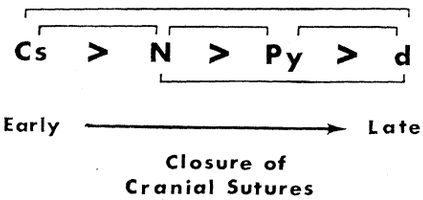

Fig. 4. Postulated dominance relationships of the genes for craniostenosis (*Cs*) and pycnodysostosis (*Py*), to their normal allelomorphs (*N*) and the deletion (*d*), with respect to closure of the cranial sutures. Brackets indicate genotypes that have been observed.

stenosis are all expressed when there is a deletion. Pycnodysostosis is recessive to the normal phenotype, however, while craniostenosis appears to be dominant to it. It is not known whether the genes for pycnodysostosis and craniostenosis are alleles, members of a pseudoallelic series, or representatives of independent loci on the short arm of the same or different G-group chromosomes. Contemporary hypotheses concerning regulatory mutations (*18*), polarity (*19*), subunit interactions (*20*), allosteric effects (*21*), and genetic duplication (*22*) provide a wealth of possible mechanisms by which closely linked mutations might have antithetical effects. In the absence of more detailed knowledge about the primary actions of the genes in question, our crude attempt at deletion mapping does not permit us to distinguish between these possibilities.

It should be emphasized that craniostenosis is in all probability a polygenic trait. Craniostenosis has, for example, been observed in other cytogenetically distinct aneuploid states (*23*). However, our observations suggest that at least one gene locus which influences this trait is present on the short arm of a G-group chromosome, and they provide additional support to existing evidence that specific genetic information affecting bone formation is carried on a G-group chromosome (*14, 15, 24*).

Autosomal deletions in man may be the cause of more or less characteristic clinical syndromes, as in the case of the B, G, and E partial monosomes. Of perhaps greater genetic significance, however, are those deletions which are in themselves compatible with a normal phenotype, but which may permit or alter the expression of genes carried on the intact homolog. In informative families, deletions of this type may permit the chromosomal localization of rare autosomal genes, as is suggested by our cases. In addition, deletions provide an opportunity to map common genetic markers. Thus, any gene transmitted by a deletion carrier along with the deletion chromosome cannot be located in the region of the deletion; similarly, any locus at which a deletion carrier is heterozygous for a co-dominant allele cannot be so located; finally, in situations where the protein product of a gene can be studied, dosage effects in deletion heterozygotes may reasonably be anticipated. By these criteria, genetic studies in the present families have permitted the exclusion of the ABO, MNS, Rh, Kidd, Lutheran, haptoglobin, hemoglobin, and lactate dehydrogenase loci from the regions involved by the deletions. It is apparent that the indications for karyotype studies should be broadened to include instances of rare recessive traits in which there is no evidence of parental consanguinity. As noted by Haldane, autosomal hemizygosity should be considered a possible alternative to homoallelism in cases of this type.

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Tetrahymena: Effect of Freezing and Subsequent Thawing on Breeding Performance

Abstract. Tetrahymena pyriformis, *syngen 1, frozen in 10 percent dimethylsulfoxide, stored for 2 months, and then thawed could conjugate normally. More significantly, they were viable, in normal numbers, through two sexual reorganizations. The strains apparently did not sustain genetic damage during the treatment. The techniques offer considerable promise for the maintenance of breeding stocks in ciliated protozoans.*

Stocks of *Tetrahymena pyriformis*, grown in various ways, gradually lose their ability to produce viable progeny at conjugation, perhaps because of accumulated micronuclear defects (*1*). Also associated with defective micronuclei is the phenomenon of genomic exclusion (*2*), in which the entire genome of one parent is lost at conjugation. Strains of *Tetrahymena* that have been maintained in laboratories by vegetative growth and many found in natural habitats are amicronucleate and incapable of sexual reorganization (*3*). Breeding stocks must be carefully selected and revitalized constantly by the time-consuming process of crossing and then testing the progeny of a new generation at least once a year. This procedure limits the number of stocks that can be maintained and restricts genetic studies on these forms.

The possibility of storing breeding stocks of *Tetrahymena* in the frozen state is intriguing since this process might eliminate the need for continued inbreeding. Viability has been maintained in four sterile strains and one genetically active strain of these ciliated protozoans frozen in 10 percent dimethylsulfoxide and subsequently stored at temperatures below −170°C (*4*). We have tested whether breeding performance of two strains of syngen 1

(5) was impaired by this treatment.

For these strains, D1-14651a and F-13652, the following modifications in the procedure used initially at American Type Culture Collection (ATCC) were beneficial: (i) a 20 percent (volume to volume) solution of dimethylsulfoxide in 1 percent proteose peptone was added to an equal volume of a 3-day-old culture; (ii) after equilibration of this mixture for 30 to 35 minutes at 35°C in a water bath, a concentrated suspension of cells, which had settled at the bottom of the tube, was collected with a pipette; (iii) vials containing 0.2-ml samples were placed in a Nitro-Freeze (6) apparatus at −23°C; (iv) after 20 minutes the vials were transferred to the vapor area of a tank refrigerated with liquid nitrogen.

To eliminate the necessity for using aseptic techniques in breeding experiments, we transferred cultures recovered from low-temperature storage from axenic 1 percent proteose peptone broth to bacterized peptone (50 ml of 1 percent proteose peptone in a 250-ml flask, inoculated with *Aerobacter aerogenes* and incubated overnight at 21° or 22°C, diluted before use as 1 part to approximately 70 parts of demineralized water). Cultures of complementary mating types were mixed in depression slides and observed for formation of mating pairs. During the first 10 or 12 hours of conjugation, individual pairs were placed in depression slides and allowed to multiply. Such pairs could be classified into three groups. Some died without dividing or after a few cell divisions. Others separated and divided normally, but could be shown by tests of mating type and serotype not to have completed the reorganization process; they retained their sexual maturity, the parental mating types, and other characteristic phenotypes of the parental strains. The remaining pairs were classified as "viable conjugants." From each culture derived from such pairs we established three sublines by isolating three single cells into separate depressions. After these lines had been serially transferred until maturity, several pairs of sister lines (derived from the same conjugating pair) of different mating types were crossed to obtain the second generation. While crosses of the first generation reveal dominant lethal mutations, the F_2 crosses should reveal recessives.

Two of the Illinois inbred series ("families") of syngen 1 (D1 and F) were used in these studies. Some subcultures of the same strains were maintained at Illinois in axenic peptone, some were sent to ATCC but were untreated, some were frozen and stored at −160°C for 3 days, and some were frozen and stored for 2 months. Sister lines of different mating types maintained at Illinois were also employed in the analysis.

In crosses to sister stocks to obtain first-generation progeny (Table 1), the frozen strains (Table 1, crosses 2, 3, 5, and 6) yielded frequencies of normal conjugants as high as those from parallel crosses with unfrozen strains (Table 1, crosses 1 and 4). Similarly, in crosses between different inbred strains, the frozen strains (Table 1, crosses 9 and 10) performed at least as well as the control crosses (Table 1, crosses 7 and 8).

Crosses were made between sublines of single pairs derived in the first experiments, so that homozygous recessives could be established (Table 2). The crosses with frozen parents were generally as satisfactory as those with unfrozen parents. One cross of frozen parents gave a very high percentage of nonconjugants, but so also did one cross in which neither parent had been frozen. Since each of these crosses was made between sister lines derived from the same pair in the original (first generation) cross, their poor performance can be accounted for by some abnormality in the individual pair involved. Certainly the available data provide no suggestion of a dramatic increase in genetic damage.

Our data suggest that collections of breeding strains of *Tetrahymena* can be maintained with minimum effort. Most of the genetic work with *Tetrahymena* has been carried out with a limited number of strains of syngen 1, because the strains of other syngens, although capable of mating, have become sterile since they were collected. For studies of other syngens, new col-

Table 1. Crosses involving cultures recovered from storage at low temperatures. Thirty pairs were examined for each cross. Abbreviations: A, unfrozen; B, frozen and stored 3 days; C, frozen and stored 2 months; P, Illinois stock; S2, Illinois stock of a sister line of different mating type; D1 and F, inbred stocks of syngen 1.

Cross		Nonconjugants (No.)	Deaths (No.)	Viable conjugants (%)
No.	Type			
1	D1-A × D1-S2†	2	10	60
2	D1-B × D1-S2	4	0	87
3	D1-C × D1-S2	3	0	90
4	F-A × F-S2	1	0	97
5	F-B × F-S2	0	0	100
6	F-C × F-S2	0	4	87
7	D1-P × F-P	5	4	70
8	D1-A × F-A	0	7	77
9	D1-B × F-B	1	0	97
10	D1-C × F-C	4	2	80

Table 2. Crosses involving first-generatiion progeny of cultures recovered from storage at low temperatures. Crosses D1-C × D1-S2, F-B × F-S2, and F-C × F-S2 are intrastrain crosses in which one parent had been frozen and one was untreated. Crosses Dl-P × F-P and D1-A × F-A are interstrain crosses in which both parents were untreated. Crosses D1-B × F-B and D1-C × F-C are interstrain crosses in which both parents had been frozen. A, unfrozen; B, frozen and stored 3 days; C, frozen and stored 2 months; P, Illinois stock; S2, Illinois stock of a sister line of different mating type. D1 and F, inbred stocks of syngen 1.

Cross	Pairs (No.)	Nonconjugants (No.)	Deaths (No.)	Viable conjugants (%)
D1-C × D1-S2				
1	30	0	0	100
2	18	0	0	100
3	30	0	1	97
4	30	1	1	93
5	29	2	2	86
F-B × F-S2				
1	30	0	0	100
2	30	0	0	100
3	18	0	0	100
4	18	0	0	100
5	30	0	1	97
6	30	0	6	80
7	30	0	10	67
8	18	0	6	67
F-C × F-S2				
1	30	0	0	100
2	30	1	0	97
3	30	1	0	97
4	24	0	4	84
5	15	0	4	73
6	24	1	8	62
D1-P × F-P				
1	30	1	1	93
2	30	0	2	93
3	30	0	3	90
4	30	2	5	77
5	24	0	7	71
6	30	26	0	13
D1-A × F-A				
1	30	0	0	100
2	30	0	0	100
3	18	0	0	100
4	30	1	0	97
5	30	0	10	67
6	18	1	6	61
D1-B × F-B				
1	30	0	0	100
2	30	0	0	100
3	30	0	1	97
4	30	0	1	97
5	30	0	1	97
6	27	0	1	96
D1-C × F-C				
1	30	0	0	100
2	27	0	0	100
3	30	0	1	97
4	18	1	0	94
5	30	0	3	90
6	48	47	1	00

lections must be made and characterized, and, as soon as possible, newly acquired strains must be frozen and stored to prevent genetic deterioration. Ideally, reservoirs of cultures should be maintained in several locations. Technical problems still remain, however. The fraction of viable cells recovered is still low, and the procedure appropriate for syngen 1 may not be applicable to other syngens.

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Siliceous Urinary Calculi in Calves: Prevention by Addition of Sodium Chloride to the Diet

Abstract. *Urinary calculi were found in all but one of 14 calves given a ration associated with the formation of siliceous calculi. No calculi were found in a similar group of 14 calves given the same ration with sodium chloride added (4 percent). It is suggested that sodium chloride prevented calculus formation by increasing water intake and urine volume, with a consequent reduction in the concentration of silicic acid in the urine.*

Urinary calculi composed largely of amorphous silica occur frequently in range cattle in the northern Great Plains of North America (*1, 2*). Obstruction of the urethra is a major cause of death among cattle in this area. During the fall and winter, when the incidence of urethral obstruction is highest, the silica content of the native prairie grasses is often higher than during spring and early summer (2, 3). In most of the grass species, however, the content of silica rarely falls below 2 percent at any time. The concentration of silicic acid in the urine of ruminants that consume mature prairie grass is often two to three times the concentration of a saturated solution (about 19 mg/100 ml at body temperature) and markedly higher than the concentration in urine when leguminous forages are consumed (*4*). The higher concentration in the urine of animals that consume prairie grass is due both to a lower urine production and a higher rate of silicic acid excretion.

Silicic acid polymerizes in simple aqueous solution when the concentration exceeds the saturation value (*5*). The rate of polymerization increases exponentially with increase in silicic acid concentration (*5*); it also increases with rising temperature and in the presence of electrolytes (*5*). Both the high concentration of silicic acid and the presence of electrolytes in the urine of ruminants that consume prairie hay would favor rapid polymerization of the silicic acid. The evidence suggests, on the other hand, that polymerization does not occur in urine when the concentration of silicic acid is below saturation (*6*). Since polymerization of silicic acid is involved in the formation of calculi, it is reasonable to assume that calculi will not form in cattle that are consuming prairie grass as long as the concentration of silicic acid in the urine is kept below 19 mg/100 ml. The experiment reported here supports this assumption. Calculus formation was prevented in calves receiving a prairie-grass hay diet supplemented with enough sodium chloride to increase the urine volume and maintain the silicic acid concentration below the saturation value.

Each member of two groups of 14 steer calves, 10 Holstein and 4 Jersey, was given pelleted prairie grass hay as desired from birth to 10 months of age. The predominant grass in the pellets was *Festuca scabrella* Torr. The pellets given to one group contained 4 percent sodium chloride but those given to the other group contained none. For the first 5 months, the Holsteins received 4.5 kg of milk per day and the Jerseys received 2.7 kg. Water was always available to both groups. The prairie hay contained 6.5 percent protein, 10.4 percent ash, 6.3 percent silica, and 4.1 percent nonsilica ash.

Water intake was measured during the last 2 weeks of the experiment, and on 2 consecutive days during this period urine samples were obtained and analyzed for silicic acid (*7*). At the end of the experiment all calves were killed, and their kidneys and bladders were examined for calculi. Calculi in the bladder were obtained by washing the contents with running water into an 80-mesh sieve and in the kidneys by opening and washing each calyx. The calculi were air-dried, weighed, and pooled for silica analysis (*7*).

The mean water intake of the animals given salt was higher than in those not given salt (Table 1). Since variations within groups in feed intake were rather large, and water intake is related to feed intake, values for water intake per unit of feed intake are also given in Table 1. There were highly significant differences between groups with respect to the mean silicic acid concentration in the urine. The concentration of silicic acid in the urine of all calves that received the salt supplement was below saturation; the con-

Table 1. Growth rate, water intake, urinary silicic acid concentration, and weight of calculi in kidneys and bladders of calves receiving prairie hay or prairie hay plus 4 percent sodium chloride. Results are given as means and standard errors.

Growth rate (kg/day)	Water intake (kg/day)	Water intake/ feed intake (kg/kg)	Urinary silicic acid (mg/100 ml)	Calculi in kidneys and bladder (mg)
		Prairie hay		
0.76 ± 0.04	19.4 ± 1.37	2.74 ± 0.11	27.2 ± 1.64	49.4 ± 15.0
		Prairie hay plus 4 percent NaCl		
0.71 ± 0.04	29.1 ± 2.21	3.67 ± 0.09	14.8 ± 0.46	0
		Probability		
>.200	<.005	<.001	<.001	<.01