CORTICOTYPE TRANSMISSION IN TETRAHYMENA¹

D. L. NANNEY

Department of Zoology, University of Illinois, Urbana

Received July 8, 1966

RECENT studies on the inheritance of cortical variations in *Paramecium aurelia* (SONNEBORN 1963; BEISSON and SONNEBORN 1965) demonstrate a "maternal" mode of transmission for these traits. Certain kinds of cortical variations, and particularly those observed in duplex cells or associated with inverted ciliary rows, are capable of indefinite persistence in vegetative propagation. When crosses are carried out between clones of distinctive form, the exconjugants characteristically produce clones with the original patterns of organization, even though they have become genically equivalent. Even when the isolated cytoplasm is massively exchanged during conjugation, the exconjugants retain their peculiarities. These observations, with others, virtually exclude any possibility that differences in either the primary structure or the state of activity of nuclear genes can be responsible for the morphological differences, but demonstrate instead that the "hereditary information" underlying the differences in cortical patterns is stored and transmitted by structures associated with the cortex itself.

The crucial questions are those concerning the mechanism of maintenance of cortical specificity. Persistent reports of DNA associated with the ciliate cortex (see e.g. RANDALL and DISBREY 1965) lend plausibility to the suggestion (see LWOFF 1950) that gene-like elements imbedded in the cortex represent peripheral "nucleic" reservoirs responsible in some way for cortical characteristics. Such a suggestion is in harmony with current interpretations concerning the autonomy of other cellular organelles such as plastids and mitochondria (see GIBOR and GRANICK 1964). However, certain observations concerning the mode of origin and patterns of transmission of cortical variants (see SONNEBORN 1950, 1963) raise serious questions concerning the adequacy, or even the utility, of this interpretation, and suggest instead that information concerning supra-molecular patterns may in some instances be maintained and transmitted through grosser structural assemblies and by mechanisms distinct from those operating in nucleic transmission.

The purpose of this communication is to describe the mode of transmission of certain cortical variants in *Tetrahymena pyriformis* and to explore its significance for the larger questions at issue.

MATERIALS AND METHODS

The strains of *T. pyriformis* were representatives of three of the inbred "families" of syngen 1, designated as A, A3 and B. Stock cultures were grown axenically in tubes containing 10 ml

¹ This study was supported by grants from the Public Health Service (GM-07779).

of 1% proteose peptone and were maintained by loop transfers once a month. When maintained in this fashion all cultures are morphologically heterogeneous. The number of ciliary rows (meridians or kineties) may vary from about 15 to 35. Duplex forms are common in older cultures. Other variations, correlated in a systematic way with meridian number, are also apparent but will not be considered here in any detail (NANNEY 1966a, b, c).

The stability of meridian numbers was assessed by means of recloned lines. Single cells were isolated into depression slides containing, in addition to the peptone medium, streptomycin and penicillin to reduce the possibility of contamination. The developing cultures were subsequently tubed in 10 ml of medium and maintained at room temperature, ca. 24°. After 2 or 3 days each tube culture was fed an additional 5 ml of culture medium and harvested 2 to 4 hours later. The cultures were fixed and stained by the Chatton-Lwoff silver impregnation technique (CORLISS 1953). The numbers of ciliary meridians were ascertained on samples of 50 cells from each culture.

Crosses were made after centrifuging the parental cultures and resuspending the cells in deionized water. Conjugating pairs were isolated into depression slides and, following pair separation, one of the exconjugants was removed to another container. The exconjugants were thereafter treated in the same manner as the vegetative clones. Approximately 20 fissions intervene between isolation and harvest. Each exconjugant clone was screened for evidence of true conjugation either through mating tests (true conjugants are immature), or serotype tests (true conjugants have different antigenic properties), or both.

RESULTS AND ANALYSIS

Vegetative Stability of Corticotypes. Recloned lines from heterogeneous cultures usually produce after 20 fissions populations which are again heterogeneous but in which the variation is much more restricted than in the parental culture. In some cases samples of 50 cells include only a single type. Significantly, the monotypic clones usually consist of cells with 18 or 19 meridians (corticotypes 18 or 19) though rarely monotypic clones of higher or lower corticotypes are observed. This observation establishes the "stability center" of the corticotypic distribution in the vicinity of 18 or 19, and permits the assessment of changes in the other clones. Thus, clones with predominantly type 17 cells usually have a few variants with 18, and clones largely composed of cells with 20 meridians usually have a few with 19. These are interpreted as clones initiated by cells with 17 (or 20) meridians which are transforming toward the stability center by the gain (or loss) of meridians.

Moving further from the center in both directions the clones become progressively more heterogeneous and the modal corticotype may no longer represent the type of the initiating cell; in these cases the "initial type" is not ascertained by the mode, but rather by the class which is most distal from the stability center. Because the stability of corticotypes decreases progressively with distance from the center, a point is eventually reached at which the initial type may not even be represented in the final distribution. In the absence of a technique for determining the corticotype of a cell whose progeny are to be examined, this difficulty cannot be overcome at the present time, even though the direction of the bias introduced is obvious.

In Table 1 are presented the distributions of corticotypes in 184 recloned cultures classified according to the presumed corticotypes of the initial cells. The frequency of the initial type is presented in the center (I) column, and the

TABLE 1

Corticotype distributions in 20-fission clones of syngen 1, T. pyriformis

Initial corticotype	Corticotype distribution														Total clones examined
	—9	—8	—7	—6	—5	—4	—3	—2	—1	<i>I</i> *	+1	+2	+3	+4	
14	..	..	..	..	..	..	..	..	..	12.0	24.0	32.0	30.0	2.0	1
15	..	..	..	..	..	..	..	..	..	3.0	87.0	9.0	0.0	1.0	2
16	..	..	..	..	..	..	..	..	..	85.7	13.4	1.0	..	..	15
17	..	..	..	..	..	..	..	..	0.9	94.7	4.4	..	..	..	9
18	..	..	..	..	..	..	..	..	1.6	89.4	8.8	0.3	..	..	28
19	..	..	..	..	..	..	..	0.1	1.0	98.3	0.6	..	..	..	20
20	..	..	..	..	..	..	..	..	8.0	92.0	..	..	..	..	6
21	..	..	..	..	..	..	0.3	2.1	22.4	75.0	..	..	..	..	20
22	..	..	..	..	..	..	0.6	7.6	48.0	43.0	0.9	..	..	..	27
23	..	..	..	..	..	..	..	4.4	59.6	36.0	..	..	..	..	5
24	..	..	..	..	..	..	4.3	26.2	51.9	17.6	..	..	..	..	17
25	..	..	..	..	..	1.8	13.8	36.4	39.8	8.2	..	..	..	..	9
26	..	..	..	..	..	1.0	14.0	42.2	34.8	8.0	..	..	..	..	10
27	..	..	..	1.3	2.0	6.0	23.3	32.0	28.0	7.3	..	..	..	..	3
28	..	..	..	..	..	4.0	12.0	28.0	34.0	22.0	..	..	..	..	1
29	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0
30	..	..	2.0	12.0	36.0	34.0	12.0	4.0	0.0	2.0	..	..	..	..	1
31	..	2.0	2.0	22.0	54.0	12.0	2.0	2.0	2.0	2.0	..	..	..	..	1
32	..	4.0	12.0	18.0	34.0	16.0	6.0	8.0	0.0	2.0	..	..	..	..	1
33	2.0	12.0	16.0	18.0	24.0	20.0	4.0	2.0	0.0	2.0	..	..	..	..	1
34	10.0	20.0	30.0	30.0	4.0	4.0	0.0	0.0	0.0	2.0	..	..	..	..	1

The initial (*I*) corticotype is identified as the modal type for clones near the stability center and as the type most removed from the center for displaced distributions.

* *I* = fraction of initial type in final population.

number of clones represented is shown on the right. Note that the stability of the initial type rises up to corticotype 19 and falls thereafter. The two exceptions (corticotypes 14 and 28) are represented by single clones. Note also that the frequency of the initial type remains constant through corticotypes 30–34. This is clearly an artefact, however, because at least one of the 50 cells has to be in the initial class in order to identify it. Clones characterized as initial types 27, 26, or 25, are probably in many cases derived from initial cells with higher numbers of meridians.

These data may be used to define more precisely the stability characteristics of the individual corticotypes. Such an attempt employing more limited data has been presented earlier (NANNEY 1966a). One may first estimate the stability simply on the basis of the fraction of cells remaining in the “initial” class. This unchanged fraction may be equated to the first Poisson term, e^{-x} , and x , the mean rate of change for the growth interval, can be estimated. Since the growth interval involves about 20 cell divisions (fissions), and since the number of variants should be approximately proportional to the number of divisions, x/F is an estimate of the rate of transformation per cell division.

One may also estimate the rate of change for a corticotype even when it is not the initial type. In a distribution such as that for initial type 27 in Table 1, 92.7%

of the population either are or have been type 26. Since 28% are still type 26, the unchanged fraction for type 26 is 28/92.7. Other estimates for the stability of type 26 can be obtained from higher distributions, and this same distribution provides estimates for the stability of a series of lower corticotypes. Estimates of stability based on the frequencies of secondary transitions (changes from a type other than the initial type) do not, however, have precisely the same significance as estimates based on primary transitions (changes from the initial type). More specifically, the derived type is not originally present in the culture, and hence does not have the entire growth interval to generate new types. To adjust the rate of change estimated for secondary transitions, account must be taken of the number of cell divisions undergone by the relevant subpopulation. Thus, if half the final population is made up of a secondary type and its derivatives, that portion of the population has undergone 19 instead of 20 fissions. If a fourth of the population is in the significant portion of the distribution, it has undergone only 18 fissions, etc. To transform an estimate of change for the growth period into an estimate for the rate per fission, x may be divided by the number of fissions. All estimates of secondary stabilities were pooled and weighted according to the size of the relevant populations. Thus, when a secondary type and its derivatives constituted more than 50% but less than 100% of the population, the fission number was set at 19; when it was more than 25% but less than 50% the fission figure used was 18, etc. The sample size (N) consists simply of the total numbers of cells in the relevant portions of the pooled populations. Summed sample sizes smaller than 20 were not tabulated. The results of this analysis are presented in Table 2.

The rates of change based on transformation from the initial types ($F = 20$) delineate the "stability curve". The probability of change for types 14 and 15 is greater than 10% per cell division, but drops to less than 1% for corticotypes 17–20. The most stable type is corticotype 19 (not 18 as was previously estimated (NANNEY, 1966a) on the basis of limited data in this region), and the rate of change is approximately 1 per 1000 cell divisions. Actually, even this rate may be overestimated, because errors in ascertainment are almost certainly made at least this often. Above the point of maximum stability the rate of change rises progressively to the limit capable of being estimated with a sample of only 50 cells.

The secondary transformations yield a picture which is quantitatively different. In the highest corticotypes for which data are available (31–33), the rate of change estimated is higher for secondary than for primary transformations. This is simply a reflection of the artificial constraints on estimates of primary transitions, which do not apply to secondary changes; 100% change can be inferred from the presence of at least one cell in a higher corticotype. The secondary changes for corticotypes 21–27 are consistently less common than the primary changes. 19-fission subclones have a rate of change which is half or less than that observed in 20-fission clones. Subclones of smaller size have even lower rates of change which approach only 10% of the rate estimated for initial types. Below the stability center the disparity between rates of primary and secondary change is not so obvious, but the data are not sufficiently numerous to exclude a difference.

TABLE 2

Estimates of rates of corticotypic transition for each of the corticotypes (T)

T	F	N	e^{-x}	x	x/F	T	F	N	e^{-x}	x	x/F
14	20	50	.120	2.12	.106	25	20	450	.082	2.50	.125
15	20	100	.030	3.51	.176		19	557	.399	.92	.048
	19	44	.253	1.37	.072		18	37	.784	.24	.013
16	20	750	.857	.15	.007		17	22	.773	.26	.015
	19	129	.798	.23	.012	26	20	500	.080	2.53	.127
17	20	450	.947	.05	.003		19	220	.391	.94	.049
	17	106	.943	.06	.003		18	68	.603	.51	.028
18	20	1400	.894	.11	.006	27	20	150	.073	2.62	.131
19	20	1000	.983	.02	.001		19	196	.311	1.17	.062
20	20	300	.920	.08	.004		18	24	.375	.98	.054
	17	430	.914	.09	.005	28	20	50	.220	1.51	.076
21	20	1300	.750	.29	.015		19	218	.174	1.75	.092
	19	757	.855	.16	.008	29	19	234	.068	2.69	.143
	16	49	1.000	.00	.000	30	20	50	.020	3.98	.199
22	20	1350	.430	.84	.042		19	195	.046	3.08	.162
	19	160	.932	.07	.004	31	20	50	.020	3.98	.199
	17	330	.833	.18	.011		19	147	.007	4.96	.261
23	20	250	.360	1.02	.051	32	20	50	.020	3.98	.199
	19	935	.648	.43	.023		19	98	.000	...	...
	17	75	.934	.07	.004	33	20	50	.020	3.98	.199
24	20	850	.176	1.74	.087		19	49	.000	...	...
	19	699	.557	.59	.031	34	20	50	.020	3.98	.199
	18	49	.715	.34	.019						

A fission interval (F) of 20 is assumed for "initial types"; an F of 19 represents a subpopulation making up 50 to 99% of the population; F of 18 represents subpopulations of 25 to 49%, etc. N is the pooled sample size for all relevant subpopulations. The term x/F is an estimate of rate of change per cell division.

This difference in the rates of primary and secondary changes has been noted before (NANNEY 1966a) and is supported by the additional data reported here. A possible interpretation is that cells displaced from the stability center move toward it not only through the discrete shifts which can be scored (gains or losses of meridians) but also through cryptic adjustments of cortical organization which facilitate the more readily discernible alterations. Thus, a cell which has just gained (or lost) a meridian may not have the same properties as another cell with the same meridian number, but which has had that meridian number for several cell generations. All secondary transitions occur in cells which have gained or lost a meridian within the 20-fission growth cycle. The initial cells, in contrast, represent a mixed population; some may have just changed their meridian numbers while others may have persisted in a particular corticotype for an extended period of time before their isolation and may have undergone changes which make them highly susceptible to meridional modifications. This interpretation would account

for much of the heterogeneity of initial transformation rates previously noted (NANNEY 1966a).

This heterogeneity introduces complications in analyses such as that attempted here. Observations on single clones, or even groups of clones from a single source, are not very reliable, and care must be taken in interpreting differences within and between strains. The data summarized in Table 1 come from several sources. The clones include lines from inbred series A, A3 and B which were established by recloning stock cultures. They also include "hybrids" of crosses between A and B and $A3 \times B$; all of these latter were established as exconjugant clones. The question arises as to whether all the different inbred series have precisely the same stability characteristics, and whether the hybrids have distinctive properties. One might also ask whether the very act of conjugation has consequences for cortical organization, either to stabilize or destabilize corticotypes. Attempts to answer these questions have been made, but convincing answers are not available. Occasionally differences are observed among the groups for particular corticotypes, but the lack of an overall pattern of differences, combined with the great variance of the individual estimates, makes interpretation hazardous. For this reason the estimates from all sources have been pooled; if differences among the

TABLE 3

Corticotype distributions in exconjugant clones in cross 1 of $A \times B$

	Corticotype								
	19	20	21	22	23	24	25	26	27-32
A parent	..	1	49	..	..	..	..	..	..
B parent	..	..	..	..	..	2	6	9	34
Progeny									
1a	..	..	50	..	..	..	..	..	..
1b	..	..	..	3	19	25	3	..	..
2a	..	1	49	..	..	..	..	..	..
2b	..	..	..	3	19	26	2	..	..
3a	..	2	48	..	..	..	..	..	..
3b	..	..	..	..	5	17	22	6	..
4a	1	3	46	..	..	..	..	..	..
4b	..	..	..	1	8	30	11	..	..
5a	..	2	48	..	..	..	..	..	..
5b	..	..	..	2	23	25	..	..	..
6a	..	8	42	..	..	..	..	..	..
6b	..	..	..	..	2	23	23	2	..
7a	..	50	..	..	..	..	..	..	..
7b	..	..	1	3	27	19	..	..	..
8a	..	1	49	..	..	..	..	..	..
8b	..	..	..	..	5	18	21	6	..
9a	..	4	46	..	..	..	..	..	..
9b	..	..	..	2	13	19	13	3	..
10a	..	10	40	..	..	..	..	..	..
10b	..	..	..	2	21	21	6	..	..

groups do exist they must be slight and a large investment of effort would be required to demonstrate their reality.

Corticotype transmission through conjugation. We have already suggested that the corticotype distributions in exconjugant clones probably differ in no systematic way from the distributions in other clones. This conclusion remains to be documented. Table 3 summarizes the observations made on ten pairs isolated from a cross between two recloned lines, one from family A and the other from family B. The A parent was almost uniformly type 21, but the B parent had the broad distribution characteristic of very high clones; the range was from 24 to 32. The exconjugant clones are arranged so that the "a" member of each pair has the lowest distribution of meridians. We note that each pair yielded one exconjugant clone with predominantly 20 or 21 meridians and one clone with a broad distribution ranging usually from 22 to 26. In no case do the distributions of the two exconjugant clones overlap. In the one pair in which the "high" distribution overlapped into the usual range of the low clones (pair 7), the low clone was uniformly type 20. The only reasonable interpretation of these distributions is that the exconjugant from the A parent yielded a low distribution and that the exconjugant from the B parent produced a high distribution.

The nine exconjugant clones with initial types of 21 produced a total of 32/450 cells with lower corticotypes; thus the fraction unchanged is 418/450 or .930. This value is higher than the .750 calculated in Table 2, suggesting a slightly greater stability either for cells undergoing conjugation, for cells conjugating with other cells of more distal corticotype, or for this genotype as opposed to others. The single clone (7a) showing only corticotype 20 was probably derived from a parental cell with 20 meridians.

The clones with high distributions, presumably derived from the B parent, consist of two with an "initial type" of 24, five with an initial type of 25, and four with an initial type of 26. The "unchanged fractions" for these three groups are .440, .110, and .085 in comparison with the values of .176, .082, and .080 in Table 2. Considering the sample sizes, the agreement is acceptable. Certainly no suggestion is available of a tendency to move toward the mate of lower corticotype. We must note, however, that in the parental B culture only 34% of the cells were in the range of 24–26, even though all ten of the exconjugant clones were observed here. Some of the exconjugant clones must have abandoned their "true" initial type completely. This, however, is also true of cells with high corticotypes which have not undergone conjugation. Moreover, conjugation in a heterogenous culture may not be entirely random; cells with lower corticotypes may have some selective advantages in mating. In any case, distinctive properties for exconjugants as opposed to vegetative cells have not yet been demonstrated.

In Table 4 are presented the results of another cross between A and B clones, in which an attempt was made to study the effects of conjugation between clones with minimal differences. The A parent was a recloned line of uniform corticotype 18; the B parent was almost uniform type 19. Again each pair produced one exconjugant clone with a low corticotype distribution and one with a high; the only exception is pair 16 in which both parents are low. This pair probably

TABLE 4

Corticotype distributions in exconjugant clones in cross 2 of A × B

Corticotypes					Corticotypes					
	17	18	19	20		17	18	19	20	21
A parent	..	50	..	..		..	..	..	..	..
B parent	..	1	49	..		..	..	..	..	..
Progeny					Progeny					
1a	3	46	1	..	9a	..	36	14	..	..
1b	1	1	48	..	9b	..	..	49	1	..
2a	1	47	2	..	10a	..	49	1	..	..
2b	..	1	48	1	10b	..	..	3	45	2
3a	..	13	34	3	11a	..	49	1	..	..
3b	..	..	48	2	11b	..	..	50	..	..
4a	..	48	1	1	12a	..	47	3	..	..
4b	..	..	49	1	12b	..	..	50	..	..
5a	..	46	4	..	13a	..	8	42	..	..
5b	..	..	50	..	13b	..	..	50	..	..
6a	..	48	2	..	14a	..	50	..	..	..
6b	..	..	50	..	14b	..	..	50	..	..
7a	..	46	4	..	15a	..	49	1	..	..
7b	..	1	49	..	15b	..	1	49	..	..
8a	..	49	1	..	16a	..	50	..	..	..
8b	..	1	49	..	16b	..	50	..	..	..

involved one of the rare 18-rowed B cells. The initial 18's in this sample give an unchanged fraction of 681/850 or .800. The stability of corticotype 18 is thus considerably lower than that (.894) in the total sample of 28 clones (including these 17) recorded in Table 2. Thus, some evidence is available to suggest a tendency to modulate the corticotype toward that of the mate, but the effects of genotype and conjugation *per se* cannot be excluded. The level of stability of the clones with initial corticotypes of 19 is similar to that observed with vegetative clones, but the changes in this sample are predominantly upward instead of downward, as is usually observed. These 19-rowed cells do not tend to modulate toward their lower corticotype mate but rather move in the opposite direction. One completely atypical clone (10b) may represent an extreme manifestation of this trend; it was not included in the data summarized in Table 2.

The final cross to be considered was between an A3 clone below the stability center and a B clone which was above (Table 5). This cross was carried out early in the investigation before staining procedures had been perfected and before much experience in counting meridians had been obtained. Moreover it was conducted under rushed circumstances which caused experimental errors; some of the pairs had to be excluded because one of the presumed exconjugant clones produced a bimodal distribution, one mode characteristic of each parent. This is the result expected when the cell removed following conjugation is not the exconjugant but one of its products. In any case the 12 pairs shown in Table 4 appear to be reliable and the cross is consistent with the later studies. Each of the pairs

TABLE 5

Corticotype distributions in exconjugant clones in a cross of A3 × B

		Corticotypes							
		16	17	18	19	20	21	22	23
Parents		37	12	1	..	..	4	39	7
Progeny	1	50	..	..	..	47	3	..	..
	2	44	6	..	1	19	30	..	..
	3	47	3	..	2	25	20	3	..
	4	47	2	1	..	7	32	11	..
	5	..	49	1	..	3	30	17	..
	6	50	..	..	..	2	30	18	..
	7	49	1	..	..	..	17	33	..
	8	28	22	..	..	4	25	20	1
	9	21	28	1	..	2	25	22	1
	10	31	19	..	..	..	27	22	1
	11	43	7	..	..	..	26	23	1
	12	48	2	..	..	2	18	29	1

again produced one exconjugant clone with a low distribution and one with a high distribution; no overlap is apparent after 20 fissions. Eleven of the low initials seems to have come from a type 16 cell and one from a 17. Two of the high initials appear to have been derived from a type 21 cell and the remainder probably came from cells with 22 meridians. The rare cells with 23 meridians could be due to miscounts.

Doublet distributions in stock and recloned cultures. One other observation made during this investigation, though somewhat peripheral to the purpose of this paper, should perhaps be recorded. We earlier (NANNEY 1966a) reported that duplex individuals with two sets of oral structures and other organelles were frequently observed in cultures with higher corticotypes. Essentially no cells of corticotype 22 or lower were doublets and nearly all of corticotype 28 or higher were doublets. In the range between 23 and 27, however, the distribution of doublets was erratic. In some cultures, for example, all cells with corticotype 25 were duplex, while in other cultures no cells of this corticotype were duplex. All these observations, as well as others on oral morphogenesis, were made on stock cultures of various ages since transfer.

TABLE 6

Comparison of corticotypic distributions of duplex cells in stock and recloned cultures

		Corticotype									
Source		20-21	22	23	24	25	26	27	28	29	30-34
Stock cultures	Number	7	10	23	62	41	7	..	..	..	..
	% duplex	14	40	70	92	93	100	..	..	..	..
Recloned cultures	Number	7	26	51	90	92	115	79	49	16	15
	% duplex	0	0	0	0	7	24	27	69	81	100

During this investigation attempts were made to obtain recloned doublet cultures, but most isolated doublets yielded cultures with few or no duplex cells. This result is not simply the consequence of modulation toward the stability center because many cells were still within the meridional range in which doublets are seen in stock cultures. The situation is somewhat clarified by the observations recorded in Table 6. Three stock cultures approximately three weeks from inoculation were stained, and 50 cells from each were examined for meridian number and duplex structure. As can be seen, duplex cells with as few as 21 meridians were observed, and over 90% of the cells with 24 meridians were duplex. In contrast, none of the type 24 cells in the recloned cultures was duplex. In both cases the incidence of duplex forms is correlated with the corticotype, but the pattern of correlation is strongly influenced by the conditions of culture.

This observation provides a platform for a cautionary comment. A number of the properties of corticotypes may vary with cultural conditions, and the conditions must be specified when the behavior is characterized. In particular, the stability patterns described here pertain to corticotype transmission in recloned cultures. If these patterns were applicable to all conditions of growth, cultures would be expected to move progressively toward their stability sinks and to remain there. Yet, as mentioned earlier, stock cultures tend to be extremely heterogeneous, and after long maintenance many are found to have mean meridian numbers which are very unstable under conditions of maximal growth. Neither the mechanism for dispersion, nor that for convergence has yet been explored in detail, but they may well be susceptible to environmental manipulation and they may provide the organism with means of adjustment to changed conditions.

DISCUSSION

Are corticotypic variants hereditary? Most clones of *Tetrahymena* are polymorphic; they contain individuals which can be distinguished by counting the numbers of ciliary rows. Many other cortical features are correlated in systematic ways with the degree of cortical compoundness (NANNEY 1966a, b). The purpose of this study was to examine the stabilities of the different corticotypes which make up the polymorphic array, and to determine if possible the physical basis for the differences among the corticotypes. Although many questions remain unresolved, the essential features of the stability patterns are clear. Under the maximal growth conditions employed cells of each of the corticotypes tend to produce at fission progeny with the same number of ciliary meridians as the parent. Changes do occur, however, at rates which can be estimated, and which are characteristic of the individual corticotypes. The most stable corticotypes for syngen 1 are those with 18 or 19 meridians, and changes occur for these only about once in each thousand cell divisions. Corticotypes below the stability center tend to modulate upward and corticotypes above 19 modulate downward, at rates which are proportional to their distance from the center.

Although the rates of change can be ascertained, a decision as to whether

corticotype differences are "hereditary" is compounded by semantic difficulties. The classical operational definition for hereditary differences at the cell level is based on a dilution test. Because the number of macromolecules in a cell is smaller than 10^{13} , and 10^{13} is smaller than 2^{50} , a cell will in the course of 50 fissions distribute its original substance completely and be replaced by individuals many of which have no material relic of their progenitor. If two cells which are different initially produce in a common environment clones whose individuals are still distinguishable after 50 fissions, the initial differences cannot be due simply to materials accumulated or synthesized during their earlier history, but must reflect an active mechanism for the maintenance of biological specificity. The differences are, by this definition, "hereditary" differences. The difficulty with such a definition is that it is too conservative. If the differences between the clones persist, they are unambiguously hereditary; but the failure of the differences to persist in *all* the members of the clones is not a sufficient basis for concluding that the differences are nonhereditary. Some gene mutations, for example, are common enough to yield some overlap between parallel clones which were originally different. To say that hereditary differences are those which *tend* to persist in the material dilution accompanying growth is appropriate; to say that differences which are not *absolutely* permanent are not hereditary is unacceptable. Thus, a definition of hereditary differences which is capable of wide applicability must include some specification of the permissible rates of change. However, the significant parameter is not a particular instantaneous rate of change, but rather the pattern of change of the rate of change.

The dilution definition is based on an implicit model. It assumes that nonhereditary differences are due to distinctive molecules passively carried in the compared cells. In the extreme case one might propose that a single critical molecule (perhaps a stable mRNA molecule) is sufficient to define the differences. Depending upon the number of such molecules, the frequency with which cells lacking the molecules will be produced will initially be relatively low. As dilution continues the rate will increase until the probability of producing a cell lacking the molecule is one half when the mother cell has only one (see for example GIBSON and BEALE 1962). If more than one molecule is required the eventual rate of loss of a distinction will be even higher. Thus, if two molecules are required for the maintenance of a trait, cells with two molecules will produce progeny of which three fourths have lost the distinction (assuming random distribution). Generally, therefore, differences due to a dilutable determinant will be characterized by a progressively increasing rate of convergence for parallel clones, approaching eventually a rate of .50 or higher.

If we now consider corticotype stabilities, and ask whether the differences between a cell with e.g. 16 meridians and another with 22 meridians are "hereditary" the answer is obvious. The clones after 20 fissions have tended to converge certainly, but the rates of convergence in a sensibly equivalent environment become slower and slower as the two populations develop, and not progressively more rapid. Although the clones have not been maintained for 50 cell divisions,

their behavior under such circumstances can be confidently predicted. Corticotypes do not conform to the predictions of the dilution model and must, therefore, be considered to be "hereditary traits".

Where are the hereditary determinants located? Having concluded that corticotypes are hereditary variants, at least within this frame of reference, we must now confront the question of how they are maintained. The first issue to be disposed of is that of the geography of the hereditary mechanism. During conjugation in *Tetrahymena* each of the mating cells produces two haploid gametic nuclei. The male and female pronuclei are in fact produced by a single mitotic division and are, hence, genically alike. The male pronuclei are exchanged and fuse with the female pronuclei to establish identical zygotic nuclei in the two members of a mating pair. From this anlage is generated the entire nuclear apparatus for the exconjugant. Exconjugants are alike in their nuclear apparatus. Yet, as we have seen, exconjugants of diverse corticotype do not become alike, but persist in the structural patterns which characterized them before conjugation. Genic differences are not responsible for the corticotypic variations.

This observation does not alone exclude all kinds of nuclear participation in the maintenance of cortical differences. Examples of uniparental transmission of nuclear differentiation are well known in ciliates (see for example SONNEBORN 1954). These, however, depend upon the capacity of old macronuclei to transmit functional information to new macronuclei through the cytoplasm. The parental distinctions for mating types and serotypes in *Paramecium* persist in the progeny only if the isolated endoplasms of the two conjugants are kept separate. Yet, in *Tetrahymena* (McDONALD 1964) every conjugation is apparently accompanied by massive cytoplasmic exchange, and corticotypic differences nevertheless persist. For this reason the locus for the information for corticotypic distinctions in *Tetrahymena* appears not to be in the nucleus (encoded in either structural or functional states) nor in the isolated cytoplasm, but must be placed in the cortex itself. The evidence from *Tetrahymena* is thus parallel to and consistent with that developed by SONNEBORN and his coworkers for cortical variants in *Paramecium* (SONNEBORN 1963; BEISSON and SONNEBORN 1965).

What is the mechanism of hereditary transmission? As mentioned in the introduction, two general mechanisms of heredity have been considered as possible bases for the perpetuation of cortical variants. The first, or "corticogenic" hypothesis views the ciliate cortex as a semiautonomous genetic system containing its own genetic determinants, associated perhaps with the kinetosomes (LWOFF 1950). The cortical pattern is believed to reflect an underlying corticogenic mosaicism; the kinetosomes underlying specific organelles are genetically differentiated. Unfortunately this interpretation explains very little. The extensive microsurgical studies on larger ciliates (see TARTAR 1961) demonstrate that the morphogenetic activities of a segment of the cortex depend far less on the origin of its components than upon the position of the segment in a larger geometrical pattern. Thus, under normal circumstances new oral structures may arise regularly from one ciliary row (the "stomatogenic kinety") whose components in each generation are linearly related. But, if this row is removed, the activities

usually associated with it are simply shifted to the adjacent kinety. Radical reconstruction of cortical elements often leads to the formation of structures which normally would never be developed from the component parts. The regions of the cortex which fail to produce particular structures are not genetically incapable of doing so.

A different perspective on these problems comes from the studies on the *Paramecium* cortex (SONNEBORN 1963). Duplex cells can be constructed simply by interfering with the separation of conjugating cells, but once developed they yield hereditarily distinct clones. One may scarcely argue that the differences between singlets and doublets are due to genetic differences among their kinetosomes. On the other hand, the pattern of organization possesses hereditary stability. Doublet cells do not yield doublet progeny because of their unique genic constitution (either nuclear or cytoplasmic) but because of their unique cortical organization. They produce doublet progeny because they are themselves doublets. Similarly segments of ciliary rows can be inserted with reversed polarity (BEISSON and SONNEBORN 1965) and these are reliably reproduced in subsequent generations. The inverted regions do not give rise to inverted regions because they have "mutated" kinetosomes, but because the cortical components are inverted; and the modified organization encodes information utilized in morphogenesis.

The conclusion which emerges from these studies, therefore, is that a cell possesses (at least) two classes of structural information: information linearly coded in nucleic templates, either nuclear or cytoplasmic, and information encoded in supra-molecular organization. The nucleic information specifies the macromolecular constituents and these in turn impose certain constraints on grosser structural assemblies. But macromolecular properties do not in fact determine all aspects of organellar and supra-organellar construction. The macromolecules can be assembled into a variety of patterns and a significant factor in cellular morphogenesis is the pre-existing supramolecular organization. The mechanism of organization and transmission employing supramolecular templates has been called "cytotaxis" (SONNEBORN 1963). Cytotaxis may exert its influence at many different levels of organization; in no case are the details of the mechanism clearly understood, but its reality seems firmly established.

SUMMARY

Populations of *Tetrahymena pyriformis* consist of classes of individuals which differ in the numbers of ciliary meridians they possess. The stabilities of the different classes (corticotypes) have been estimated and the corticotypes are shown to be "hereditary". Breeding studies demonstrate that the physical basis for the hereditary differences is associated with the cortex itself and not with the nucleus. The mechanism of perpetuation of patterns of cortical organization is apparently cytotactic rather than cytotenic.

LITERATURE CITED

- BEISSON, J., and T. M. SONNEBORN, 1965 Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. Proc. Natl. Acad. Sci. U.S. **53**: 275-282.
- CORLISS, J. O., 1953 Silver impregnation of ciliated protozoa by the Chatton-Lwoff technique. Stain Technol. **28**: 97-100.
- GIBOR, A., and S. GRANICK, 1964 Plastids and mitochondria: Inheritable systems. Science **145**: 890-897.
- GIBSON, I., and G. H. BEALE, 1962 The mechanism whereby the genes M_1 and M_2 in *Paramecium aurelia*, stock 540, control growth of the mate-killer (μ) particles. Genet. Res. **3**: 24-50.
- LWOFF, A., 1950 *Problems of Morphogenesis in Ciliates*. Wiley, New York.
- MCDONALD, B. B., 1966 The exchange of RNA and protein during conjugation in Tetrahymena. J. Protozool. **13**: 277-285.
- NANNEY, D. L., 1966a Corticotypes in Tetrahymena. Amer. Naturalist **100**: 303-318. — 1966b Cortical integration in Tetrahymena: an exercise in cytogeometry. J. Exptl. Zool. **161**: 307-318. — 1966c Corticotypic techniques in Tetrahymena taxonomy. J. Protozool. (in press).
- RANDALL, J., and C. DISBREY, 1965 Evidence for the presence of DNA at basal body sites in *Tetrahymena pyriformis*. Proc. Royal Soc. London B **162**: 473-491.
- SONNEBORN, T. M., 1950 The kinetosome in cytoplasmic heredity. J. Heredity **41**: 222-224. — 1954 Patterns of nucleo-cytoplasmic integration in Paramecium. Proc. 9th Intern. Congr. Genet. (Caryologia **6** Suppl. Vol.): 307-325. — 1963 Does preformed cell structure play an essential role in cell heredity? pp. 165-219. *The Nature of Biological Diversity*. Edited by J. M. ALLEN. McGraw-Hill, New York.
- TARTAR, V., 1961 *The Biology of Stentor*. Pergamon Press, New York.