The Essential Metabolites of a Strain of *Paramecium aurelia* (Stock 47.8) and a Comparison of the Growth Rate of Different Strains of *Paramecium aurelia* in Axenic Culture

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SUMMARY: *Paramecium aurelia*, var. 4, stock 47.8 (sensitive) grown in an axenic medium required the following amino acids: DL-tyrosine, L-phenylalanine, L-tryptophan, DL-methionine, DL-threonine, L-leucine, DL-isoleucine, L-lysine, L-histidine, L-arginine, and DL-serine. DL-valine, L-proline and glycine were synthesized at a slow rate. Tyrosine, methionine and serine were required by stock 51.7 (sensitive). Other essential metabolites required by stock 47.8 (s) were thiamine, riboflavin, folic acid, nicotinic acid, pantothenic acid, and possibly pyridoxal. 2,6-diaminopurine inhibited growth at concentrations as low as 1-4 $\mu\text{g./ml.}$ The growth rates (expressed as final population density/ml./transfer) of various stocks in identical media are different. An apparent correlation was found to exist between the growth rates and the chromosome number of these stocks, those with the largest chromosome number attaining the highest population density.

Paramecium aurelia was successfully established in axenic culture by van Wagtendonk & Hackett (1949). The heat-sterilized medium was composed of equal parts of a 0.5% yeast autolysate (Basamin-Busch) and an autoclaved, 24 hr. culture of *Aerobacter aerogenes* in lettuce extract. Subsequent work led to the development of a medium consisting of salts, B-vitamins, a plant extract, a yeast extract, proteose-peptone and guanylic and cytidylic acids (van Wagtendonk, Miller & Conner, 1952). The use of this medium made it possible to show that folic acid, riboflavin, thiamine and a steroid were absolute growth requirements for stock 57.1 (s) (van Wagtendonk, Conner, Miller & Rao, 1953; Conner, van Wagtendonk & Miller, 1953).

Additional stocks of *Paramecium aurelia* have now been established in axenic culture and maintained through more than a hundred serial subcultures. The specific amino acid and vitamin requirements of stock 47.8 (s), and a comparison of the growth of various stocks and their requirements are reported here.

METHODS

Five different stocks of *Paramecium aurelia*, variety 4, were examined in these experiments: 47.8 (s), 51.7 (s) and its opposite mating type 51.8 (s), 32.7 (s), and 29.7 (s). None of these stocks contained kappa. Axenic cultures of stock 51.7 (s) were available from earlier experiments, while the other stocks

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were freed from associated bacterial forms by treatment with penicillin after the method of van Wagtendonk & Hackett (1949). The organisms were cultured in 5 ml. of the test media in 15 × 150 mm. Pyrex tubes covered with metal caps. All experimental series were incubated at 27° for 5-day periods for each subculture. At the end of each 5-day incubation period an inoculum (0.1–0.3 ml.) was transferred to the next serial subculture. The total number of paramecia was determined by a direct count of measured samples from each tube and the counts from duplicate tubes were averaged to give the final population number. The growth obtained in the experimental tubes was expressed as a percentage of the control in each series, the control being arbitrarily taken as 100. All experiments were repeated at least once.

The method of preparation of the required unidentified factor(s) from yeast reported by van Wagtendonk *et al.* (1953) was modified. A 1/4 (w/v) suspension of Fleischmann's 'active dry' yeast was prepared by continuously stirring the yeast in distilled water at 50° for 2–3 hr. The suspension was autoclaved for 20 min. at 120° and centrifuged to collect a clear amber supernatant fluid. The supernatant fluid was again autoclaved in a section of cellulose dialysis tubing with one end left open to be tied off immediately after sterilization. This technique allowed for the exhaustive dialysis of large batches at room temperature without incurring contamination. The non-dialysable yeast fraction (NDF) was added aseptically to the basal medium given in Table 1. All the components of the medium may be autoclaved together in final concentration except the NDF and the B-vitamins which must be sterilized separately and added aseptically. All solutions were prepared with double distilled water; the pH value of the medium was 6.8–7.0.

Table 1. *Composition of basal medium for growth of Paramecium aurelia, var. 4, stock 47.8*

A non-dialysable yeast fraction (NDF) was added aseptically to this medium to give a complete medium. The B-vitamins were sterilized separately and added aseptically. The other components were autoclaved together in final concentration.

	Final concn. ($\mu\text{g./ml.}$)		Final concn. ($\mu\text{g./ml.}$)
MgSO ₄ ·7H ₂ O	40.0	Stigmasterol	0.5
Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	10.0	Ca panthothenate	2.0
MnCl ₂ ·4H ₂ O	0.1	Nicotinamide	2.0
ZnCl ₂	0.02	Pyridoxal.HCl	2.0
CaCl ₂ ·2H ₂ O	20.0	Riboflavin	2.0
CuCl ₂ ·2H ₂ O	2.0	Folic acid	1.0
FeCl ₃ ·6H ₂ O	0.5	Thiamine	6.0
K ₂ HPO ₄	570.0	Nitrogen source: (1 % proteose-peptone or amino acids as given in Table 2)	
KH ₂ PO ₄	570.0		
Ethylenediamine tetraacetic acid	16.0		
Sodium acetate	570.0		
Sodium pyruvate	570.0		

Conner & van Wagtendonk (1955) showed that the steroid requirement of *Paramecium aurelia* stock 51.7, although fairly specific with regard to

molecular configuration, may be satisfied by any one of several steroids. A preliminary report on the need for steroids by stock 47.8 was published by Conner, (1955), and a steroid requirement for *P. multimicronucleatum* has been shown by Johnson & Miller (to be published). Thus, stigmasterol was included in all the media used in these experiments.

RESULTS

The requirement for a non-dialysable factor (NDF) from yeast

Studies are in progress to identify the unknown factor(s) present in the essential fraction from yeast. This component is an absolute requirement for all stocks of *Paramecium aurelia* thus far tested. Although the active yeast fraction used in these experiments remained quite complex, sufficient purification was obtained in the dialysis step alone to allow its use in the determination of several other essential metabolites. Upon dialysis of the initial aqueous extract from the yeast approximately 75 % of the total solids were removed, and a tenfold purification of the activity on a weight basis was effected. The average weight of several NDF preparations was 12.5 mg./ml. The response of stocks 47.8 and 51.7 to different concentrations of the NDF, in the complete medium with amino acids is given in Fig. 1. Concentrations below 0.5 mg./ml. would not support continued growth of the organisms. The non-dialysable yeast fraction gave a very faint ninhydrin reaction, a positive biuret reaction and a positive Benedict's test for reducing sugar after strong acid hydrolysis.

Nitrogen requirements

As shown in Table 3 the nitrogen requirements of *Paramecium aurelia* can be met by proteose-peptone. This material is satisfactory for routine growth and in its presence the requirements for stigmasterol, folic acid, riboflavin, thiamine and the NDF can be shown. However, it became desirable to replace proteose-peptone with known compounds in order to determine whether it was masking additional vitamin requirements, and to allow for a more specific analysis of the nitrogen requirements.

An acid hydrolysis of proteose-peptone was carried out by refluxing it for several hours with hydrochloric acid. The acid was removed by vacuum distillation and with the aid of Amberlite IR-45 (OH), whereupon the hydrolysate was resolved on a two-dimensional paper chromatogram. The following amino acids were identified: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine. The presence of a small amount of cystine was also indicated.

Several preliminary experiments with stock 47.8 to test whether proteose-peptone could be replaced by its acid hydrolysate or by a mixture of the amino acids identified in the chromatogram indicated that either would permit growth provided tryptophan was also added. Different concentrations of the complete amino acid mixture and of some of the individual amino acids were tested to determine their approximate optimum concentrations. Table 2 lists the

Fig. 1. The response of two stocks of *Paramecium aurelia* to varying concentrations of the non-dialysable yeast fraction (NDF) in the complete basal medium with amino acids. Concentrations of the NDF below 0.5 mg./ml. would not support continued growth of the organisms. ○, stock 47.8; ●, stock 51.7. The final population was determined 5 days after inoculation.

Table 2. *Mixture of amino acids used to replace proteose-peptone in the basal medium for the growth of Paramecium aurelia, var. 4, stock 47.8*

	Final concn. (μ g./ml.)		Final concn. (μ g./ml.)
L-Tryptophan	50	DL-Serine	200
DL-Methionine	150	L-Phenylalanine	75
DL-Threonine	150	L(-)-Tyrosine	50
DL-Isoleucine	150	L-Proline	50
L-Leucine	150	L-Alanine	25
L-Lysine	125	L-Aspartic acid	50
L-Histidine	50	L(+)-Glutamic acid	75
L-Arginine	100	Glycine	25
DL-Valine	75	L(-)-Hydroxyproline	100
		L-Cystine	50

concentrations which supported the most favourable growth. These concentrations were used throughout the experiments described below.

The results of experiments with organisms of stock 47.8 to compare the growth obtained with proteose-peptone, its hydrolysate, and amino acids as the nitrogen source are given in Table 3. These results clearly demonstrate that: (1) free amino acids were utilized and served as the major nitrogen source;

(2) the absence of specific single amino acids (tryptophan and tyrosine in this initial series) prevented or markedly limited continued growth; (3) the presence of one or more of the normally non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine and proline) markedly stimulated growth. The absence of cystine or hydroxyproline did not impair growth.

Table 3. *A comparison of the growth of Paramecium aurelia, var. 4, stock 47.8 in media containing different nitrogen sources*

The nitrogen sources were tested in the complete basal medium using the NDF at a concentration of 2.5 mg./ml. A, 1 % proteose peptone; B, an acid hydrolysate of proteose peptone (PP-H); C, PP-H plus tryptophan; D, a mixture of 18 amino acids contained in the PP-H plus tryptophan, as given in Table 2, (AA); E, AA minus hydroxyproline and cystine; F, AA minus tyrosine; G, AA minus alanine, proline, glycine, aspartic acid and glutamic acid.

Medium	Number of serial subcultures								
	1	2	3	4	5	6	7	8	9
	Percentage organisms/unit volume of medium as compared with proteose peptone taken as 100 %								
A	100	100	100	100	100	100	100	100	100
B	34	37	20	10	0	—	—	—	—
C	32	32	55	67	38	57	61	—	—
D	44	42	67	58	40	52	76	82	85
E	42	41	69	50	51	59	58	80	80
F	26	25	38	23	16	9	6	0	—
G	30	35	31	26	23	35	43	44	44

Using the single omission technique, extended series were designed to determine the need by organisms of stock 47 and 51 for each of the following amino acids: serine, methionine, phenylalanine, tyrosine, tryptophan, threonine, isoleucine, leucine, lysine, histidine, valine, and arginine (Table 4). Both stocks require methionine, serine and tyrosine; stock 47.8 also showed the need for histidine.

Two to four mg. NDF/ml. were used in the medium for these initial experiments. The results indicated that either the NDF, at the concentration used, still contained a sufficient amount of protein to mask the detection of all required amino acids, or that the organisms could synthesize most of the amino acids at a slow rate. Subsequent experiments with stock 47.8, in which the concentration of NDF was decreased to 1 mg./ml. (w/v) confirmed the former hypothesis. The final population in the control (the complete amino acid medium) decreased thereby from an average of about 10,000 organisms/ml. to about 4000/ml. (Fig. 1). However, the modification allowed for a more complete analysis of the amino acid requirements. Data obtained from these experiments are presented in Table 5. The single omission of each of the seventeen amino acids showed that eleven of them: tryptophan, methionine, threonine, isoleucine, leucine, lysine, histidine, arginine, serine, phenylalanine and tyrosine were absolute growth requirements for stock 47.8, for in each case no growth was obtained in the third serial subculture.

The need for an exogenous source of valine, proline and glycine for optimum growth is also clear (Table 5). In the first transfer of *Paramecium aurelia* from a stock culture to a valine-less medium, growth was limited to 49 % of the control followed by the stabilization of growth, through seven transfers at a level of 31 % of the control. A more gradual growth decline was apparent in a medium lacking proline or glycine, and the number of organisms became stabilized at 50–40 % of the control through seven subcultures. The omission of alanine, glutamic acid, or aspartic acid, singly, resulted in some growth decline in the initial transfers, but then all such cultures stabilized at numbers equal to or greater than the control. When proline, glycine, alanine, glutamic acid and aspartic acid together were omitted from the medium, growth was limited to about 40 % of that obtained in the complete medium.

Table 4. *A comparison of two stocks of Paramecium aurelia, var. 4, in a complete medium and in media lacking in amino acids*

The complete medium was composed of the basal medium given in Table 1 plus the amino acid mixture given in Table 2 minus hydroxyproline and cystine. All media contained 4 mg./ml. of the non-dialysable yeast fraction (NDF).

Medium	Stock 47.8. No. of 5-day serial subcultures				Stock 51.7. No. of 5-day serial subcultures			
	1	2	3	4	1	2	3	4
	Percentage organisms/unit volume of medium as compared with complete medium taken as 100 %							
Complete	100	100	100	100	100	100	100	100
Complete								
Minus phenylalanine	50	43	41	78	89	86	45	81
Minus tyrosine	69	24	27	0	81	17	0	—
Minus tryptophan	65	30	45	63	89	62	65	100
Minus methionine	19	0	10*	0	45	0	17*	0
Minus threonine	42	38	32	50	78	69	45	57
Minus isoleucine	31	38	36	50	78	24	37	43
Minus leucine	54	43	52	53	100	45	50	65
Minus lysine	85	73	67	78	74	100	62	89
Minus histidine	58	70	47	0	96	100	89	89
Minus arginine	81	51	54	50	74	96	62	51
Minus valine	62	73	54	75	81	79	70	81
Minus serine	38	0	30*	16	59	0	47*	0
Minus alanine, glycine, proline, aspartic acid and glutamic acid	44	48	49	45	87	79	78	74

* Reinoculated.

B-vitamin requirements

The replacement of the complex proteose-peptone component of the earlier medium by amino acids made a re-examination of the B-vitamin requirements for *Paramecium aurelia* possible. Initial experiments with stock 47.8 in the amino acid medium, using high NDF concentrations, clearly showed that this stock required folic acid, riboflavin and thiamine. No indication for the requirement of choline, biotin or vitamin B₁₂ was apparent in any of the

Table 5. *Amino acid requirements of Paramecium aurelia*, var. 4, stock 47.8

The complete medium was composed of the basal medium given in Table 1 plus the amino acid mixture given in Table 2 minus hydroxyproline and cystine. All media contained 1 mg./ml. of the non-dialysable fraction (NDF).

Medium	No. of 5-day serial subcultures			
	1	2	3	4
	Percentage organisms/unit volume of medium as compared with complete medium taken as 100 %			
Complete	100	100	100	100
Minus tyrosine	35	0	—	—
Minus phenylalanine	54	9	0	—
Minus tyrosine minus phenylalanine	43	3	0	—
Minus tyrosine + 3 × phenylalanine	40	18	0	—
Minus tryptophan	32	9	0	—
Minus methionine	24	0	—	—
Minus threonine	30	6	0	—
Minus isoleucine	30	15	0	—
Minus leucine	35	9	0	—
Minus lysine	59	18	0	—
Minus histidine	32	3	0	—
Minus arginine	70	12	0	—
Minus serine	12	0	—	—
Minus valine	49	33	34	33*
Minus proline	78	64	57	51*
Minus glycine	73	45	31	37*
Minus alanine	92	85	100	100*
Minus aspartic acid	57	70	100	100*
Minus glutamic acid	76	97	100	100*
Minus proline, alanine, glycine, aspartic acid and glutamic acid	70	51	43	43*

* Carried through three more transfers without change in the final population density.

experiments. That pantothenate, nicotinamide and pyridoxal might be required was, however, suggested by the inconsistent results which at times showed definite suboptimal growth in the absence of any one of these three vitamins. When using a lower NDF concentration (0.75 mg./ml.) a clearer response to the absence of these vitamins was given. Table 6 shows the results obtained upon the omission of each of these factors both singly and in combination. In addition to folic acid, riboflavin and thiamine, pantothenic acid and nicotinamide are essential metabolites for stock 47.8, while a clearer indication for the requirement or perhaps the suboptimal synthesis of pyridoxal is apparent.

Inhibition of growth by 2, 6-diaminopurine

Hamilton (1953) reported that *Paramecium aurelia*, stock 51.7, grown on cultures of living *Aerobacter aerogenes*, directly utilized adenine, guanine and 2,6-diaminopurine for polynucleotide synthesis. Also a requirement for guanylic acid and cytidylic acid by *Paramecium multimicronucleatum* in axenic culture was reported by Johnson (1952). Young (1955) and Young &

van Wagtenonk (1956) found that *P. aurelia*, stock 51.7 (s), required a uridine diphosphate conjugate for growth in an axenic medium. In the course of testing several nucleic acid derivatives for growth-promoting activity it was observed that 2,6-diaminopurine inhibited the growth of organisms of stocks 51.7 and 47.8 in the range of 1–4 $\mu\text{g./ml.}$ The response of these stocks to increasing concentrations of 2,6-diaminopurine are given in Fig. 2.

Table 6. *B-vitamin requirements of Paramecium aurelia, var. 4, stock 47.8*

The complete medium was composed of the basal medium given in Table 1 plus the amino acid mixture given in Table 2. All media contained 0.75 mg./ml. of the non-dialysable yeast fraction (NDF).

Medium	Number of 5-day serial subcultures			
	1	2	3	4
	Percentage organisms/unit volume of medium as compared with the complete medium taken as 100 %			
Complete	100	100	100	100
Minus folic acid	0	0*	10*	0
Minus riboflavin	34	0	29*	0
Minus thiamine	100	14	0	100*†
Minus pantothenic acid	54	12	0	65*‡
Minus nicotinamide	46	17	0	86*‡
Minus pyridoxal	88	35	49	34§
Minus biotin	79	93	100	100§
Minus choline	94	86	100	100§

* Reinoculated. † No growth in the next subculture. ‡ No growth in the third subculture. § Carried through three additional subcultures without change in the final population density.

Growth rate differences between stocks 47.8, 32.7, 51.7, 51.8, and 29.7 in axenic culture

Differences in growth rate, and thus in final population densities, were immediately obvious among the different stocks when cultivated in an axenic medium. All of the stocks examined have been carried through more than 100 successive transfers and a direct comparison of the growth rates has been made from time to time. The differences in growth rate persist even in the most complex axenic medium employed. The rate of growth in an identical medium always followed the pattern: stock 47 > stock 32 > stock 51 > stock 29. Table 7 shows a direct comparison of these stocks carried simultaneously in the complete amino acid medium containing the optimum concentration of NDF. Both mating types of stock 51 were compared to determine whether a mating type difference might possibly effect a growth rate difference. An interesting comparison can be made between the difference in growth rate of these five stocks and the reported micronuclear chromosome numbers (Dippell, 1954) for these same stocks. The average rate of growth of the stocks parallels roughly the chromosome number. No difference in growth rate was apparent between the two mating types 7 and 8.

Fig. 2. Inhibition of the growth of *Paramecium aurelia* in various axenic media by 2,6-diaminopurine. ●, Stock 47.8 in a proteose peptone-whole yeast extract (4 mg./ml.) medium; ○, stock 47.8 in a proteose peptone + B-vitamins + NDF (2 mg./ml.) medium; ◐, stock 51.7 in a proteose peptone + B-vitamins + NDF (2 mg./ml.) medium. The final population was determined 5 days after inoculation.

Table 7. Differences in final population densities in successive subcultures and in chromosome numbers of five stocks of *Paramecium aurelia*, var. 4

The corresponding subcultures of the different stocks were grown simultaneously in the basal medium plus the amino acid mixtures shown in Table 2 and with a concentration of 1.5 mg./ml.

Stock	No. of 5-day serial subcultures								Mean	Chromosome no.
	1	2	3	4	5	6	7	8		
	Final population $\times 10^{-2}$ organisms/ml.									
47.8	66	90	100	64	76	69	68	60	75	47-51
32.7	—	—	—	40	31	30	32	32	33	44-46
51.7	19	27	38	26	29	20	28	25	27	41-45
51.8	—	29	32	22	20	27	21	25	25	41-45
29.7	15	16	25	21	16	21	16	18	19	33-37

DISCUSSION

Specific amino acid requirements have been reported for only two other ciliates, *Tetrahymena pyriformis* (Kidder & Dewey, 1951) and *Glaucoma scintillans* (Fuller, 1948). Although the basal requirements of *Paramecium*

multimicronucleatum and *P. aurelia* appear to be the same in crude medium, Johnson (1952) was unable to substitute free amino acids for Proteose-peptone in his sterile medium for *P. multimicronucleatum*. The reason for this might be due to the use of an inadequate mixture of amino acids or to the low concentration of B-vitamins in the medium. Another factor which has contributed to the successful establishment of *P. aurelia* in an amino acid medium is the supplement of the essential sterol in a purified uniformly available form.

The replacement of Proteose-peptone with amino acids in the axenic medium for *Paramecium aurelia* constitutes a major step toward the goal of obtaining this organism in a complete chemically defined medium. The fact that growth falls to zero within the first few subcultures when any one of the reported eleven essential amino acids is absent from the medium indicates the lack of ability to synthesize these acids. The more than 75 % diminution of growth in the absence of valine suggests that this amino acid can be synthesized only at a limiting rate by *P. aurelia*; this may also be the case for proline and glycine. A comparative nutritional study of several vertebrates has shown that valine is required by nearly all. Proline has been reported to be required by *Trichomonas foetus* (Weiss & Ball, 1947), *Glaucoma scintillans* (Fuller, 1948), glycine by *Trichomonas foetus*, and by *Aedes aegypti* (Goldberg & de Meillon, 1948). The absolute requirement for serine by *P. aurelia* is paralleled in *Tetrahymena pyriformis*, strain E, as reported by Elliott (1949). A closer examination of the proper concentrations and metabolic interrelationships of the amino acid requirements of *Paramecium aurelia* must await the fuller understanding of other metabolites such as the carbon sources, salts, and other growth factors.

The inability of *Paramecium aurelia* to oxidize phenylalanine to tyrosine is concluded from the fact that an exogenous source of both amino acids is essential for continued growth. Most animals can meet their tyrosine needs when supplied with a source of phenylalanine, although apparently the reverse reaction does not occur; only tyrosine will spare phenylalanine in such cases. Until recently a one-step oxidation was postulated for the conversion of phenylalanine to tyrosine. However, studies of auxotrophic mutants of *Escherichia coli*, K12, strain W (Simmonds, Dawling & Stone, 1954) strongly support the view that the conversion of phenylalanine to tyrosine is indirect, the two amino acids being synthesized from a common precursor by reversible reactions.

The essential NDF component of the present medium remains complex. It contains polysaccharides, some protein, and apparently some non-dialysable vitamin conjugates. The yeast fraction contributes essential nucleic acid derivatives (Young, 1955; Young & van Wagtendonk, 1956). This is supported by Johnson's (1952) demonstration of the need for guanylic and cytidylic acid in *Paramecium multimicronucleatum*.

Little is known of the requirements for recognized vitamins in ciliates other than *Tetrahymena pyriformis*. Kidder & Dewey (1951) recorded that thiamine, riboflavin, the vitamin B₆ group, pantothenic acid, folic acid and nicotinamide are required for *T. pyriformis*. *Colpoda duodenaria* (Tatum, Garnjobst & Taylor, 1942; Garnjobst, Tatum & Taylor, 1943) requires relatively high

concentrations of thiamine, riboflavin, pantothenic acid, nicotinamide and pyridoxine. *Paramecium aurelia* requires thiamine, riboflavin, pantothenic acid, nicotinamide and folic acid. The organism apparently can synthesize pyridoxal (and the other members of the B₆ group) at a suboptimal rate.

The observations on the growth inhibition of two stocks of sterile *Paramecium aurelia* by 2,6-diaminopurine in a very low concentration are in direct contradiction with those reported by Hamilton (1953) for *P. aurelia* grown in living *Aerobacter aerogenes* cultures. Although further work must be done to clarify the phenomenon, this single observation emphasizes the need for employing axenic cultures for many of the studies designed to analyse protozoan metabolism.

The differences in growth rate of stocks 47.8, 32.7, 51.7, 51.8 and 29.7 cannot be satisfactorily explained. The difference is evident in each medium investigated and can therefore not be due to an unknown essential metabolite for the slower growing stocks. If the chromosome differences are indeed due to aneuploidy, as suggested by Dippell (1954), and if the apparent correlation between the chromosome number of the stocks and their rate of growth is valid, the following explanation might be offered. The stocks would differ by having different numbers of whole gene sets through the duplication of whole chromosomes or of parts thereof, rather than differing at the same single locus, or at a few loci through mutations. The differences in growth rates might then be explained by a greater or lesser synthetic capacity according to the number of like sets of genes present through ploidy in part of the genome.

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