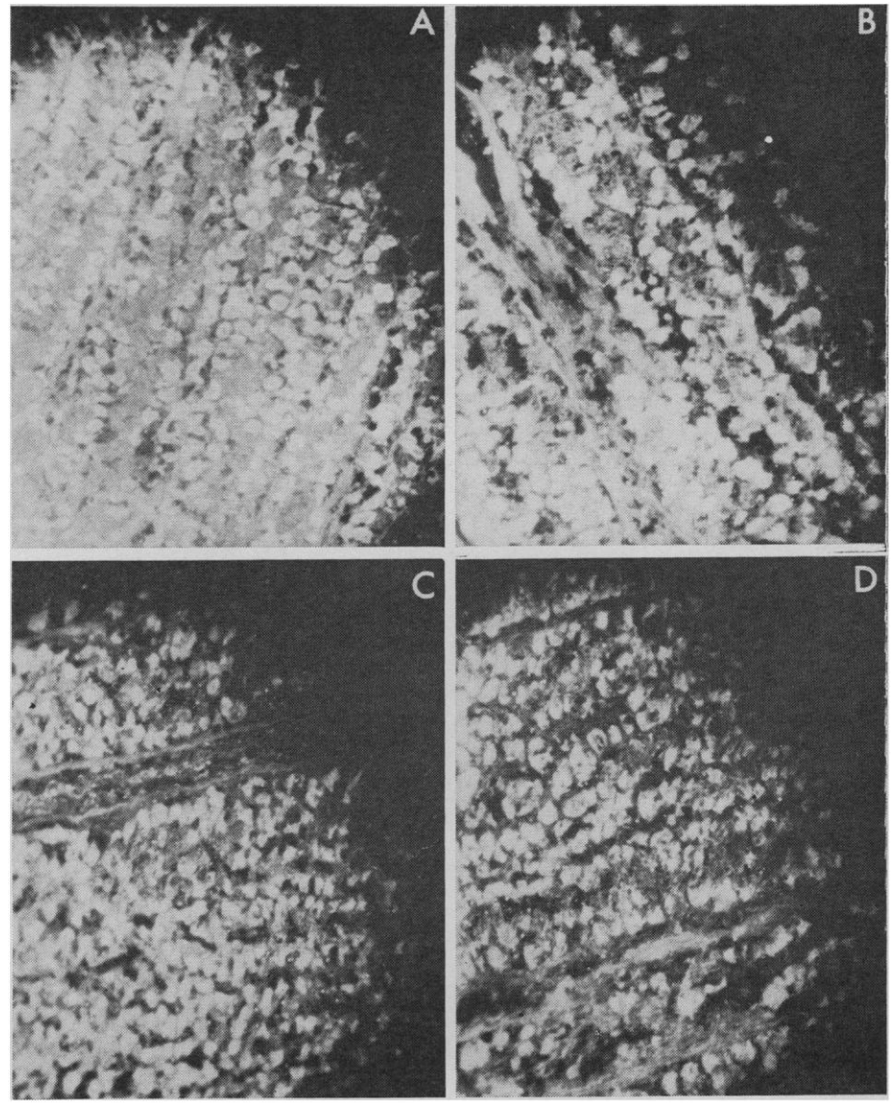

Fig. 2. Gastric parietal cell immunofluorescence of gastric juice and serum from a patient with atrophic gastritis. (*A*) Serum with antiserum to IgG. (*B*) Gastric juice with antiserum to IgG. (*C*) Serum with antiserum to IgA. (*D*) Gastric juice with antiserum to IgA.

Table 1. Autoantibodies in gastric juice, as shown by immunofluorescence. 0, negative; ±, equivocal; W+, weakly positive; +, positive; ++, strongly positive.

To gastric parietal cell		To thyroid acinar cell	
Serum	Gastric juice	Serum	Gastric juice
Pernicious anemia			
+	+	0	0
+	+	0	0
++	+	+	0
++	0	W+	0
Atrophic gastritis			
++	++	W+	0
+	±	++	0
+	±	+	0

positive staining with the conjugated antiserum to IgG; in three out of four subjects positive staining occurred with antiserums to both IgG and IgA (Fig. 2). With whole human serum, both of these conjugates gave single, appropriate lines of precipitation in immunoelectrophoresis. To confirm this apparent absence of a contaminating antibody to IgG in the conjugated antiserum to IgA, serums and gastric juices were absorbed with sufficient rabbit antiserum to human Fc (*5*) to suppress completely all IgG antibody detectable by immunofluorescence. The conjugated antiserum to IgA was absorbed with an amount of human IgG (*6*) sufficient to neutralize any contaminating antiserum to IgG present in the conjugated antiserum to IgA. Neither of these maneuvers separately, or combined, suppressed staining with the conjugated antiserum to IgA. These preliminary results show that the gastric parietal cell antibody in both serum and gastric juice is of both IgG and IgA type, the former probably predominating.

In human saliva, colostrum, and lacrimal secretions the predominant immunoglobulin is IgA, whereas in serum, IgG is the major γ-globulin. In bile and in small intestinal secretions, IgG predominates but the ratio of IgG to IgA is lower than in serum. It has been suggested that either a preferential secretion or local production in adjacent lymph nodes may account for some of the globulins present in these fluids (*2*), and the work of Tomasi *et al.* (*7*) suggests that there may be local synthesis of IgA in the salivary gland; this is predominantly 11*S* γ-globulin, a polymer which has been found in saliva and colostrum but is not present in serum. No similar studies of gastric secretion have been reported. Our own data do not help to resolve the question whether parietal-cell antibodies are elaborated in the gastric mucosa or not, since IgG and IgA components are present in both gastric juice and serum. Synthesis in the gastric mucosa might be one explanation of why the parietal cell antibody is found in gastric juice but the thyroid acinar cell antibody is not. An alternative explanation is that some factors in the gastric mucosa determine that of the two autoantibodies only the parietal cell antibody will pass across the gastric mucosa to the gastric lumen (*8*).

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9. We thank Dr. R. Wistar for preparation of antiserums to human serums, Cohn's fraction II, human Fc piece, and purified human γG globulin. Type-specific antiserums to γG and γA globulin were supplied by Hyland Laboratories. This investigation was supported by NIH grant AM-06971-02 and by a USPHS postdoctoral fellowship grant (J.M.F.).

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Second Mutant Gene Affecting the Amino Acid Pattern of Maize Endosperm Proteins

Abstract. *The mutant* floury-2 *results in the production of maize endosperm proteins with an altered amino acid pattern. The lysine concentration is high, approximately equal to that in mutant* opaque-2, *and the methionine concentration is higher than in any other stock tested. Other mutants of similar phenotype,* opaque-1, floury-1, *and* soft-starch *do not cause major changes in amino acid pattern.*

The drastic change in amino acid pattern of endosperm proteins effected by the *opaque-2* mutant of maize has been reported (*1*). The effectiveness of *opaque-2* maize in supporting the growth of weanling rats has also been reported (*2*).

That maize endosperm proteins are deficient in lysine and tryptophan encourages the search for stocks with higher contents of these amino acids. The rationale for investigating originally the amino acid pattern of *opaque-2* and other mutants of like phenotype—pronounced opacity of the endosperm in contrast with the translucence of normal endosperms—was based on the conjecture that such mutants might lack the ability to produce the zein fraction of endosperm proteins. If so, and if there were compensatory synthesis increasing the amount of the other major protein fractions (acid-soluble and glutelin), an increased lysine and tryptophan content would result since the lysine and tryptophan content of zein is very low (*3*). None of the mutants is completely blocked in the ability to produce zein (*4*), but the amount present in *opaque-2* is significantly reduced (*1*). In addition starch-gel electrophoresis shows that four components of normal zein are missing from *opaque-2* zein (*4*).

Five different mutants of similar phenotype, *opaque-1* (o_1), *opaque-2* (o_2), *floury-1* (fl_1), *floury-2* (fl_2), and *soft-starch* (*h*) were available. Their isolation and mode of inheritance has been reported by Emerson, Beadle, and Fraser (*5*). The opaque and floury mutants were tested initially, and *h* was tested subsequently. We now present the amino acid patterns for all five mutants and show that in addition to *opaque-2*, the *floury-2* mutant also has an altered amino acid pattern and higher lysine and methionine concentrations.

The seeds analyzed were mature, air-