the M_5 lines than for the original cultivar. The daily seedling mortality, as measured by the slope of the probit regression line (*3*), was lower for these mutant lines than for the original cultivar.

A screening of 20,000 M_2 tomato seedlings revealed 120 resistant individuals. In 23 M_3 progenies, 117 resistant seedlings were found, from which 12 M_4 lines were selected. A trial, with ten replications, of four pooled M_4 lines showed a 25 percent reduction in seedling weight of these lines, grown in soil treated with diphenamid, whereas the reduction in seedling weight of the original cultivar was 40 percent (Table 1).

These results indicate that increased resistance of crops to herbicides may be obtained by selection from mutagenically treated populations. This could eventually improve the efficiency of herbicides, and also restrict their applications to those which are satisfactory from the public health viewpoint. The resistant mutants will also provide suitable plant material for studies of the inheritance and the mechanisms of selectivity. A better understanding of selectivity could assist in deliberate synthesis of new herbicides with specific characters.

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References and Notes

1. V. S. Comstock and R. N. Andersen, *Crop Sci.* **8**, 508 (1968); J. Francisco Pereira, W. E. Splittstoesser, H. J. Hopen, *Weed Sci.* **19**, 647 (1971); A. Karim and A. D. Bradshaw, *Weed Res.* **8**, 283 (1968); M. Krol and M. Ruszkowski, *Hodowla Rosl. Aklim. Nasienn.* **11**, 677 (1967); F. R. Miller and R. W. Bovey, *Agron. J.* **61**, 282 (1969); J. D. Nalewaja and R. E. Bothun, *Crop Sci.* **9**, 160 (1969); G. E. Russel, *J. Int. Inst. Sugar Beet Res.* **3**, 32 (1968).
2. M_2, M_3, M_4, and M_5 are the second, third, fourth, and fifth generations descending from the seeds treated with the mutagen.
3. D. J. Finney, *Probit Analysis* (Cambridge Univ. Press, New York, ed. 3, 1971), pp. 20–49.
4. Supported in part by the Central Research Fund of the Hebrew University.

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Genetic Determination of Phenotypic Variation in Sickle Cell Trait

Abstract. *Two genetic sources of variation influence the percentage of sickle cell hemoglobin found in heterozygotes. One factor is strongly related to the percentage of hemoglobin S in the carrier parent and appears to be determined by sickle hemoglobin isoalleles, whereas the other is related to racial background and may well be polygenic.*

In heterozygous carriers of sickle cell trait, the proportion of abnormal hemoglobin varies from person to person but is usually less than 0.5 (*1*). To investigate the causes of individual variation, replicate measurements were made of the proportion of sickle hemoglobin (HbS) in 272 heterozygous parents and children in 67 families that were segregating for sickle cell trait. The subjects were members of a migrant population from northeastern Brazil that was of triracial origins (*2*). A single blood sample was drawn from each family member. The red cells were washed three times with saline and then frozen in a glycerol citrate solution and stored at below −60°C until hemoglobin typing and quantitation were performed. The proportions of hemoglobins A, S, and A_2 were estimated by the starch gel electrophoresis method of Sunderman (*3*). Duplicate determinations of each hemolysate were made on separate gels, and yielded a standard error for the percentage of HbS of ± 0.9 percent.

When the percentage of HbS was expressed as the proportion of β-chain-containing hemoglobin, that is, HbS/(HbA + HbS), a negatively skewed frequency distribution was observed with a range of 27 to 50 percent and a major mode at 44 percent (Fig. 1). Analysis of the results from 184 children in the sample showed that the variance among families was more than three times larger than the variance within families ($F = 3.42$; d.f. 66,117) while the within-family variance was, in turn, more than eight times as great as the variation between replicate determinations ($F = 8.72$; d.f. 117,184). Both of these differences were significant at the 0.001 confidence level, and suggest that a major component of the observed variation is genetically determined.

Linear regression analysis showed that there was a highly significant relationship between the weighted mean value of the percentage of HbS in children and the percentage observed in their carrier parents ($b = 0.44 \pm 0.10$, 65 d.f., $P < .001$). This parent-offspring regression leads to a heritability estimate of 0.88, which implies that 88 percent of the variation in the proportion of HbS is additive. The intraclass correlation coefficient among siblings was 0.47, giving a heritability estimate of 0.94. The close agreement of these two estimates provides little evidence that the observed genetic influence includes a major dominance component.

The effects of age, sex, and a racial-admixture index on the percentage of HbS were also investigated by multiple regression analysis, and only in the case of race was a highly significant effect observed ($b' = 0.282 \pm 0.079$, 268 d.f., $P < .001$) (*4*). Each individual in the study had been classified into one of seven racial admixture groups by a single trained observer who had no knowledge of the hemoglobin typing results. The classification was based on a subjective evaluation of abdominal skin pigmentation, hair color and type, and physiognomy. Analysis of the blood typing results showed that the categories did, in fact, correspond to increasing degrees of Negro ancestry (*5*). The overall effect of the racial variable was to reduce the average percentage of HbS in carriers classified as

Table 1. Effect of racial class on the percentage of HbS in sickle cell heterozygotes.

Statistical test	Regression coefficient ± S.E.	Degrees of freedom	P
Among-sibships regression of racial class on percent of HbS	− 0.43 ± 0.14	65	< .01
Within-sibship regression of racial class on percent of HbS	− 0.51 ± 0.23	119	< .05
Regression of parental racial class on percent of HbS	− 0.05 ± 0.13	75	> .05

"preto," or black, in comparison with carriers classified "blanco," or white, by about 2.5 percent.

When the racial-admixture score was regressed on the percentage of HbS within sibships, children with a higher admixture score were found to have a significantly lower proportion of sickle hemoglobin than did their lighter colored siblings ($P < .05$). The magnitude of the among-family regression coefficient was similar to that of the within-families regression, and was significant at the 0.01 level. The regression of parental racial score on parental percentage of HbS had the same sign but was not significant at the 0.05 confidence level (Table 1).

These data indicate that there are at least two different sources of variation in the percentage of HbS found in subjects with sickle cell trait. The first component appears to be related to the proportion of HbS found in the carrier parent and accounts for approximately 25 percent of the total among-family variance. Itano (*6*) suggested that differences among heterozygotes in the percentage of HbS results from HbS isoalleles which produce their primary or secondary gene product with varying efficiency. If this were true, in HbAS × HbAA matings the percentage of HbS in heterozygous offspring would be determined by the HbA allele received from the normal parent; hence, one would not expect to observe a significant relation between the percentage of HbS in the children and that in their carrier parent. As noted previously, we found a very highly significant parent-offspring regression.

A marked increase in the proportion of sickle cell hemoglobin can be seen with coexistent β thalassemia (*7*). Low proportions of abnormal hemoglobin are often encountered with α-chain hemoglobin variants, a finding which has been interpreted as indicating the presence of a duplication of the α-chain locus in some individuals (*8*). Duplication of nonidentical alleles is known to exist at the γ-chain locus in man (*9*) and, as has been previously suggested (*10*), a similar mechanism could account for the marked reduction in the proportion of sickle hemoglobin that is found in some subjects with sickle cell trait. Our data are consistent with the possibility that HbS isoalleles contribute to the among-family variance. However, isoallelic

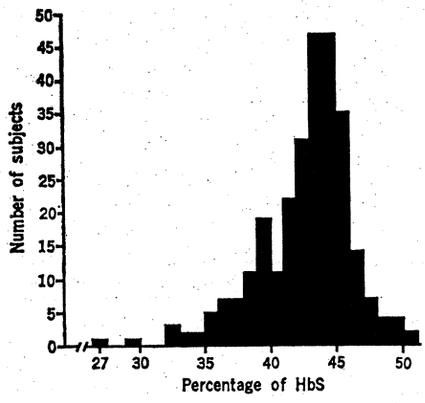

Fig. 1. Distribution of the percentage of HbS in 272 subjects with sickle cell trait. Each observation is the average of duplicate measurements.

variation and variation resulting from allelic duplication cannot readily be distinguished in the present study.

The second genetic effect appears to be related to the racial background of the individual; the regression on the racial index accounted for approximately 12 percent of the among-family variance, and may well reflect a polygenic system. It seems plausible to assume that in populations where sickle cell trait has been maintained by selection, genetic modifiers may have accumulated which tend to adjust the percentage of HbS to a physiologically optimal level. The present study dealt with a hybrid population in which the effect of these modifiers was unmasked.

There are several recognized genetic causes for variation of clinical severity in patients who appear to have homozygous sickle cell anemia (*11*). Whether the two genetic factors identified in this study have any influence on the homozygous phenotype is at present unknown.

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References and Notes

1. J. V. Neel, I. C. Wells, H. A. Itano, *J. Clin. Invest.* **30**, 1120 (1951); H. A. Itano, in *Abnormal Haemoglobins in Africa*, J. H. P. Jonxis, Ed. (Davis, Philadelphia, 1965), p. 3; C. V. Tondo and F. M. Salzano, *Amer. J. Hum. Genet.* **14**, 401 (1952).
2. H. Krieger, N. E. Morton, M. P. Mi, E. Azevedo, A. Freire-Maia, N. Yasuda, *Ann. Hum. Genet.* **29**, 13 (1965). From an analysis of the blood typing results, Krieger *et al.* estimated the population had 59 percent Caucasian, 30 percent Negro, and 11 percent American Indian ancestry. The entire population sample was composed of 2129 parents and 4766 children in 1068 families. For a detailed description of the population and the ascertainment criteria, see N. E. Morton (*12*).
3. Hemolyzates containing approximately 5 g of hemoglobin per 100 ml were prepared by a sixfold dilution of the glycerolized packed red cells with distilled water. The hemolyzate was centrifuged at 30,000*g* for 15 minutes and subjected to electrophoresis in an eight-slot starch gel with a tris-EDTA-borate buffer system (*13*) (EDTA = ethylenediaminetetraacetic acid). After electrophoresis, the hemoglobin bands were cut out of the gel in four horizontal slices made across the gel at equal intervals between and beyond the hemoglobin A, S, and A_2 bands. The resulting strips were then separated into individual sets of three slabs by vertical cuts. The slabs containing the hemoglobin A and S bands were each dissolved in 10 percent NaOH to a final volume of 50 ml, and the A_2 band to a final volume of 10 ml. The hemoglobin concentrations of the resulting solutions were then measured with an AutoAnalyzer by the benzidine-peroxide procedure described by Sunderman (*14*).
4. The regression coefficient for sex was of borderline significance: females had 0.8 percent more HbS than males ($t = 1.96$, d.f. $= 268$, $P < .05$). When a within-sibship analysis was performed to remove the among-family variation, the effect of sex, adjusted for race, did not approach significance ($t = 1.17$, d.f. $= 118$). Therefore, the data give no strong evidence for an effect of sex.
5. H. Krieger and his colleagues (*2*) estimated that the seven racial admixture groups had the following proportions of Negro ancestry: blanco, 17 percent; amarelo claro, 25 percent; amarelo escuro, 32 percent; mulato claro, 33 percent; mulato medio, 41 percent; mulato escuro, 47 percent; and preto, 69 percent. Estimates of Indian admixture in the seven groups ranged from 5 percent (preto) to 23 percent (amarelo escuro).
6. H. A. Itano, *Amer. J. Hum. Genet.* **5**, 34 (1953); J. V. Neel, I. C. Wells, H. A. Itano, *J. Clin. Invest.* **30**, 1120 (1951).
7. D. J. Weatherall, *Thalassaemia Syndromes* (Blackwell, London, 1965).
8. H. Lehman, *Brit. Med. J.* **4**, 748 (1968).
9. T. H. J. Huisman and W. A. Schroeder, *Amer. J. Hum. Genet.* **22**, 505 (1970); D. Kabat, *Science* **175**, 134 (1972); T. H. J. Huisman, W. A. Schroeder, A. M. Dozy, J. R. Shelton, J. B. Shelton, E. M. Boyd, G. Apell, *Ann. N.Y. Acad Sci.* **165**, 320 (1969). Normal human newborn infants produce two types of Hbγ chains, $^{G}\gamma$ and $^{A}\gamma$, which differ by a single amino acid substitution. During the first 3 months of life there is an ontogenetic shift from predominant $^{G}\gamma$ to $^{A}\gamma$ production. It is not yet clear whether polymorphism exists with respect to presence or absence of the duplicated γ gene in man. Polymorphic duplication of the α-chain locus has been documented in a variety of mammals including the goat (*15*), deer (*16*), horse (*17*), mouse (*18*), and man (*19*). Duplication of the β-chain locus is polymorphic in sheep (*20*) and has been reported in association with a Lepore hemoglobin in at least two human families (*21*).
10. W. E. Nance, *Science* **141**, 123 (1963). If bivalent alleles exist, the $\beta^{A}\beta^{S}/\beta^{A}$, $\beta^{S}\beta^{A}/\beta^{A}$, or $\beta^{S}/\beta^{A}\beta^{A}$ genotypes could account for heterozygotes who show an unusually low proportion of HbS. Furthermore, $(\beta^{A}\beta^{S})/\beta^{A}$ subjects could not have classical homozygous sickle cell anemic offspring regardless of whom they marry. This fact would have important practical consequences for genetic counseling as well as theoretic significance with respect to the forces which maintain the polymorphism. Morton and his colleagues (*22*) could find no evidence for the existence of a common bivalent ($\beta^{A}\beta^{S}$) allele in a segregation analysis of the present data. However, their test was not a powerful one since it depended upon the observation of an overall disturbance in the observed segregation ratio resulting from offspring of rare heterozygotes with the $(\beta^{A}\beta^{S})/\beta^{S}$ genotype. These individuals might be expected to produce more HbS than HbA, but we found no example of such a phenotype in the population sample.
11. Recognized genocopies of sickle cell anemia include rare abnormal hemoglobins which also show the sickling phenomenon (*23*), rare hemoglobin variants which have an electro-

phoretic mobility similar to that of HbS but do not show the sickling phenomenon (*24*), coexistent β thalassemia (*7*), coexistent α-chain mutations which modify the sickling properties of HbS (*25*), and the HbS/F genotype (*7*).
12. N. E. Morton, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 69 (1964).
13. W. E. Nance, A. Claflin, O. Smithies, *Science* **142**, 1075 (1963).
14. F. W. Sunderman, *Amer. J. Clin. Pathol.* **40**, 227 (1963).
15. T. H. J. Huisman, J. B. Wilson, H. R. Adams, *Arch. Biochem. Biophys.* **121**, 528 (1967).
16. T. H. J. Huisman, A. M. Dozy, M. H. Blunt, F. A. Hayes, *ibid.* **127**, 711 (1968).
17. J. V. Kilmartin and J. B. Clegg, *Nature* **213**, 269 (1967).
→ D. B. Rifkin, M. R. Rifkin, W. Konigsberg, *Proc. Nat. Acad. Sci. U.S.A.* **55**, 586 (1966); K. Hilse and R. A. Popp, *ibid.* **61**, 930 (1968).
19. S. R. Hollan, J. G. Szelenyi, B. Brimhall, M. Duerst, R. T. Jones, R. D. Koler, Z. Stocklen, *Nature* **235**, 47 (1972); R. K. Abramson, D. L. Rucknagel, D. C. Shreffler, *Science* **169**, 194 (1970).
20. S. H. Boyer, *J. Hered.* **58**, 279 (1967).
21. Y. Ohta, K. Yamaoka, I. Sumida, T. Yanase, *Nature New Biol.* **234**, 218 (1971); P. Dherte, H. Lehmann, J. Vandepitte, *Nature* **184**, 1133 (1959).
22. N. E. Morton, M. P. Mi, N. Yasuda, *Amer. J. Hum. Genet.* **18**, 233 (1966).
23. H. M. Ranney, *N. Engl. J. Med.* **282**, 144 (1970); L. E. Pierce, C. E. Rath, K. McCoy, *ibid.* **268**, 862 (1963); R. M. Bookchin, R. P. Davis, H. M. Ranney, *Ann. Intern. Med.* **68**, 8 (1968).
→ H. A. Itano, *Proc. Nat. Acad. Sci. U.S.A.* **37**, 775 (1951); P. Sturgeon, H. A. Itano, W. R. Bergren, *Blood* **10**, 389 (1955).
25. A. P. Kraus, T. Miyaji, T. Iuchi, L. M. Kraus, *J. Lab. Clin. Med.* **66**, 886 (1965).
26. This work was supported by PHS grants HL-15166 and GM-1056, and grants from the John A. Hartford Foundation and the James Whitcomb Riley Association.

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Contingent Negative Variation as an Indicator of Sexual Object Preference

Abstract. *The contingent negative variation (CNV) was recorded in the interval between paired visual exposures of male nudes, female nudes, and sexually "neutral" silhouettes. Groups of 12 male and 12 female subjects viewing 50 randomized presentations from each stimulus category responded with averaged CNV amplitudes proportional to the predicted degree of sexual interest in the stimulus classes.*

Clinical and basic research in the area of normal and deviant human sexuality has been hampered by the lack of objective and reliable methods for assessing sexual object preferences. While measures of penile volume changes have demonstrated their value in studies of the male (*1*), and recent studies of vaginal blood flow appear promising in the female (*2*), there exists no single technique which is applicable to both sexes and is adequately sensitive to discriminate between stimuli representing preferred and nonpreferred sexual objects (*3*). Further, the intrusive, time-consuming, and cumbersome aspects of current methods have posed serious obstacles to their acceptance and broader application. We now report the successful application of a well-studied electrocortical measure, the contingent negative variation (CNV), as an indicator of sexual object preference in a group of putatively normal males and females. In addition, the method to be described appears potentially useful in analyzing preference and interest patterns in response to a broad range of visual stimuli.

The contingent negative variation utilized in the present study—also sometimes referred to as the "expectancy wave" or "E-wave"—was first described by Walter (*4*) and has been the subject of extensive neuro-psychophysiological study. The CNV shows itself as a relatively slow surface-negative shift in the baseline of the scalp-recorded electroencephalogram (EEG), which is revealed by computer averaging of epochs of EEG defined by repetitive stimuli. The CNV typically occurs in the period between the presentation of a conditional or "warning" stimulus (S1) and a succeeding unconditional or "imperative" stimulus (S2). The CNV seems to reflect a state of anticipation, expectancy, or preparation for the contingent (S2) stimulus, in that its appearance and amplitude are enhanced in situations which promote the interest and attention of the subject with regard to S2. Experimentally, CNV is facilitated by requiring some response by the subject to S2. Such response, however, is not a necessary condition for CNV, since stimulus qualities of S2 may, in and of themselves, function efficiently to command the subject's interest and attention. Conversely, conditions which promote distraction and inattention serve to inhibit CNV. Likewise, there is evidence to indicate that both high and low levels of autonomic arousal, as represented by anxiety or boredom, are inhibitory to CNV (*5*).

The literature reports only one previous study relating an electrocortical response to sexual stimuli. Lifshitz (*6*) utilized the visual averaged evoked potential (AEP), and noted qualitative differences in the AEP waveforms of certain individual male subjects when presented with female in contrast with nonsexual visual stimuli. However, no attempt was made to assess the differential effect of preferred and nonpreferred sexual object stimuli.

In devising our experimental procedure we reasoned that a subject would greet an opportunity to view a preferred sexual stimulus with a heightened state of anticipation or expectancy, which would express itself in relative CNV enhancement, when such opportunities were contrasted with those to view nonpreferred or nonsexual stimuli. In order to avoid the need for subjects to learn or be conditioned to an extraneous and potentially confounding set of S1 stimuli, it was decided that a brief, initial presentation of the visual stimulus should serve the function of the S1 "warning" stimulus for each trial. Consequently, each trial consisted of two presentations of a back-projected colored slide. The first presentation, serving as S1, lasted 500 msec. The second presentation, serving as S2, appeared after a delay of 1500 msec and remained on for 2000 msec. The subsequent trial was initiated after a fixed delay of 9.45 seconds for equipment cycling. The experiment consisted of 150 trials, 50 trials drawn from each of three categories of stimuli: female nudes, male nudes, and sexually "neutral" figures. To control for the ordering of categories, habituation to the procedure, and possible expectancy effects due to the fixed intertrial interval, trials representing the three categories were presented in a fixed randomized order.

The photographic subjects were males

Table 1. Data for contingent negative variation: amplitude in microvolts.

Stimulus sex	Male subjects (N = (12)		Female subjects (N = (12)		Total
	1st 25 trials	2nd 25 trials	1st 25 trials	2nd 25 trials	
Opposite sex	9.38	9.38	12.72	10.84	10.58
Same sex	7.83	8.02	11.24	8.46	8.89
"Neutral"	6.36	9.15	10.34	6.50	8.09
Mean values	7.86*	8.85*	11.43†	8.60†	9.19

* The mean of these values is 8.36. † The mean of these values is 10.02.