GENETIC STUDIES ON THE MATING-TYPE INTERCONVERSION IN *SACCHAROMYCES* YEASTS

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CONTENTS

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PREFACE

For a long time, "Biology" meant the description of the distinctive features of individual organisms: It was "Natural History" rather than "Science". In considering "What is Science?", the history of physics and chemistry show that science has aimed not at describing differences between individual phenomena but at discovering the similarities, in other words, the fundamental principles (or universal rules) by which all phenomena can be explained. It is not surprising, then, that it was a physicist rather than a biologist who first applied this approach to the most fundamental problem in Biology, "What is life?" or "What are the fundamental features of life?" This movement in Biology which grew around an experimental physician, Max Delbrück of the California Institute of Technology in U. S. A. in 1938, has resulted in a central branch of modern biology, molecular genetics, in only three decades. At present, it is no exaggeration to say that the fundamental features of life, for example, the structure and function of the gene, the self-duplicating process of genetic structures, the expression of genetic information and its regulation and so forth, have been generally outlined. In this field, it was natural that prokaryotic microorganisms such as viruses and bacteria, which were recognized to be the most simple form of life, were selected as biological materials to study the fundamental features of life. Although a large number of problems remain concerning detailed biochemical reactions which occur in the fundamental phenomenon of life, I believe it is time to start testing the many concepts of molecular genetics established from studies on bacteria

and viruses, and furthermore, through those studies to approach the biological phenomena unique to higher organisms, development and differentiation, memory and learning, muscle contraction and the nervous system, and so on. Of eukaryotic microorganisms, yeasts are one of the most suitable materials for the study of pure biological science, because they are intermediate in biological complexity between bacteria and the higher fungi, and present many advantages for genetic studies including rapid growth, clonability, and ease of handling and storage: They are also adaptable to replica plating, micromanipulation, and an array of biochemical procedures.

Ultimately, the purpose of science should be to serve man: It should not be pursued for its own sake nor to satisfy scholar's private curiosity. Beyond this, there is no limitation on the subject of the scientists research. But the engineer, especially the bioengineer, must consider whether the biological materials he deals with are suitable for his "Technology". From this point of view, yeast is one of the most important organisms. For many centuries yeast has served man, e.g., in baking, wine-making and several brewing-industries, and nowadays is even used for biomass-production. Therefore, studies on yeasts themselves as objects of "Technology" should provide more useful information for man, and in particular for the fermentation industry. When one considers how microbial genetics can contribute to fermentation industry, i.e., how genetics can be applied for improvement of industrial microorganisms, two major techniques can be imagined. One aims at improvement of the intrinsic ability of microorganisms by mutagenesis, the other at combining useful genes or strengthening metabolic activity

of microorganisms by hybridization or polyploidization. Mutagenesis has been successful in the improvement of industrial microorganisms, as typified by the fermentative production of penicillin; but hybridization or polyploidization as practical techniques have not yet met with success. Nevertheless, improvement of yeast strains used in fermentation industry must be done by hybridization techniques because the nuclear phase of their vegetative cells is generally diploid or polyploid, and recessive mutation induced by mutagenesis cannot be expressed phenotypically. Thus I started this study in the hope that an understanding of the genetic system controlling mating-type interconversion in yeasts would provide new ideas for the improvement of hybridization techniques and the development of more logical procedures for the breeding of yeasts, and that clues to the unique mechanism of sex differentiation of higher organisms might be obtained from yeasts.

In Chapter I, the relationship between genotypes and phenotypes for homothallism is elucidated through identification of three kinds of homothallic genes. Chapter II deals with the location of these three homothallic genes by a novel procedure for mapping (required by the complex corelation of the genotypes and phenotypes in homothallism) along with the conventional method. In Chapter III, it is demonstrated that the homothallic genes can function in vegetatively growing diploid cells, if homozygous for mating type, as well as soon after spore germination. Using this finding, tetraploids were produced from diploid cells by the action of homothallic genes. Finally, several models of mating-type interconversion are discussed in relation to regulatory interconnections between the homothallic genes and several mutations

which affect the mating type-directed phenomena, and the possibility of breeding higher polyploid cells is considered.

CHAPTER I

THE GENETIC SYSTEM CONTROLLING HOMOTHALLISM IN *SACCHAROMYCES* YEASTS

INTRODUCTION

It may be fairly said that yeast genetics began with Winge's discovery of the alteration of the nuclear phase of Saccharomyces cerevisiae. He showed that standard vegetative cells of S. cerevisiae are diploid, produced by copulation of two spores or gamates derived from an ascospore. The diploid nuclei undergo reduction (meiosis) at spore formation to produce four haploid ascospores under appropriate conditions (Winge 1935). This was followed by the observation that when an ascospore is isolated from the ascus by micromanipulation and cultured individually, copulation occurs between two cells and the cell fusion give rise to a zygote from which large ellipsoidal diploid vegetative cells are produced (Winge and Lausten 1937). Strains which exhibit this property are nowadays called homothallic. In 1943, Lindegren and Lindegren (1943) observed an alternative form of behaviour, in strains which are nowadays called heterothallic. In these strains, cells in an isolated spore culture never show cell fusion but produce small, round and clustered haploid cells. By pairing the haploid cultures derived from a single ascus in all possible combinations, they demonstrated that there are two kinds of mating-types, designated a and α, which control the mating response: Mixing cultures of like mating type gave no reaction, whereas mixing cultures of opposite mating-types gave distorted premating cells and, within a few hours, zygotes. The reversion is attained by sporulation with meiosis. This mating-type specificity was found out to be genetically controlled by a single pair of alleles, a and α on chromosome III (Lindegren and Indegren 1943; Hawthorne and Mortimer 1968; Mortimer

and Hawthorne 1969). The discovery of these heterothallic strains promoted the rapid growth of yeast genetics, and interest in yeast has increased greatly. At present, the importance of yeasts as bilogical material not only for genetic but also biochemical and physiological studies is widely recognized, since they grow rapidly, colonize and are easily handled, stored, harvested, and are also adaptable to replica plating and micromanipulation.

However, in the early days, it seems that attention was not given to the reason for the difference between homothallic and heterothallic strains. It was Winge and Roberts in 1949 who first mentioned this problem (Winge and Roberts 1949). They demonstrated that thallism is genetically controlled, by showing Mendelian segregation with respect to thallism on analysis of hybrids obtained by pairing spores of heterothallic S. cerevisiae and homothallic S. chevalieri; the hybrids produced asci in which two spore clones remained haploid and displayed a mating specificity and two spore clones became diploid after a few generations and showed no further mating response. Thus, they supposed a gene, D, for diploidization or homothallism, and thought that the apparent lack of mating specificity in two of the four spore clones was due to the segregation of that gene. It can be said that genetic studies on homothallism versus heterothallism began in this year.

The next step in genetic studies on homothallism was made by Takahashi et al. (Takahashi, Saito and Ikeda 1958; Takahashi 1958). They reported complementary gene system in S. cerevisiae consisting of HM1, HM2 and HM3 in which the HM1, HM2 hm3 and hm1, HM2 HM3 genotypes

gave homothallic character to a culture. The D gene from S. chevarieri was considered to be different from the S. cerevisiae complementary HM gene system since the D gene and HM genes segregated independently (Takahashi and Ikeda 1959). But it was not until 1963 at the XI International Congress of Genetics that Hawthorne gave the first clear explanation of homothallic gene-action (1963a, b). According to his idea, a homothallic gene produces homothallism by accelerating the mutation of a mating-type allele to the alternative form in a certain portion of cells and forming zygotes between a and α cells within a culture. In S. lactis, Herman and Roman (1966) found two unlinked, independently homothallic genes, Ha and Hα, which gave specific effects on the mating type allele, i.e., one locus is effective in changing the mating type from a to α, and the other from α to a. Additional genes for homothallism were described for S. oviformis (Takano and Oshima 1967, 1970b; Oshima and Takano 1971). A gene designated HOα could bring about the change of α to a, but the change from a to α required the presence of a modifier gene, HM, in addition to the HOα gene.

Allelism tests between homothallic genes and gene systems of different origins, i.e. the D gene from S. chevalieri, Takahashi's complementary HM gene system from S. cerevisiae, and the gene system consisting of HOα and HM from S. oviformis, revealed that these genes or gene systems are included in the HOα and HM gene system (Takano and Oshima 1970a). A homothallic strain having the HOα HM genotype showed a perfect homothallic life cycle, i.e., all 4 spore cultures from each ascus gave rise to homothallic diploids (the Ho type of homothallism; Santa Maria and Vidal 1970), while a homothallic culture originated from a spore of

the α HOα hm genotype showed a 2 homothallic: 2a segregation in each ascus. Each homothallic segregant again showed a 2 homothallic: 2a segregation (the Hq type of homothallism; Santa Maria and Vidal 1970). in 1970, however, Santa Maria and Vidal reported another type of meiotic life cycle which gave a 2 homothallic: 2α segregation in each ascus in a strain of S. norbensis (the Hp type of homothallism). This observation suggests an additional genetic factor(s) for controlling homothallism in Saccharomyces yeasts. This possibility was tested by the genetic analysis of various hybrids prepared by crosses of S. norbensis strain SBY 2535 with strains having several different genotypes for the homothallic genes (Oshima and Takano 1972). Results of the genetic analyses showed that the HOα and HM gene system should be revised to a system consisting of HO, HMα, and HMa genes where the two genes HO and HMα correspond to the previous HOα gene and the HMa to the previous HM gene. For the conversion of the a mating-type allele to α, both HO and HMa genes are required, while the conversion of α to a requires the HO and HMα genes. A strain containing the ho allele is a stable heterothallic clone according to this system.

Two types of semi-homothallic life cycle designated as the Hp and Hq types of homothallism have been explained by this model as follows (Oshima and Takano 1972). The homothallic diploid clone showing the Hp type segregation has the a HO hmα HMa/α HO hmα HMa genotype for the homothallic genes. Upon self-sporulation, meiotic division occurs and each ascus contains two a and two α spores at haplophase. Since all these haploid spores are the same for homothallic genes, i.e., the HO hmα HMa genotype, the two a spores give rise to diploid cultures due to

cell fusion subsequent to the conversion of the mating-type allele from a to α by the mutagenic action of the HO and HMa genes in some fraction of cells within a few generations after the spore germination. The latter two α spores, however, are able to produce stable haploid cultures because the HO hmα HMa genotype is not effective for the conversion of α to a. The homothallic diploid segregants will again show a 2 homothallic: 2α segregation by the same mechanism. Similarly the Hq type strain has the a HO HMα hma/α HO HMα hma genotype. In this case, the two α spores formed in each ascus produce diploid cells by self-fertilization and two a spores give rise to stable haploid cultures by single-spore culture.

They also observed in the same report (Oshima and Takano 1972) that 15 of the 77 asci tested showed a 4 homothallic: 0 heterothallic segregation in the crosses of α Hp (an α haploid segregant from the Hp type homothallic diploid) to a Hq (an a haploid from the Hq diploid). The expected genotype of this hybrid should be α HO hmα HMa/a HO HMα hma. Eight asci which showed enough potency of sporulation were selected from these 15 asci and subjected to further analysis. It was found that the four homothallic spore-cultures from each tetrad of these hybrids did not always show a 2 Hp : 2 Hq segregation. Three asci showed a 4 Ho : 0 Hp : 0 Hq segregation, four asci showed a 2 Ho : 1 Hp : 1 Hq segregation and the remaining ascus showed a 0 Ho : 2 Hp : 2 Hq segregation. These data suggest that the genetic activities of HMα and HMa are each controlled by at least two duplicated loci, or there might exist some other mechanism which gives rise to homothallism in the culture. To elucidate this problem, I started this study in the autumn of 1971 as an

undergraduate student, with genetic characterization of the Ho segregants derived from the hybrids of the α Hp to a Hq cross.

In Chapter I, data are described which strongly suggest that the 4 Ho 0 Hp : 0 Hq and 2 Ho : 1 Hp : 1 Hq segregations from the α Hp to a Hq cross were not attributable to independent duplicated genes for the HMα and HMa functions but to the homothallic phenotype (the Ho type) of the HO hmα hma clone or the HMa and HMα functions of the hmα and hma alleles, respectively.

MATERIALS AND METHODS

Organisms: The strains used in this chapter are descendants from the above mentioned crosses (Oshima and Takano 1972) between heterothallic haploid clones having the a HO_{α} hm (now designated a HO HMα hma) genotype and the haploid α clones originated from Saccharomyces norbensis SBY 2535 which was kindly supplied by Dr. Santa Maria of the Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain. According to the description by Santa Maria and Vidal (1970), S. norbensis SBY 2535 is a spontaneous mutant derived from S. norbensis SBY 2314, and is distinguished from its original strain by the higher viability of ascospores at the dissection of asci. The original strain (SBY 2314) was diploid by its cell size (3.2 - 7.8 × 3.2 - 10 μm), cell shape (round or oval, single or in pairs), and the ability to sporulate without preceding cell fusion (Santa Maria 1963). We confirmed these facts with the strain SBY 2535 and observed that this strain is prototrophic

on Burkholder's synthetic minimal medium. It sporulated well on sodium acetate sporulation medium (Fowell 1952) and produced abundant four-spored asci. Results of tetrad dissection of asci showed virtually a 2 homothallic : 2α segregation in the 29 asci tested so far except for 4 irregular asci (3 of them showed a 1 homothallic : 3α segregation and another, a 0 homothallic : 4α segregation). Some of the homothallic segregants from SBY 2535 were subjected to further tetrad analyses and it was observed that these clones showed a 2 homothallic : 2α segregation in every ascus. These observations were well in accord with the descriptions by Santa Maria and Vidal (1970).

The Ho type homothallic strains carrying the D gene, C-1728b-4C and 1932-2A, allelic to the $\underline{HO}_{\alpha}$ $\underline{HM}$ genotype, i.e., the $\underline{HO}$ $\underline{HM\alpha}$ $\underline{HMa}$ genotype by the new knowledge (Takano and Oshima 1970a; Oshima and Takano 1972), were those strains which were kindly supplied by Dr. T. Takahashi of the Brewing Science Research Institute, Suita, Japan and Dr. D. C. Hawthorne of the University of Washington, Seattle, U. S. A. Many other heterothallic haploid stocks for yeast genetics were also used as standards for the determination of mating types. Those strains selected from our stock cultures and the D strains were marked with several authentic auxotrophic genetic markers.

Media: The nutrient medium consisting of yeast extract (Daigo Eiyo Chemicals & Co., Ltd.), 0.5%; polypeptone (Daigo Eiyo Chemicals & Co., Ltd.), 1% dextrose, 4%; KH_2PO_4, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.2%; with or without agar 2% in tap water was used for general use and for the stock cultures. Modified Burkholder's medium supplemented with various amino acids and nucleic acid bases, 0.2mM each (except for L-tryptophane,

0.4mM; DL-threonine, 5mM) was used as synthetic medium for test of the auxotrophic genetic markers.

Techniques: The procedure of Fowell (1952) was followed to promote spore formation; cell's culture for one to five days in nutrient medium were smeared on Fowell's agar on which sporulation was achieved after two days incubation. Tetrad dissection was carried out by the method of Johnston and Mortimer (1959) with the aid of a micromanipulator. Mating types were determined by the mass mating technique with a and α mating-type haploid culture (Lindegren and Lindegren 1943). A clone from a single-spore culture, which did not show zygote formation with either a or α standard cultures, was designated as a non-mater. Almost all non-maters were identified as the homothallic clones by their ability to sporulate, by their cell size which is similar to diploid cells (Takano, Yoshizumi and Terashima 1966), and by the characteristics of cell shape and budding (Townsend and Lindegren 1954). For the isolation of a hybrid by the single-cell isolation technique, a mating culture in a few ml of nutrient medium was subcultured in nutrient or minimal medium, where selective growth of a hybrid cell was achieved by a difference in growth rate from the parental clones. Another hybridization technique, crosses by conjugating a spore to a spore, was carried out under the micromanipulator. For analysis of the nutritional requirement, fresh cells from a slant culture were suspended in 2ml of sterile water and one drop of this suspension containing about 10^4 cells was spotted on agar plate of Modified Burkholder's medium. The requirement was judged by no development of visible colonies after 3 days incubation at 30°C.

RESULTS

Genetic characterization of the Ho segregants derived from the hybrids of the α Hp to a Hq cross: In order to test the genetic behavior of the Ho segregants from the α Hp to a Hq cross, allelism tests were carried out among the four tetrad clones in each one of three asci, i.e., C-18-2, C-18-16, and C-24-15, in which all tetrad clones showed the Ho type of homothallism, as described in a previous paper (Oshima and Takano 1972) and briefly summarized in the Introduction. In a typical experiment, four homothallic clones from a given ascus were sporulated. Spores derived from each homothallic clone were sporulated. Spores derived from each homothallic clone were isolated and crossed with spores from another clone of the same tetrad by spore-to-spore contact on a thin agar film of nutrient medium with the aid of a micromanipulator. Thus, six different hybrids were obtained by crossing the homothallic tetrad clones derived from the same ascus. Results of the tetrad analyses of these hybrids are summarized in Table I-1. It is evident that the Ho clones in each of 3 original asci, C-18-2, C-18-16, and C-24-15, consisted of two different clones, respectively. The C-18-2A clone should have the same genotype as C-18-2D because the hybrid of this combination showed a 4 homothallic : 0 heterothallic segregation in all of the 25 asci dissected. Similarly, the genotype of C-18-2B is the same as C-18-2C and differed from C-18-2A and C-18-2D. Though one ascus showed a 3 homothallic : 1a : 0α segregation, the genotype of C-18-16A must be the same as C-18-16C and different from C-18-16B and C-18-16D. It is also clear that C-24-15A and C-24-15B have the same

Table I-1. Tetrad segregation from hybrids obtained by intra-ascus crosses in asci C-18-2, C-18-16, and C-24-15

Ascus no.	Cross[a]	Segregation in asci (Homothallic[b] : a : α)								
		4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
C-18-2	A × B	3	6	1	6	4*[c]	1*	3*	1*	0
	A × C	4	5	3	9	2*	0	1	0	1
	A × D	25	0	0	0	0	0	0	0	0
	B × C	25	0	0	0	0	0	0	0	0
	B × D	7	4	4	10	0	0	0	0	0
	C × D	13	1	2	6	2*	1*	0	0	0
C-18-16	A × B	13	3	2	5	0	1*	0	0	1
	A × C	24	1	0	0	0	0	0	0	0
	A × D	0	6	3	12	2*	2*	0	0	0
	B × C	8	2	2	10	0	0	0	0	3
	B × D	25	0	0	0	0	0	0	0	0
	C × D	11	2	3	7	1*	0	0	0	1
C-24-15	A × B	25	0	0	0	0	0	0	0	0
	A × C	7	4	3	11	0	0	0	0	0
	A × D	6	3	6	7	1*	0	1*	0	1
	B × C	8	3	2	12	0	0	0	0	0
	B × D	11	4	3	7	0	0	0	0	0
	C × D	25	0	0	0	0	0	0	0	0

[a] Each cross was made by the spore-to-spore mating method.

[b] Three types of homothallism, Ho, Hp, and Hq, were included in this category.

[c] The asci belonging to the ascus-types which could not be expected by hypothesis II were re-examined with the stock cultures. However, these asci marked with asterisks were not examined again, because they had been discarded at the time of re-examination.

genotype and which differes from that of C-24-15C and C-24-15D.

Next, we performed allelism tests among six homothallic clones from these three asci, C-18-2, C-18-16, and C-24-15, using two clones having the different genotypes in each respective ascus, as shown in Table I-2. Results clearly showed that these clones were divisible into two classes. One, which we tentatively called Type I, consisted of C-18-2A, C-18-16B, and C-24-15C (which also included C-18-2D, C-18-16D, and C-24-15D, according to the data listed in Table I-1). The other, Type II, consisted of C-18-2B, C-18-16A, and C-24-15A (C-18-2C, C-18-16C, and C-24-15B should also be included in this class).

Allelism tests were also performed with these Type I and Type II clones with the homothallic strain of the D gene. The D gene should be redesignated HO HMα HMa, as described above. Results of the tetrad analysis of hybrids prepared by spore-to-spore crosses between the D strains and the Type I or Type II clones are summarized in Table I-3. The hybrids obtained by crosses of the D strains and the Type II clone showed a 4 homothallic : 0 heterothallic segregation in all asci so far tested (41 asci), while the stable haploid clones of both mating types, a and α, were segregated from the hybrids prepared by crosses of the D strains and the Type I clone. These facts clearly indicated that the genotype of the Type II clone is the same as that of the D strain and that the Type I clone should bear another genotype which also gives the homothallic phenotype to the culture.

Some of the Ho clones of Type I and Type II were back crossed to the α Hp and the a Hq strains by spore-to-cell contact. These data are summarized in Table I-4. It was observed that the same ascus-types were

Table I-2. Tetrad segregation from hybrids obtained by inter-ascus crosses among the segregants of asci C-18-2, C-18-16, and C-24-15

Cross[a]	Segregation in asci (Homothallic[b] : a : α)								
	4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
C-18-2A × C-18-16A	14	2	3	7	0	0	0	0	0
× C-18-16B	25	0	0	0	0	0	0	0	0
× C-24-15A	9	1	3	7	0	0	0	0	1
× C-24-15C	27	0	0	0	0	0	0	0	0
C-18-2B × C-18-16A	26	0	0	0	0	0	0	0	0
× C-18-16B	6	3	0	10	0	0	0	0	0
× C-24-15A	25	0	0	0	0	0	0	0	0
× C-24-15C	11	5	4	5	0	0	0	0	0
C-18-16A × C-24-15A	22	0	0	0	0	0	0	0	0
× C-24-15C	8	3	3	2	0	0	0	0	0
C-18-16B × C-24-15A	6	0	3	4	0	0	0	0	0
× C-24-15C	25	0	0	0	0	0	0	0	0

[a] Each cross was made by the spore-to-spore mating method.

[b] Three types of homothallism, Ho, Hp, and Hq, were included in this category.

Table I-3. Tetrad segregations from hybrids obtqined by crossing the Type I and Type II class of the Ho strains to the homothallic strains carring the D gene

Cross[a]	Segregation in asci (Homothallic[b] : a : α)								
	4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
1932-2A[c] × C-18-2A[d]	9	3	3	2	0	0	0	0	0
1932-2A × C-18-2B[e]	25	0	0	0	0	0	0	0	0
C1728b-4C[c] × C-18-2A	34	19	12	13	0	0	0	0	3
C1728b-4C × C-18-2B	16	0	0	0	0	0	0	0	0

[a] Each cross was made by the spore-to-spore mating method.

[b] Three types of homothallism, Ho, Hp, and Hq, were included in this category.

[c] The homothallic strains carrying the D gene.

[d] Type I class of the Ho strain.

[e] Type II class of the Ho strain.

Table I-4. Tetrad segregations from the hybrids prepared by crossing the Ho clones of Type I and Type II to the α Hp or *a* Hq strains

Cross[a]	Expected genotype[b]	Segregation in asci (Homothallic[c] : a : α)								
		4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
α Hp × Type I	α *HO hmα HMa/a HO hmα hma*	5	0	22	0	0	3	0	0	0
α Hp × Type II	α *HO hmα HMa/a HO HMα HMa*	3	0	60	0	0	21	0	0	0
a Hq × Type I	*a HO HMα hmα/α HO hmα hma*	3	39	0	0	26	0	0	0	0
a Hq × Type II	*a HO HMα hmα/α HO HMα HMa*	17	78	0	0	31	0	0	0	0

[a] Each cross was made by the spore (of the Ho clone)-to-cell (of the α Hp or a Hq strain) mating method.

[b] Expected genotypes according to hypothesis II.

[c] Three types of homothallism, Ho, Hp, and Hq, were included in this category.

segregated by tetrad analyses of the hybrids. If both Types of the Ho clones were back crossed to the α Hp strain, hybrids never segregated the haploid clone of a mating type and contained two or fewer α clones in each ascus. On the other hand, hybrids prepared by crossing them with the a Hq strain did not segregate the haploid clone of α mating type and two or fewer a clones were observed in each ascus. Thus, virtually no difference was observed in the ascus-types of segregants. However, the data listed in Table I-4 showed some difference in the ratios of the ascus-types with respect to their combination. This observation suggests a linkage relationship of the mating-type locus to HMα and will be discussed later.

The observations described above could most probably be explained by the following alternatives. First, there are duplicated genes for the HMα and HMa functions, respectively, and the homothallic gene system consists of five independent loci, HO, HMα1, HMα2, HMa1, and HMa2 (hypothesis I). For example, the Ho type homothallic diploid of Type I should be homozygously marked with the HO, HMα1, hmα2, HMa1, and hma2 genes. The Type II and also the D strains should be marked with the HO, hmα1, HMα2, hma1, and HMa2 genes homozygously. The haploid α Hp and a Hq clones used in this study should have the α HO hmα1 hmα2 HMa1 HMa2 and a HO HMα1 HMα2 hma1 hma2 genotypes respectively. Another explanation (hypothesis II) is that each of the three kinds of homothallic genes consists of a single pair of alleles, i.e., HO/ho, HMα/hmα and HMa/hma, respectively. However, a spore having the HO hmα hma genotype would give rise to an Ho type homothallic culture. In this explanation, the Type I clone should be homozygous for the HO, hmα, and hma alleles

while Type II and the D strains have the HO HMα HMa genotype at their haplophase nucleus. The haploid α Hp clone and the a Hq clone should have the α HO hmα HMa and a HO HMα hma genotype, respectively.

Test of the hypotheses: According to these alternative hypotheses, different segregation patterns would be expected for each hypothesis with respect to the type of ascus and to the type of homothallic clone in certain crosses. However, among the crosses between the Hp strain of S. norbensis SBY 2535 to various strains having different genotypes, i.e., ho_{α} hm, ho_{α} HM, HO_{α} hm, and HO_{α} HM (here we have followed the earlier symbols; Takano and Oshima 1970a), some of the combinations would be expected to segregate in the same of similar ways (see Table AII-1). The most significant differences in the segregation patterns would be expected in the crosses of α Hp to a Hq, particularly in the crosses mentioned in the previous paper (Oshima and Takano 1972) and in the Introduction, in which duplicated genes were suggested for HMα and HMa according to hypothesis I. Another combination, crosses between the Ho type clones of Type I and Type II, should also be useful for testing these hypotheses The expected frequencies of the ascus-types in these combinations, listed in Table I-5, were calculated, assuming that the hypothetical homothallic genes and the mating-type locus were segregated independently of each other. The HO hmα hma genotype should give rise to the heterothallic phenotype according to hypothesis I and the Ho type of homothallism according to hypothesis II. According to hypothesis II, 4 homothallic 0 heterothallic asci will be restricted to three types of asci, i.e., 4 Ho : 0 Hp : 0 Hq, 2 Ho : 1 Hp : 1 Hq, and 0 Ho : 2 Hp : 2 Hq, while nine ascus-types would be expected from hypothesis I. In the other

Table I-5. The ascus-types of the segregants derived from the diploids prepared by crossing the α Hp to *a* Hq strains and the Type I to Type II of the Ho type homothallic strains

Ascus	Observed[a]	Expected frequency[b]	
		Hypothesis I	Hypothesis II
		$\frac{\alpha}{a}\ \frac{HO}{HO}\ \frac{hm\alpha1}{HM\alpha1}\ \frac{hm\alpha2}{HM\alpha2}\ \frac{HMa1}{hma1}\ \frac{HMa2}{hma2}$	$\frac{\alpha}{a}\ \frac{HO}{HO}\ \frac{hm\alpha}{HM\alpha}\ \frac{HMa}{hma}$
		or	or
Spore A B C D		$\frac{s^c}{s}\ \frac{HO}{HO}\ \frac{HM\alpha1}{hma1}\ \frac{hm\alpha2}{HM\alpha2}\ \frac{HMa1}{hma1}\ \frac{hma2}{HMa2}$	$\frac{s^c}{s}\ \frac{HO}{HO}\ \frac{hm\alpha}{HM\alpha}\ \frac{hma}{HMa}$
Ho Ho Ho Ho	14/25	36	6
Ho Ho Ho Hp	0	72	0
Ho Ho Ho Hq	0	72	0
Ho Ho Hp Hq	10/25	144	8
Ho Ho Hp Hp	0	6	0
Ho Ho Hq Hq	0	6	0
Ho Hp Hp Hq	0	12	0
Ho Hp Hq Hq	0	12	0
Hp Hp Hq Hq	1/25	1	1
Ho Ho Ho a	0	144	0
Ho Ho Hp a	17/17	84	4
Ho Ho Hq a	0	48	0
Ho Hp Hq a	0	28	0
Ho Ho Ho α	0	144	0
Ho Ho Hp α	0	48	0
Ho Ho Hq α	6/6	84	4
Ho Hp Hq α	0	28	0
Ho Ho a α	8/10	196	8
Ho Hp a α	0	28	0
Ho Hq a α	0	28	0
Hp Hq a α	2/10	4	4
Ho Ho a a	NT[d]	19	0
Ho Ho α α	NT	19	0
Ho a a α	---[e]	16	0
Ho a α α	---	16	0
a a α α	19/735	1	1
Total		1296	36

Legend for Table I-5

[a] From the pooled tetrad clones of the diploids prepared by crosses between α Hp to a Hq or between the Ho clones of Type I and Type II, the tetrad clones showing 4 homothallic : 0 heterothallic, 3 homothallic : 1 heterothallic, and 2 homothallic : 1a : 1α segregations in which all the homothallic clones had high enough levels of sporulation were collected. Each homothallic diploid segregant was sporulated and the 4-spored asci were dissected. The type of the homothallism was decided by the segregation pattern of 10 to 20 asci in each homothallic clone. The results are indicated as the observed number of asci per total number of asci tested in each category.

[b] The expected frequency of each ascus type was calculated assuming that the hypothetical genes were unlinked to each other or with the mating-type locus.

[c] Mating by spore-to-spore fusion was employed for crossing between the Type I and Type II of the Ho strains.

[d] Not tested.

[e] No proper tetrad was observed in this ascus-type; for details, see text.

classes of asci showing the 3 homothallic : 1a and 3 homothallic : 1α segregations, four possible ascus-types would be expected from hypothesis I and only one ascus-type, 2 Ho : 1 Hp : 1a or 2 Ho : 1 Hq : 1α, respectively, would be expected from hypothesis II in each class. In asci showing the 2 homothallic : 1a : 1α segregation the ascus-types would be expected to be 2 Ho : 1a : 1α or 1 Hp : 1 Hq : 1a : 1α from hypothesis II. It would not be expected to find asci showing 2 homothallic : 2a : 0α, 2 homothallic : 0a : 2α, 1 homothallic : 2a : 1α, or 1 homothallic : 1a : 2α segregations from hypothesis II, while the occurrence of these ascus-types would be expected by hypothesis I, as shown in Table I-5.

Hypotheses I and II were tested according to the above criteria. We were able to collect 25 asci showing a 4 homothallic : 0 heterothallic segregation, 17 asci showing a 3 homothallic : 1a segregation and 6 asci of a 3 homothallic : 1α segregation in which all the homothallic clones had high enough levels of sporulation for further analysis from the pooled asci dervied from hybrids prepared by the crosses of an α Hp × a Hq combination (217 asci) and from the diploids prepared by crossing between the Ho clones of Type I and Type II (518 asci). All the homothallic clones were sporulated and the four-spored asci were dissected. Results of the tetrad analyses, as listed in Table I-5, indicated that all the clones in 14 of 25 asci showing a 4 homothallic : 0 heterothallic segregation were identified as the Ho type homothallic strains; the other 10 asci showed a 2 Ho : 1 Hp : 1 Hq segregation and the remaining ascus was consisted of two Hp and two Hq clones. We were not able to observe the other six ascus-types which could be expected

from hypothesis I. All 17 tested asci which showed a 3 homothallic : 1a segregation fell into an ascus-type of 2 Ho : 1 Hp : 1a. Similarly, all 6 asci showing a 3 homothallic : 1α segregation were identified as a 2 Ho : 1 Hq : 1α type. Although, we tested only 10 asci which showed a 2 homothallic : 1a : 1α segregation, each one showed the ascus-type either of a 2 Ho : 1a : 1α (8 asci) or a 1 Hp : 1 Hq : 1a : 1α (2 asci) segregation.

On the other hand, we had observed a significant number of asci which showed the 2 homothallic : 2a : 0α, 2 homothallic : 0a : 2α, 1 homothallic : 2a : 1α, and 1 homothallic : 1a : 2α segregations from the hybrids prepared by α Hp to a Hq crosses or by crosses between the Ho clones of Type I and Type II, as shown in a previous paper (Oshima and Takano 1972; Table 1) and also in Table I-1 of this chapter. These ascus-types would be expected from hypothesis I but never from hypothesis II. I tried to isolate the HO hmα hma clone from some of the asci showing a 1 homothallic : 3 heterothallic segregation according to hypothesis I. For example, the haploid clones of a mating type from an ascus showing a 1 homothallic : 2a : 1α segregation should have one of the following four genotypes, i.e., a HO HMα1 HMα2 hma1 hma2, a HO HMα1 hmα2 hma1 hma2, a HO hmα1 HMα2 hma1 hma2, or a HO hmα1 hmα2 hma1 hma2. These four genotypes of the a clones can be classified by the genetic analyses of the hybrids prepared by crossing them to the α clone of the same asci which should have the α HO hmα1 hmα2 HMa1 HMa2 genotype. Among those crosses, the diploids obtained by crossing the a HO hmα1 hmα2 hma1 hma2 clone should segregate two α clones in each ascus while some of the a clones might converted to the homothallic diploids. If

the cross was made with an a clone having either one of the other three genotypes, it should also be possible to isolate the a HO hmα1 hmα2 hma1 hma2 clone by repeated back crosses of the haploid a segregants to the α HO hmα1 hmα2 HMa1 HMa2 haploid. These possibilities were attempted using the haploid clones from a tetrad showing a 1 homothallic : 2a : 1α segregation which occurred in the cross of C-18-2A × C-18-2C (Table I-1). Another set of tetrad clones obtained by the additional dissections (those data were not included in Table I-1) of the asci which originated from diploid of the C-18-16C × C-18-16D cross could also be analyzed. However, we observed no haploid clone showing the segregation patterns expected from hypothesis I as discussed in the above argument. Further pedigree analyses of all these haploids according to the above possibility always resulted in inconsistencies in their genotypes. It was also observed that some clones which previously had been classified as heterothallic haploids in tetrads of a 1 homothallic : 3 heterothallic segregation in Table I-1 were identified as diploids due to the recognition of their low but significant potencies of sporulation by careful re-examination of the stock cultures. These asci were classified as an ascus-type of 2 homothallic : 1a : 1α and data listed in Table I-1 were revised according to these observations. We did not re-examine the asci showing the 2 homothallic : 2a, 2 homothallic : 2α segregation of some of the asci with a 1 homothallic : 3 heterothallic segregation which were observed in the previous study (Oshima and Takano 1972; Table 1) and in Table I-1 (marked with asterisks) in this Chapter, because they had been discarded at the time of re-examination. These types of ascus were never observed in the latter experiments made by careful examination

as shown in similar experiments listed in Tables I-2 and I-3. These results suggest that the occurrence of asci showing the 2 homothallic : 2a : 0α, 2 homothallic : 0a : 2α and 1 homothallic : 3 heterothallic segregation could be attributable to errors in microscopic inspection or they might be irregular asci. In another report (Takano, Kusumi, and Oshima 1973), the conversion-insensitive α allele, α-inc, were identified in S. diastaticus. If there occurred such a conversion-insensitiveness or a slow converting mating-type allele in the present strains, this might also give tetrads showing such segregations which could not be expected from hypothesis II. In any case, we should now exclude the segregation data showing 1 homothallic : 2a : 1α, 1 homothallic : 1a : 2α, 2 homothallic : 2a : 0α, and 2 homothallic : 0a : 2α segregations as irregular asci. In addition, we observed only two genotypes, HO HMα1 hmα2 HMa1 hma2 and HO hmα1 HMα2 hma1 HMa2, among 12 Ho type homothallic clones from 3 asci showing 4 homothallic : 0 heterothallic segregation from the α Hp to a Hq crosses, while we could expect two other different genotypes, i.e., HO HMα1 hmα2 hma1 HMa2 and HO hmα1 HMα2 HMa1 hma2, according to hypothesis I. All these observations excluded the idea proposing four independent loci for the HMα1, HMα2, HMa1, and HMa2 genes and strongly supported hypothesis II.

In conclusion, all the observations of the genetic controlling system for homothallism in Saccharomyces can be explained with three kinds of homothallic genes, each of which consists of a single pair of alleles, HO/ho, HMα/hmα, and HMa/hma, respectively. A spore having the HO hmα hma genotype will give rise to a homothallic diploid culture the same as the strain having the HO HMα HMa genotype. A spore having the

a HO hmα HMa genotype will give rise to a homothallic diploid having the Hp type life cycle and the culture originated from a spore having the α HO HMα hma genotype will give a diploid culture showing the Hq type life cycle. The other genotypes, a HO HMα hma, α HO hmα HMa, and the ho allele combined with either of the alleles at HMα and HMa loci will give a heterothallic character to the culture. In a previous publication (Takano, Kusumi, and Oshima 1973), it had been supposed that a spore having the combined genotype of the inactive alleles of HMα and HMa, i.e., the hmα hma genotype, would give rise to a stable heterothallic haploid culture. This prediction would be wrong and would contradict the present conclusion if a spore had the HO genotype.

Linkage relations between the HMα and the mating-type loci: Before interbreeding with S. norbensis SBY 2535, all of our strains had been marked with the HMα allele homogeneously. With these strains, no linkage relationship was detected in any gene pairs among the HO, HMa, and the mating-type loci (e.g., Takano and Oshima 1967). However, the data listed in Table I-4 suggest some linkage between HMα and the mating-type locus. According to the present conclusion, the haploid α Hp clone has the α HO hmα HMa genotype and the Type II class of Ho is of the HO HMα HMa genotype. The genotype of the diploid hybrid prepared by crossing by cell (α)-to-spore fusion between them would be α HO hmα HMa/a HO HMα HMa, because it was supposed that only the spore having the a mating type could contribute in mating with an α cell (Oshima and Takano 1971). Thus, this type of hybrid should be doubly heterozygous for both the HMα and the mating-type loci, as indicated in Table I-4. Another combination, a cross between the haploid a Hq to the Type I class of the

Ho strain should also be doubly heterozygous for both the HMα and the mating-type loci, while this combination has the hma allele homozygously. Pooled data of the tetrad distribution of this combination showed 47 : 6 : 99 in the ratio of the parental ditype : non-parental ditype : tetratype asci. These data indicate a loose linkage [approximately 64 stranes by the D_1 formula (Shult and Lindegren 1956)] between the HMα and the mating-type loci on chromosome III.

DISCUSSION

For the explanation of the mating-type differentiation, Oshima et al. have proposed the hypothesis (Oshima and Takano 1971; Takano, Kusumi, and Oshima 1973) that the elementary structure of the mating-type locus for both the a and α alleles is essentially the same. The association of some kind of controlling element with this locus would cause the differentiation of two mating-type alleles. The mating-type locus on chromosome III would act as an affinity site for a controlling element. The HMα and HMa genes produce the specific controlling element; the association of an HMα-element with the mating-type locus would form the a mating-type allele and the association of an HMa-element with the mating-type locus would give rise to the α mating-type allele.

To expand this hypothesis to cover the present observations, I should make a revision of the molecular mechanism of the mating-type differentiation, or another speculation on the genetic function of the hmα and hma alleles. It seems not impossible, but difficult, to explain

the molecular mechanism in Ho homothallism caused by the HO hmα hma genotype by assuming the presence of inactive alleles of the HMα and HMa genes. On the other hand, another idea has been suggested by Naumov and Tolstorukov (1973) from a genetic analysis of homothallic strains, including a similar Hp strain originated from Santa Maria. According to their suggestion, the hmα allele has the same function as the HMa gene and hma has the same function as the HMα allele. I have recognized that their idea is well in accord with all of our observations and is also attractive to explain the molecular mechanism described above without any modification. However, whether the hmα and hma genes exert their function positively or whether they have no positive function for the conversion of the mating-type alleles is not yet clear; in other words, whether the Ho type of homothallism in the HO hmα hma clone is caused by the same mechanism or by another occurring in the HO HMα HMa clone remains for further study. This problem will be discussed again in Chapter III.

ABSTRACT

There are four types of life cycles in Saccharomyces cerevisiae and its related species. A perfect homothallic life cycle (the Ho type) is observed in the classic D strain. Two other types show semi-homothallism; one of them shows a 2 homothallic diploid : 2α heterothallic haploid segregation (the Hp type) and another, a 2 homothallic : 2a segregation (the Hq type). In the segregants from these Ho, Hp, and Hq diploids, each homothallic segregant shows the same segregation pattern as its parental diploid. The fourth type has a heterothallic life cycle showing a 2a : 2α segregation and the diploids are produced by the fusion of two haploid cells of opposite mating types. The diploids prepared by the crosses of α Hp (an α haploid segregant from the Hp diploid) to a Hq (an a haploid from the Hq diploid) segregated two types (Type I and II) of the Ho type homothallic clone among their meiotic segregants. Genetic analyses were performed to investigate this phenomenon and the genotypes of the Ho type homothallic clones of Type I and Type II. Results of these genetic analyses have been most adequately explained by postulating three kinds of homothallic genes, each of consisting of a single pair of alleles, HO/ho, HMα/hmα, and HMa/hma, respectively. One of them, the HMα locus, was proved to be loosely linked (64 stranes) to the mating-type locus. A spore having the HO hmα hma genotype gives rise to an Ho type homothallic diploid (Type I), the same as in the case of the D strain which has the HO HMα HMa genotype (Type II). A spore having the a HO hmα HMa or α HO HMα hma genotype will produce an Hp or Hq type homothallic diploid culture, respectively.

The other genotypes, a HO HMα hma, α HO hmα HMa, and the genotypes combined with the ho allele give a heterothallic character to the spore culture. A possible molecular hypothesis for the mating-type differentiation with the controlling elements produced by the HMα and HMa genes is proposed.

CHAPTER II

MAPPING OF THE HOMOTHALLIC GENES, *HMα* AND *HMa*, IN *SACCHAROMYCES* YEASTS

INTRODUCTION

In Saccharomyces cerevisiae and related species there are four different types of life cycle, one perfectly homothallic (the Ho type), two semi-homothallic (the Hp and Hq types), and one heterothallic. These differences are most adequately explained by postulating three kinds of homothallic genes, each consisting of a single pair of alleles, HO/ho, HMα/hmα, and HMa/hma, respectively (see Chapter I). An Ho type homothallic diploid is derived from a spore having either the HO hmα hma (Type I) or the HO HMα HMa (Type II) genotype. The classic D strain described by Winge and Roberts (1949) has the same genotype as the Type II strain. Spores having the a HO hmα HMa or α HO HMα hma genotype also give rise to diploid culture showing respectively Hp or Hq type segregation in subsequent tetrad analysis. The other genotypes, a HO HMα hma, α HO hmα HMa and those combined with the ho allele give a heterothallic character to the spore culture.

In a previous genetic study of homothallism, any linkage between these three homothallic genes and other loci could not be detected, except for loose linkage between HMα and the mating-type locus (Chapter I). This might be caused by two major difficulties attributable to special features of the genetic system in homothallism. One of the difficulties is due to the complex correspondence of the genotype to phenotype in homothallism. The second concerns the mating-type alleles which, in general, must be heterozygous, α/a, in a diploid and the function of two homothallic genes, HMα/hmα and HMa/hma, is specific to the mating-type alleles. These facts make it impossible to determine allelic 2 : 2

segregation for the HMα and HMa genes and for the mating-types, respectively, in some asci. Furthermore, to map the HMα and HMa loci, diploid heterozygus for three loci, i.e., HMα/hmα (or HMa/hma), α/a, and X/x (a standard marker to be tested for linkage to the HMα or HMa locus) should be analyzed. These difficulties inevitably required a novel algebraic procedure in three factor analysis for mapping homothallic genes.

This chapter deals with the theoretical equations to express frequencies of the ascus types for three genes on the same chromosome and mapping of the homothallic genes using those equations. Results showed that the HMα and HMa loci are located at a point approximately 45 stranes (corresponds 40 centimorgans [cM]) distal to the thr4 locus and 65 stranes (53 cM) distal to the his4 locus on chromosome III, respectively. On the other hand, the HO gene has failed to show linkage to 25 markers disctributed over 17 chromosomes so far tested by conventional tetrad analysis.

MATERIALS AND METHODS

Organisms: The principal strains used are listed in Table II-1. All diploids were constructed using these strains or using descendants from these diploids. The gene symbols are those proposed by Plischke et al. (1976). Map positions of various genetic markers used in these experiments are described by Mortimer and Hawthorne (1973). The genotype of all heterothallic strains obtained from other workers was confirmed to be ho HMα HMa by genetic analysis of hybrids prepared by crosses

Table II-1. Genotypes and sources of the principal strain used.

Strain	Genotype			Source or reference[b]	Thallism
	Homothallic gene	Mating type	Genetic marker[a]		
T-1068-43B	*ho HMα hma*	α	*ade1 arg4 his4 leu2 lys2 met2 thr4 trp1*	-----	Hetero
X3382-3A	*ho HMα HMa*	*a*	*ade1 arg4 aro7 asp5 cdc14 gal1 his2 his6 pet17 trp1*	R. K. Mortimer	Hetero
X3144-11A	*ho HMα HMa*	α	*arg9 his6 ilv3 leu2 met14 pet8 pet19 rad1 trp1*	R. K. Mortimer	Hetero
C3137-3D	*ho HMα HMa*	*a*	*ade6 lys9 ura3*	T. Takahashi	Hetero
C3461-2D	*ho HMα HMa*	α	*ade2 his8 lys7 met14*	T. Takahashi	Hetero
X963-18C	*ho HMα HMa*	α	*ade8 his8 met2*	C. Lindegren	Hetero
136	*ho HMα HMa*	*a*	*ade1 ade2 gal1 his7 lys2 rna1 tyr1 ura1*	R. K. Mortimer	Hetero
473	*ho HMα HMa*	*a*	*ade1 ade2 cdc5 gal1 his7 lys2 tyr1 ura1*	L. H. Hartwell	Hetero
T-1059-18B	*HO HMα hma*	*a*	*ade1 gal1 his4 leu2 thr4*	-----	Hetero
SBY 2535-14C[c]	*HO hmα HMa*	α		J. Santa Maria	Hetero
C1728b-4C	*HO HMα HMa*	Non-mater	*ade2 leu1*	T. Takahashi	Homo
1932-2A	*HO HMα HMa*	Non-mater	*arg4 lys2 met4*	D. C. Hawthorne	Homo
C-18-16D	*HO hmα hma*	Non-mater	*arg4 lys2*	-----	Homo

[a] The terminology of genetic symbols follows that proposed by Plischke et al. (1976). It was confirmed that those markers segregated as expected from the map constructed by Mortimer and Hawthorne (1973).

[b] The strains for which no source was indicated were selected from our stock culture for Yeast Genetics.

[c] A spore culture derived from S. norbensis strain SBY 2535 which was provided by J. Santa Maria (see Chapter I).

between these strains and several authentic Ho strains of the HO HMα HMa genotype. Tetrad analysis showed a 2 homothallic : 2 heterothallic segregation in every ascus tested so far and a 4 homothallic : 0 heterothallic segregation when the several homothallic diploid segregants from the above crosses were dissected after self-sporulation

Media and Techniques: All media, except for sporulation, and techniques were described in Chapter I. Medium of McClary, Nulty, and Miller (1959) consisting of anhydrous sodium acetate (0.82%), dextrose (0.1%), yeast extract (0.25%), and agar (2%) was used for testing ability to sporulate. Since cells showed better growth on this medium than on Fowell's medium (1952), it was possible to pick up enough cells from a colony on the replicated plate for microscopic inspection of sporulation.

Detection of linkage by the conventional procedures: Due to the complex correspondence of genotypes to phenotypes in homothallism, three factor analysis is necessary for mapping the homothallic genes. However, scoring of the tetrad distributions of the HO/ho gene relative to known genetic markers is possible, because the HO allele is epistatic to both the a and α mating-type alleles. For example, if a spore having the HO HMα HMa genotype is crossed to a heterothallic cell having the a (or α) ho HMα HMa genotype and the resultant diploids are subjected to tetrad analysis, all tetrad spores having the HO allele will give rise to homothallic diploid clones. The spore cultures having the ho allele will show stable heterothallism irrespective of the mating-type of spores and will develop into haploid clones. The tetrad distribution of the HO locus and known genetic markers can thus be determined by observing

segregation of diploid versus haploid ascosporal clones and the reference markers in each ascus

If the HMα and HMa genes were not linked to the mating-type locus, it would be possible to detect their linkage to standard genetic markers by randam spore analysis of heterothallic segregants from the following crosses: s HO hmα hma (s; a spore from the type I homothallic strain) × a HO HMα hma (a cell from the Hq haploid strain; see Chapter I) and s HO hmα hma × α HO hmα HMa (a cell from the Hp haploid strain), or s HO HMα HMa (a spore from the type II homothallic strain) × a HO HMα hma and s HO HMα HMa × α HO hmα HMa. The diploid will segregate heterothallic clones having solely the same genotype for homothallism and mating type as the respective heterothallic parental strain.

If there is direct linkage between HMα or HMa and the mating-type locus (in fact, a loose linkage was detected between HMα and the mating-type locus as described in Chapter I and between HMa and the mating-type locus as described in this chapter), a linkage analysis with the aid of standard markers on chromosome III is necessary to determine whether the HMα or HMa locus is mapped on the left or right hand side of the mating-type locus. Thus, it is essential to perform three factor analysis of three direct linked genes.

Equations for three factor analysis in three linked genes: Twelve ascus-types, abbreviated as X1 to X12, are expected from a hybrid of triply heterozygous cross, XYZ/xyz, as illustrated in Table II-2. Their frequencies of occurrence, denoted as x_1 to x_{12}, are obviously related to the linkage among the X, Y, and Z genes and to the gene arrangements

Table II-2. Twelve possible segregations in asci from triply heterozygous (*XYZ/xyz*) diploid.

Ascus type	Spore			
	A	B	C	D
X1	*XYZ*	*XYZ*	*xyz*	*xyz*
X2	*XYZ*	*XYz*	*xyZ*	*xyz*
X3	*XYz*	*XYz*	*xyZ*	*xyZ*
X4	*XYZ*	*Xyz*	*xYZ*	*xyz*
X5	*XYZ*	*XyZ*	*xYz*	*xyz*
X6	*XYz*	*XyZ*	*xYZ*	*xyz*
X7	*XYz*	*Xyz*	*xYZ*	*xyZ*
X8	*XYZ*	*Xyz*	*xYz*	*xyZ*
X9	*XYz*	*XyZ*	*xYz*	*xyZ*
X10	*Xyz*	*Xyz*	*xYZ*	*xYZ*
X11	*Xyz*	*XyZ*	*xYz*	*xYZ*
X12	*XyZ*	*XyZ*	*xYz*	*xYz*

on chromosome(s). The principles for establishing the linear order of three genes have been proposed by Shult and Lindegren (1955) and Shult and Desborough (1960). However, their procedure cannot be directly applied to the present case, because some of the 12 ascus-types in a three point cross with homothallic genes are indistinguishable by direct inspection of phenotype. Thus, to solve the present case, a novel algebraic procedure for calculation of the frequencies of ascus types in the case of three genes on the same chromosome is required.

If three genes, X, Y, and Z, are located on the same chromosome in that order and the tetrad distribution, i.e., ratio of the parental ditype (PD) : nonparental ditype (NPD) : tetratype (T) asci, between the X-Y and Y-Z gene-pairs in the segregants from the XYZ/xyz diploid are denoted as $a_1 : a_2 : a_3$ and $b_1 : b_2 : b_3$, where $a_1 + a_2 + a_3 = 1$ and $b_1 + b_2 + b_3 = 1$, then an X1 ascus will be given when both X-Y and Y-Z gene-pairs are segregated as PD. If crossing-over occurs in a Poisson manner, it might be expected that the tetrad distributions $a_1 : a_2 : a_3$ and $b_1 : b_2 : b_3$ are mutually independent. Thus, the frequency of X1 ascus, x_1, will be the product of a_1 and b_1 as shown by equation 1. In the same manner, frequencies of X2, X3, X4, X9, X10, X11, and X12 will be as expressed by their respective equations. Total frequency of four ascus-types, X5, X6, X7, and X8, is derived by multiplying the frequency of the T asci between X-Y gene-pair (a_3) and that of the Y-Z gene-pair (b_3), and each ascus-type will be expected to occur with equal frequency assuming no chromatid interference. Hence frequency of each ascus type, X5, X6, X7, and X8, will be given by $a_3 \cdot b_3/4$. Thus, the equations for three linked genes are:

Equation:

$$x_1 = a_1 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

$$x_2 = a_1 \cdot b_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

$$x_3 = a_1 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 3$$

$$x_4 = a_3 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 4$$

$$x_5 = a_3 \cdot b_3/4 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 5$$

$$x_6 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 6$$

$$x_7 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 7$$

$$x_8 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8$$

$$x_9 = a_3 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 9$$

$$x_{10} = a_2 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 10$$

$$x_{11} = a_2 \cdot b_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 11$$

$$x_{12} = a_2 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 12$$

Since map, distances were not calculated in centimorgans but on a no interference model of crossing-over throughout this thesis, the unit of distance between X and Y may be calculated either as $D_1 = -50 \ln(a_1 - a_2)$ or $D_2 = -33.3 \ln(1 - 3a_3/2)$ (Shult and Lindegren 1956a, 1956b; Desborough and Lindegren 1959). The unit of map distance has been termed "strane" which is equal to 50× the average number of cross-over per meiosis. It corresponds closely to centimorgans over short distances but deviates markedly, becoming greater as the distances increase. For reference, map distances in "cM" calculated using the equation derived by Perkins (1949) are shown in parentheses. The considerable shrinkage distortion characteristic of map based on centimorgans is avoided by the use of stranes (Lindegren, Lindegren, Shult, and Desborough 1959). Thus, a given distance between two genes can be

converted to the expected values of tetrad distribution by the equations:

$$a_3 = \frac{2}{3}[1 - \exp(-D_{XY}/33.3)] \quad \ldots\ldots\ldots\ldots \quad 13$$

$$a_2 = \frac{1}{2}[1 - a_3 - \exp(-0.02D_{XY})] \quad \ldots\ldots\ldots \quad 14$$

$$a_1 = 1 - (a_2 + a_3) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 15$$

$$b_3 = \frac{2}{3}[1 - \exp(-D_{YZ}/33.3)] \quad \ldots\ldots\ldots\ldots \quad 16$$

$$b_2 = \frac{1}{2}[1 - b_3 - \exp(-0.02D_{YZ})] \quad \ldots\ldots\ldots \quad 17$$

$$b_1 = 1 - (b_2 + b_3) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 18$$

where D_{XY} and D_{YZ} are map distances from X to Y and Y to Z respectively.

To confirm the above equations experimentally, tetrad data from diploids triply heterozygous for three linked auxotrophic markers, i.e., his4, leu2, and thr4 on chromosome III, in which frequencies of occurrence of the 12 ascus-types and the tetrad distributions corresponding to both the X-Y and Y-Z gene pairs can be determined directly, were collected. The collected data were compared with expected frequencies of ascus types calculated by the equations using two tetrad distribution data (Table II-3). It is clear that observed and expected values showed good fit ($70\% > P > 50\%$).

RESULTS

Detection of direct linkage between the mating-type locus and the HO, HMα, or HMa locus: Direct linkage of homothallic genes to the mating-type locus has been tested by tetrad analysis of six different combinations of crosses in which a certain homothallic gene, HO, HMα,

Table II-3. Observed and theoretical ratios of ascus types from triply heterozygous hybrids for the his4, leu2, and thr4 markers.

Ascus type	Number of asci	
	Observed	Expected
X1	360	375.0
X2	629	617.5
X3	63	59.4
X4	131	116.2
X5	43	47.8
X6	40	47.8
X7	45	47.8
X8	52	47.8
X9	15	18.4
X10	1	0.7
X11	1	1.1
X12	0	0.1
Total	1380	1379.6
χ^2	5.83	
d.f.[a]	8	
Probability (%)	70>P>50	

Legend for Table II-3

Observed data were collected from various crosses according to the ascus types as described in Table II-2. The his4, leu2, and thr4 markers correspond to X, Y, and Z of Table II-2, respectively. Expected frequencies of the ascus types, x_1 to x_{12}, were calculated from equations 1 to 12 with the tetrad distributions for the his4 - leu2 (a_1, a_2, and a_3) and leu2 - thr4 (b_1, b_2, and b_3) gene-pairs from the entire collected data after normalizing their genotypes.

[a] Each term of the ascus type in which expected occurrence of asci is less than five was not included in the calculation of χ^2 statistics.

or HMa, was heterozygous while the other two homothallic genes were homozygous (Table II-4). No direct linkage between the mating-type and HO loci was indicated, since the PD and NPD asci showed essentially equal frequencies of occurrence. Furthermore, the HO gene is not linked to a centromere, because the observed frequency of T asci with the mating-type locus which is located on chromosome III near to the centromere (20 stranes) was 577/1057 = 0.64.

On the other hand, direct linkage was indicated between the HMα and mating-type loci as described in Chapter I. Pooled data of the tetrad distribution show a PD : NPD : T ratio of 63 : 13 : 138. These data indicate a loose linkage (approximately 73 stranes by the D_1 formula or 57 cM) between them on chromosome III. Direct linkage between the HMa and mating-type loci has not been detected previously. However, further collection of tetrad data showed a PD : NPD : T ratio of 116 : 54 : 272. These data suggest a loose linkage (approximately 98 stranes by the D_1 formula or 65 cM) between these two loci.

Mapping of the HMα locus on chromosome III: To determine whether the HMα locus is located on the left arm (distal to the his4 locus; assumption I) or the right arm (distal to the thr4 locus; assumption II) three factor analyses were performed using the his4, leu2, and thr4 loci as standard markers. Diploids having the genotype heterozygous for the HMα locus and homozygous for the HO allele (HO/HO) and HMa locus (HMa/HMa or hma/hma) were constructed. They were also heterozygous for the his4, leu2, and thr4 markers. Six ascus-types (Y1, Y2, Y3, Y4, Y5, and Y6; Table II-5) are expected by direct inspection of the phenotype of each spore-culture with respect to thallism and the

Table II-4. Pooled data of the tetrad distribution for the mating-type locus and the *HO*, *HMα*, or *HMa*.

Cross	Gene-pair	Tetrad distribution			χ^{2} [a]	Probability
		PD	NPD	T		(%)
a HO HMα hma × α *ho HMα hma*	α - HO	156	147	523		
a ho HMα HMa × *s HO HMα HMa* [b]		38	39	154		
		194	186	677	3.48	20>P>10
s HO HMα HMa × α *HO hmα HMa* [b]	α - HMα	21	3	60		
a HO HMα hma × *s HO hmα hma* [b]		42	10	78		
		63	13	138	35.50	P<1
a HO HMa hma × *s HO HMa HMa* [b]	α - HMa	91	40	207		
s HO hmα hma × α *HO hmα HMa* [b]		25	14	65		
		116	54	272	31.31	P<1

[a] Chi-square statistics were calculated with regard to the observed and expected tetrad distributions assuming no linkage between the two genes.

[b] Crosses were made by the spore-to-cell mating method.

Table II-5. Observed and theoretical frequencies of the ascus-types or spore-types from the triply heterozygous diploid for the mating-type, HMα, and his4, leu2, or thr4 genes.

Ascus type	Phenotype of spore[a] A	B	C	D	his4 Obsd.[b]	his4 I[c]	his4 II[c]	leu2 Obsd.	leu2 I	leu2 II	thr4 Obsd.	thr4 I	thr4 II
Y1	homo +	homo +	homo -	homo -	12	12.0	12.0	11	13.7	13.7	9	8.9	8.9
Y2	homo +	homo +	homo -	hetero -	30	10.7	24.1	27	12.0	19.3	7	13.5	8.1
Y3	homo +	homo -	homo -	hetero +	52	70.5	57.1	68	80.4	73.1	51	46.3	51.8
Y4	homo +	homo +	hetero -	hetero -	3	0.2	2.2	3	0.2	1.2	1	0.9	0.1
Y5	homo -	homo -	hetero +	hetero +	18	37.3	20.0	18	42.5	30.3	26	18.6	27.4
Y6	homo +	homo -	hetero +	hetero -	22	6.4	21.7	29	7.2	18.5	7	12.8	4.8
	Total				137	137.1	137.1	156	156.0	156.1	101	101.0	101.1
	χ^2					87.62	2.11		101.23	14.93		9.14	0.22
	Probability (%), d.f. = 4[d]					P<1	80>P>70		P<1	P<1		10>P>5	P>99

Spore type	Phenotype of spore	his4 Obsd.	his4 I	his4 II	leu2 Obsd.	leu2 I	leu2 II	thr4 Obsd.	thr4 I	thr4 II
Z1	homo +	164	122.6	155.3	179	139.4	159.9	92	105.6	94.4
Z2	homo -	216	256.6	223.9	250	292.4	271.9	186	173.9	187.8
Z3	hetero +	110	151.4	118.7	133	172.7	152.1	110	96.4	107.6
Z4	hetero -	58	17.4	50.1	62	19.6	40.1	16	28.1	14.2
	Total	548	548.0	548.0	624	624.1	624.0	404	404.0	404.0
	χ^2		126.13	2.66		118.49	18.41		9.71	0.35
	Probability (%), d.f. = 3		P<1	50>P>30		P<1	P<1		5>P>2	95>P>90

standard genetic markers, while 12 ascus-types are expected with respect to their genotypes. The lack of correspondence between the numbers of phenotypic and genotypic classes is due to the occurrence of three different kinds of homothallic spores by sporulation of the above diploids, i.e., the Ho type with the a HO HMα HMa, α HO HMα HMa, a HO hmα hma, and α HO hmα hma genotypes; and Hp with the a HO hmα HMa or Hq with the α HO HMα hma genotype, depending on the genotype of the diploid. These homothallic spores are classified in the same category, i.e., homothallic spores, by phenotypic examination of the primary segregants of diploids.

In the calculation of the theoretical frequency of each ascus-type, values of the map distances between his4, leu2, or thr4 and the mating-type locus were deduced as 45, 27, and 30 stranes (40, 25, and 28 cM), respectively; the centromere distance of the mating-type locus was 20 stranes (19 cM) from the previous data (Takano and Oshima 1970; and the unpublished data) as shown in Fig. II-3. With these given values of map distances for the standard markers and with the various map distances between HMα and the mating-type locus, expected tetrad distributions were calculated by equations 13, 14, 15, 16, 17, and 18 given in Materials and Methods according to the assumptions I and II. Using these expected values of tetrad distributions, we could calculate the frequencies of 12 ascus-types (x_1 to x_{12}) with respect to their genotype and then those of the six categories of phenotype (y_1 to y_6). In a typical calculation using thr4 as the standard marker, the gene-arrangement by assumption I is HMα - (centromere) - mating type - thr4, and by assumption II is (centromere) - mating type - thr4 - HMα. In these cases, the PD : NPD : T frequencies (a_1, a_2, and a_3) were calculated

between the HMα - mating type region for assumption I and the mating type - thr4 region for assumption II. Similarly, the PD : NPD : T frequencies (b_1, b_2, and b_3) were calculated for the regions of mating type - thr4 (assumption I) and thr4 - HMα (assumption II). Under assumption I, the expected frequencies of Y1 to Y6 asci from a diploid prepared by crossing a spore of the HO hmα hma thr4 genotype and a heterothallic haploid cell of the a HO HMα hma THR4 genotype by the spore-to-cell mating method are as follows:

$$y_1 = x_{10} + x_{11} + x_{12}$$

$$y_2 = x_6 + x_7 + x_9$$

$$y_3 = x_4 + x_5 + x_8$$

$$y_4 = x_3$$

$$y_5 = x_1$$

$$y_6 = x_2$$

Under assumption II, those values are as follows:

$$y_1 = x_3 + x_7 + x_{10}$$

$$y_2 = x_8 + x_9 + x_{11}$$

$$y_3 = x_2 + x_4 + x_6$$

$$y_4 = x_{12}$$

$$y_5 = x_1$$

$$y_6 = x_5$$

We performed six different calculations. Three of the calculations were made based on assumption I and three were based on assumption II, each using one of the three standard markers, his4, leu2, and thr4.

It is also possible to compare the theoretical values with the observed ones with respect to the frequencies (z_1 to z_4) of four categories of spore-type, Z1, Z2, Z3, and Z4 (Table II-5) in random spore cultures using the data for analysis with respect to the ascus types. If the distance between HMα and the mating-type locus was taken as 73 stranes(57 cM) using the data listed in Table II-4, the expected frequency calculated for each ascus or spore type was as listed in Table II-5. Lower χ^2 values were generally obtained with assumption II than with assumption I, and the minimal χ^2 values were obtained by placing HMα on the right side at a distance of 78, 77, or 79 stranes from the mating-type locus by the frequencies of ascus types, and 76, 67, or 73 stranes by spore types using his4, leu2, or thr4 as standard, respectively (Fig. II-1). Thus, HMα is located distal to the thr4 locus at a distance of 67 to 79 stranes from the mating-type locus.

Mapping of the HMa locus on chromosome III: Tetrad distribution with respect to the HMa and the mating-type loci showed a PD : NPD : T ratio of 116 : 54 : 272 (Table II-4). This ratio deviates significantly from the expected value for two non-linked genes. Map distance from this ratio was calculated as 98 stranes (65 cM) by the D_1 formula. From the fact that no direct linkage was detected between the HMα and HMa genes in previous studies and the result that HMα is located distal to the thr4 locus, it was expected that HMa might be located distal to the his4 locus (assumption III) rather than distal to thr4 (assumption IV).

To test the above possibility, diploids homozygous for the HO allele and the HMα locus and heterozygous for HMa, and his4, leu2, or

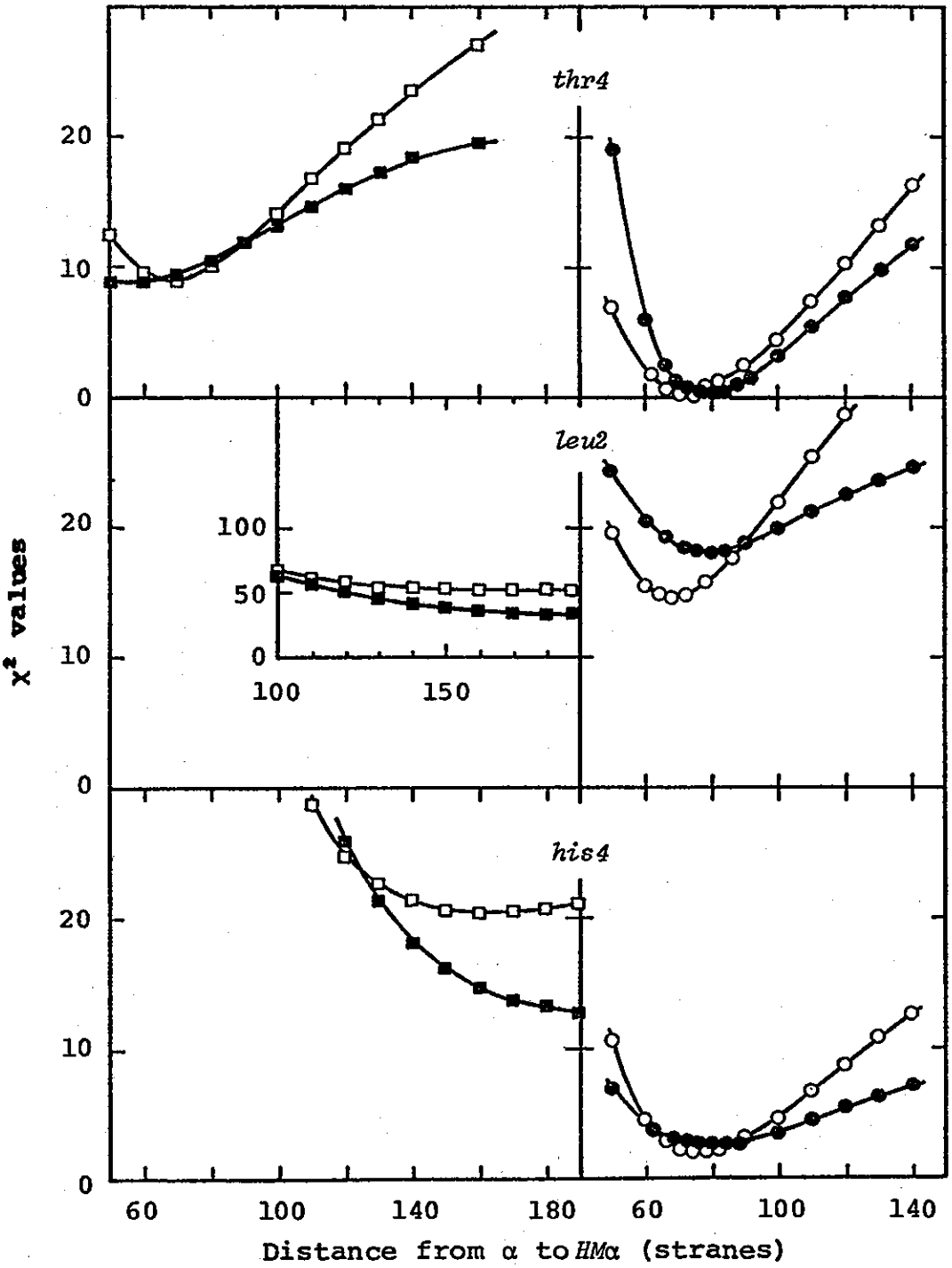

Fig II-1. Chi-square tests for the position of the HMα locus on chromosome III.

Fig. II-1 Chi-square tests for the position of the HMα locus on chromosome III. Chi-square values were plotted with respect to the observed and expected values at the various positions of the HMα gene on chromosome III using his4, leu2, and thr4 as the standard for three factor analysis. Symbols: □ and ■, χ^2 values by assumption I; and ○ and ●, by assumption II. Open and closed symbols indicate the values calculated from the data with respect to ascus types and spore types, respectively.

thr4 were constructed. They were sporulated and four-spored asci were dissected. The expected frequencies of six different ascus-types and four spore-types were calculated taking the distance between HMa and the mating-type locus as 98 stranes. The correspondence of these frequencies and of the values expected with various HMa to mating-type locus distances under assumptions III and IV with observed frequencies were tested by χ^2 statistics in the same manner as the case of HMα. Lower χ^2 values were generally obtained with assumption III but not with assumption IV, except for the χ^2 value of the ascus type with leu2 as the standard (Table II-6). Minimal χ^2 values were more critical under assumption III than IV (Fig. II-2). The distance between HMa and the mating-type locus for which minimal χ^2 value was obtained were 107, 135, or 94 stranes with the data of ascus type and 103, 116, or 112 stranes with those of spore phenotype using his4, leu2, or thr4 as the standard genetic marker, respectively. These values, 94 to 135 stranes, are consistent with 98 stranes given by calculation from the data listed in Table II-4.

The possibility that the HMa gene is located on a chromosome other than chromosome III was tested by scoring the distribution of standard genetic markers in the heterothallic segregants by random spore analysis with diploids prepared by spore-to-cell mating between a spore having the HO HMα HMa genotype and a cell having the a HO HMα hma genotype. Several diploids heterozygous for the ade1 (chromosome I), gal1 (II), lys2 (II), trp1 (IV), ura3 (V), cdc14 (VI), leu1 (VII), arg4 (VIII), his6 (IX), ilv3 (X), met14 (XI), asp5 (XII), lys7 (XIII), lys9 (XIV), ade2 (XV), aro7 (XVI), met2 (XVII), and met4 (XVII) genes were

Table II-6. Observed and theoretical frequencies of the ascus-types or spore-types from the triply heterozygous diploid for the mating-type, HMa, and his4, leu2, or thr4 genes.

Ascus type	Phenotype of spore[a] A	B	C	D	his4 Obsd.[b]	his4 III[c]	his4 IV[c]	leu2 Obsd.	leu2 III	leu2 IV	thr4 Obsd.	thr4 III	thr4 IV
Y1	homo +	homo +	homo -	homo -	30	30.2	30.2	21	19.7	19.7	27	27.3	27.3
Y2	homo +	homo +	homo -	hetero -	141	135.0	117.7	96	92.0	86.5	114	117.4	126.2
Y3	homo +	homo -	homo -	Hetero +	25	32.4	49.7	12	17.3	22.8	37	34.2	25.4
Y4	homo +	homo +	hetero -	hetero -	40	49.3	30.8	23	34.3	26.7	28	35.2	46.8
Y5	homo -	homo -	hetero +	hetero +	2	0.8	3.4	0	0.4	1.0	2	1.7	0.5
Y6	homo +	homo -	hetero +	hetero -	27	17.4	33.3	21	9.4	16.3	32	24.2	13.8
	Total				265.	265.1	265.1	173	173.1	173.0	240	240.0	240.0
	χ^2					9.04	20.82		19.86	8.11		4.33	38.14
	Probability (%), d.f. = 4[d]					10>P>5	P<1		P<1	10>P>5		50>P>30	P<1

Spore type	Phenotype of spore	his4 Obsd.	his4 III	his4 IV	leu2 Obsd.	leu2 III	leu2 IV	thr4 Obsd.	thr4 III	thr4 IV
Z1	homo +	474	478.6	440.3	313	318.6	304.8	407	418.2	439.8
Z2	homo -	282	279.1	317.4	183	176.0	189.8	278	268.0	246.4
Z3	hetero +	56	51.4	89.7	33	27.4	41.2	73	61.8	40.3
Z4	hetero -	248	250.9	212.6	163	170.0	156.2	202	212.0	233.6
	Total	1060	1060.0	1060.0	692	692.0	692.0	960	960.0	960.1
	χ^2		0.52	25.07		1.83	2.38		3.19	37.40
	Probability (%), d.f. = 3		95>P>90	P<1		70>P>50	50>P>30		50>P>30	P<1

Legend for Table II-6

[a] Homo and hetero indicate homothallic and heterothallic clones, respectively. Three types of homothallism, Ho, Hp, and Hq, were included in the "homo" category. Symbols + and - indicate a pair of alleles for the his4, leu2, or thr4 marker.

[b] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

[c] The theoretical values were calculated assuming the HMa gene is located on left (assumption III) or right (assumption IV) side of the mating-type locus at a distance of 98 stranes according to the calculation by the D_1 formula using the data listed in Table II-4.

[d] Each term of the ascus type in which expected occurrence of asci is less than five was not included for the calculation of χ^2 statistics.

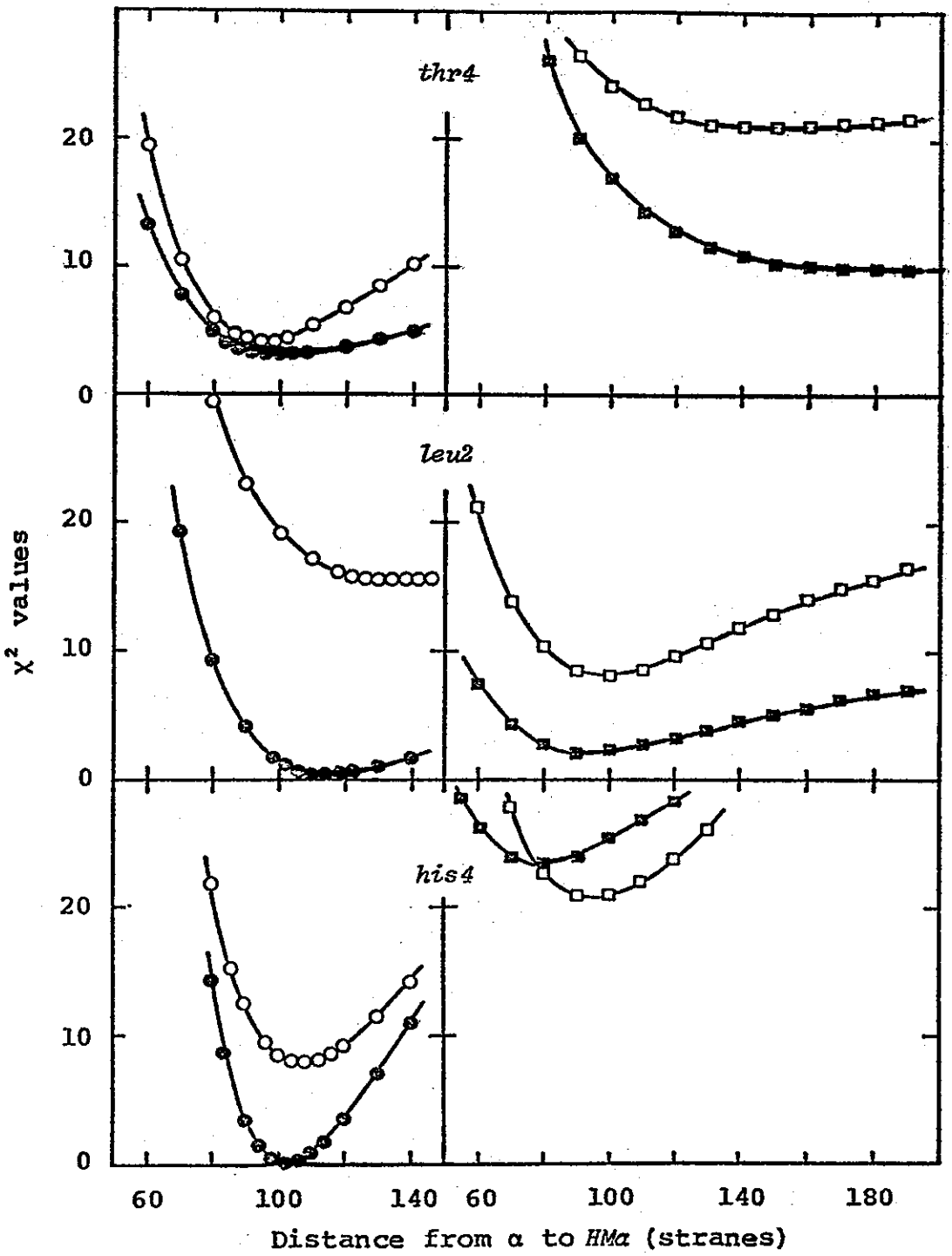

Fig. II-2. Chi-square tests for the position of the HMa locus on chromosome III.

Fig. II-2. Chi-square tests for the position of the HMa locus on chromosome III. Chi-square values were plotted with respect to the observed and expected values at the various positions of the HMa gene on chromosome III using his4, leu2, and thr4 as the standard for three factor analysis. Symbols : O and ●, χ^2 values by assumption III; and □ and ■, by assumption IV. Open and closed symbols indicate the values calculated from the data with respect to ascus types and spore types, respectively.

prepared covering 16 chromosomes other than chromosome III. Though detailed data are omitted here, we could not detect any significant deviation from 1 : 1 ratio in the frequencies of the parental and recombinant clones among the heterothallic segregants.

Linkage relationship between the HO and various markers on the 16 chromosomes: It is clear that the HO gene is not located on chromosome III (Table II-4). To determine which chromosome bears the HO gene, 25 standard markers, including the markers whose linkage to HMa was tested by random spore analysis as described above along with tyr1 (II), his7 (II), ade8 (IV), ura1 (XI), cdc5 (XIII), rna1 (XIII), pet17 (XV), and his8 (XV), were subjected to linkage analysis. Several diploids heterozygous for the above markers and having the HO HMα HMa/ ho HMα HMa genotype were constructed. Tetrad dissection of asci following sporulation of those diploids showed a 2 homothallic : 2 heterothallic segregation as the HO gene is epistatic to both of the a and α mating-type alleles, and also 2+ : 2- segregation for the genetic markers. All the tetrad distributions between HO and the standard markers indicate that these markers segregate independently from HO, except for tyr1 on chromosome II and lys7 on chromosome XIII. Tetrad distribution between HO and tyr1 showed a PD : NPD : T ratio of 29 : 13 : 83. This value is subsignificant (5% > P > 2% under the assumption that tyr1 and HO were not linked). However, we could not detect linkage of HO to lys2 (PD : NPD : T ratio of 5 : 5 : 33) and his7 (135 : 114 in parental class : recombinant class by random spore analysis) which are located on the left and right side of tyr1, respectively, on the same chromosome. In combination of lys7 and HO, we observed a PD : NPD : T ratio

of 48 : 25 : 191 ($P < 1\%$). On the other hand, two standard markers on the same chromosome of lys7, i.e., cdc5 and rna1, showed PD : NPD : T ration of 13 : 16 : 55 and 21 : 19 : 85 with HO, respectively. Linkage data of lys7 and tyr1 to the respective reference markers, cdc5 and his7, on the same chromosome essentially accord with those published by Mortimer and Hawthrone (1973), i.e., the PD : NPD : T ratio for cdc5 - lys7 was 13 : 4 : 22 ($2\% > P > 1\%$) and tyr1 - his7 showed 153 : 96 ratio ($P < 1\%$) in parental : recombinant classes by random spore analysis. In conclusion, the data strongly suggest no direct linkage of HO to all the 25 markers so far tested, including tyr1 and lys7, nor to the mating-type locus.

DISCUSSION

Because of the complex correlation of the genotypes and phenotypes in homothallism and the presence of direct linkage between the HMα and HMa loci to the mating-type locus, exact placement of HMα and HMa on chromosome III inevitably involved complicated algebraic treatment of three factor tetrad data. This principally came from the facts that the determination of the allelic 2 : 2 segregation of the homothallic gene in asci is not always possible and the mating-type loci is always heterozygous in the diploids. Furthermore, the special restrictions in the homothallic system reduced the 12 expected genotypic categories of asci to 6 kinds of ascus-types by phenotypic classification. This might reduce the reliability of the three point analysis. However,

the map distances from the mating-type locus for minimum χ^2 values based on assumptions II and III agreed closely for his4, leu2, and thr4 standard markers, while assumptions I and IV gave to meaningful values (Fig. II-1 and II-2). The results of calculations using leu2 as the standard were not critical for the mapping of either HMα or HMa loci, while the data with the his4 or thr4 marker which are the most distant from the gene to be mapped were much more critical. These results indicate that this procedure is not accurate for calculation of an absolute distance between two genes but enough for a comparison of two hypotheses as in the present case. In a typical case, the HMα gene might be linked to his4 at approximately 30 stranes under assumption I and 120 stranes under assumption II, giving a difference between the alternatives of 90 stranes. However, if the leu2 marker is used as standard, the expected difference between the two assumptions might be approximately 50 stranes. Hence comparison of the two assumptions is more clear with his4 as the standard than leu2. This might be one of the major reasons why the leu2 marker showed ambiguous results (Figs. II-1 and II-2; Tables II-5 and II-6).

The position of the HMα and HMa loci calculated above can be summarized as illustrated in Fig. II-3. The HMα gene is probably located on the right arm at a distance of approximately 95 stranes (65cM) from the centromere, and the HMa locus at approximately 90 stranes (64 cM) on the left arm. According to the data of Mortimer and Hawthorne (1966), the MAL2 gene was placed at a point outside thr4. They showed a ratio of the tetrad distribution of 137 : 4 : 145 (PD : NPD : T) between MAL2 and thr4. The data correspond to approximately 38 and 48 stranes by

Fig. II-3. Mapping the HMα and HMa loci on chromosome III. Roman letters indicate the site of the HMα and HMa loci deduced from three factor analyses using the his4, leu2, and thr4 markers as standard. Points calculated from the ascus types are placed below the line and those from the spore types above. Map distances are expressed in stranes.

the D_1 and D_2 formula, respectively. These values indicated that HMα might be placed near to the MAL2 locus, possibly distally, although the exact mapping between them must await further study.

ABSTRACT

Two of the three homothallic genes, HMα and HMa, showed direct linkage to the mating-type locus at approximately 73 and 98 stranes (57 and 65 centimorgans [cM]), respectively, whereas, the other, HO, showed no linkage to 26 standard markers distributed over 17 chromosomes including the mating-type locus. To determine whether the HMα and HMa loci located on the left or right side of the mating-type locus, equations for three factor analysis of three linked genes were derived. Tetrad data were collected and were compared with expected values by χ^2 statistics. Calculations indicated that the HMα gene is probably located on the right arm at 95 stranes (65 cM) from the centromere and the HMa locus at approximately 90 stranes (64 cM) on the left arm of chromosome III.

CHAPTER III

TETRAPLOID FORMATION THROUCH THE CONVERSION OF THE MATING-TYPE ALLELES AND THE CO-DOMINANCE OF THE HMα/hmα AND HMa/hma ALLELES

INTRODUCTION

It has been reported that homothallic genes act during cell growth within a few generations of spore germination (Takano and Oshima 1967). Consequently, conversion of one mating-type allele to the other occurs and the activity of the homothallic genes is blocked as soon as heterozygosity of the mating-type alleles is established by zygote formation between a converted and an unconverted cell. Whether the homothallic genes are able to act during vegetative growth cycles as well as soon after spore germination is not tested yet. A technical difficulty in the experimental approaching to this question lies in the infeasibility of constructing and maintaining cells containing homothallism genes that also display an a or α mating type. Typically, when ascospores carrying such a genetic combination are produced, their subsequent germination soon leads to diploidization, making it impossible to determine whether the homothallic genes act during vegetative growth. However, this difficulty is overcome by inducing reciprocal mitotic recombination between the centromere and the mating-type locus in a diploid cell. This event generates homozygosity for the mating-type locus in vegetatively growing a/α diploid cells. If homothallic genes are active during vegetative growth, it would be expected that a/a or α/α sectors will produce either a/α diploid state if only one of the a or α alleles is altered, or an a/a/α/α tetraploid state if both the sex alleles are switched and a cell containing a converted allele mates with an unconverted cell.

On the other hand, it was found that the HO hmα hma and HO HMα

HMa strains are completely homothallic as described in Chapter I. It is difficult to explain the molecular mechanism in the Ho homothallic cells having the HO hmα hma genotype by assuming the absence of the activities of the HMα and HMa genes. To explain this finding, Naumov and Tolstrokovs (1973) suggested that the hmα allele have the same function as the HMa gene, and that the hma allele is functionally equivalent to the HMa allele. The results described in Chapter I strongly support this idea. However, conclusive evidence has not been obtained, nor has dominance-recessiveness between each pair of alleles of the HMα and HMa genes been tested yet.

This Chapter describes evidence that the homothallic genes act during vegetative growth as well as after spore germination: Switching occurred from an a/a or α/α diploid cell to an a/α diploid or an a/a to α/α and vice versa. It is suggested that the hma and hmα alleles have the same or equivalent functions as the HMα and HMa alleles respectively, in other words, the HMα/hmα or HMa/hma alleles display co-dominance.

MATERIALS AND METHODS

Organisms: The strains used are listed in Table III-1. All strains were selected from our stock sulture for Yeast Genetics, and were purified by single cell isolation with the aid of a micromanipulator. Two heterothallic haploid strains, T-1059-18B(a) and C-435-39C (α), were used as the standards for the determination of mating type.

Table III-1. Genotypes of strains used.

Strain	Genotype			Thallism
	Homothallic gene	Mating type	Genetic markers[a]	
DR-14	$\frac{ho}{ho}\ \frac{HM\alpha}{HM\alpha}\ \frac{hma}{hma}$	$\frac{a}{\alpha}$	$\frac{+}{ade1}\ \frac{+}{arg4}\ \frac{his4}{his4}\ \frac{leu2}{leu2}\ \frac{lys2}{lys2}\ \frac{+}{met2}\ \frac{thr4}{thr4}\ \frac{+}{trp1}$	Hetero
C-18-2C	$\frac{HO}{HO}\ \frac{HM\alpha}{HM\alpha}\ \frac{HMa}{HMa}$	$\frac{a}{\alpha}$	$\frac{lys2}{lys2}\ \frac{trp1}{trp1}\ \frac{phoc}{phoc}$	Homo (Ho)[b]
C-18-16D	$\frac{HO}{HO}\ \frac{hm\alpha}{hm\alpha}\ \frac{hma}{hma}$	$\frac{a}{\alpha}$	$\frac{arg4}{arg4}\ \frac{lys2}{lys2}\ \frac{phoc}{phoc}$	Homo (Ho)[b]
C-436-5B	$\frac{HO}{HO}\ \frac{hm\alpha}{hm\alpha}\ \frac{HMa}{HMa}$	$\frac{a}{\alpha}$	$\frac{arg4}{arg4}\ \frac{phoc}{phoc}$	Homo (Hp)[b]
T-1055-16D	$\frac{HO}{HO}\ \frac{HM\alpha}{HM\alpha}\ \frac{hma}{hma}$	$\frac{a}{\alpha}$	$\frac{arg4}{arg4}\ \frac{gal1}{gal1}\ \frac{lys2}{lys2}\ \frac{phoc}{phoc}\ \frac{thr4}{thr4}\ \frac{ura3}{ura3}$	Homo (Hq)[b]
Dr-106	$\frac{HO}{HO}\ \frac{HM\alpha}{HM\alpha}\ \frac{hma}{hma}$	$\frac{a}{\alpha}$	$\frac{+}{ade1}\ \frac{arg4}{+}\ \frac{+}{his4}\ \frac{+}{leu2}\ \frac{+}{lys2}\ \frac{+}{met2}\ \frac{+}{thr4}\ \frac{trp1}{+}$	
C-415	$\frac{HO}{HO}\ \frac{HM\alpha}{HM\alpha}\ \frac{hma}{HMa}$	$\frac{a}{\alpha}$	$\frac{ade1}{+}\ \frac{gal1}{+}\ \frac{his4}{+}\ \frac{lys2}{+}\ \frac{+}{lys1}\ \frac{+}{met14}\ \frac{thr4}{+}$	
C-452	$\frac{HO}{HO}\ \frac{hm\alpha}{hm\alpha}\ \frac{hma}{HMa}$	$\frac{a}{\alpha}$	$\frac{arg4}{+}\ \frac{lys2}{+}\ \frac{+}{thr4}$	
T-1059-18B	*HO HMα hma*	*a*	*ade1 gal1 his4 leu2 thr4*	Hetero
C-435-39C	*HO hmα HMa*	α	*his4 leu2 lys7*	Hetero

[a] The terminology of genetic symbols follows that proposed by Plischke et al. (1976)

[b] See Chapter I for the notations.

Media: Suboptimal synthetic complete medium (SSC) was prepared by supplementing Burkholder's minimal medium with nutrient materials (amino acids and nucleic acid bases) at 1/100 of the concentrations described in Chapter I. This medium was used for determination of frequency of prototroph formation in combination with complementary genetic markers.

Techniques: Occurrence of cells showing a or α mating type from a/α diploid cells was detected by the prototroph recovery method consisting of the modified sedimented aggregation and the molten soft agar (0.7%) overlay techniques (Campbell 1973). Diploid a/α cells to be tested and standard a or α haploid cells were cultivated in nutrient broth with shaking for 24 hr at 30°C. The cells were harvested, washed with sterile distilled water and resuspended in 0.15M NaCl solution to give cell density of approximately 2×10^7 cells per ml for the a/α diploid strain and 2×10^8 cells per ml for both the a and α standard haploids. Equal volumes (0.5ml) of the cell suspensions of the a/α diploid strain and the a or α haploid strain were mixed in small tubes; the cells were immediately sedimented by centrifugation at 3,000 rpm for 5 min. After 2 hr incubation at 30°C, the sedimented mixture was resuspended gently, diluted and plated on SSC medium. All platings were performed with molten soft agar, and all plates were incubated at 30°C. The limited concentration of supplements in SSC medium permits the resolution of zygotic colonies, but prevents the non-zygotic colonies from reaching more than microcolony status. Thus, after 3 to 5 days of incubation, the colonies prominently visible on the plates were scored assuming they were derived from the zygotes between diploid

cells having mating ability and a or α cell of the standard haploid. On the contrary, non-zygotic colonies were still quite small. Mitotic recombination was induced by exposing cells of each strain on nutrient agar plates to ultraviolet-light (approximately 90 percent survival) according to Roman and Jacob (1958). Replica plating was performed following the description of Lederberg and Lederberg (1951).

RESULTS

Appearance of mating types among a/α diploid cells: Cells showing a or α mating type appear spontaneously among a/α diploid cells during vegetative growth, by mitotic recombination between the mating-type locus and the centromere on chromosome III, mitotic gene conversion, non-disjunction of chromosome III or by mutation. These events are enhanced by treatment with various recombinogens or mutagens (Roman and Jacob 1958; Zimmermann, Kern and Rasenberger 1975). Cells showing mating potency can be detected by scoring the appearance of prototrophic zygotes on an appropriate selective medium with standard haploid cells having appropriate complementary genetic markers. Here their occurrence was detected by the prototroph recovery method according to Campbell (1973) with slight modification. Results clearly indicated that switches in both directions, i.e., from the a/α non-mater cells to the cells showing a or α mating-type, occurred with the frequency of approximately 10^{-4} to 10^{-5} in all the strains tested, although characteristic biases in the frequencies were suggested in some strains,

particularly in the Hp and Hq homothallic strains (Table III-2).

Isolation of clones showing mating response from a/α diploids: To see whether the homothallic genes are effective in diploid cells showing mating type during their vegetative growth, diploid a/α cells of Hp type of homothallic strain were irradiated with a low dose (approximately 90 percent survival) of ultraviolet-light to accelerate mitotic recombination. The irradiated cells were plated on nutrient agar and the plates were incubated at 30°C for 3 days. Several colonies appearing on the plates were picked up and the isolates were tested for their mating reaction with the standard strain having a or α mating type. In the Hp type of homothallic strains, C-436-5B, 5 clones showing α mating type were obtained from 600 colonies examined, while no clones having a mating ability were isolated.

Similar results had been obtained by I. Takano and T. Oshima, Central Research Institute of Suntory Ltd. (personal communication; Takano et al. 1977). According to their results, in heterothallic strains, both a and α mating-type clones were obtained with frequencies of 0.3% to 3.8%, while in the Ho type of homothallic strain neither a or α clones could be isolated from 1,000 colonies examined. In the Hp type of homothallic strain, on the other hand, 25 clones showing solely α mating type from 1,550 colonies tested and 42 clones showing a mating type from 1,880 colonies were obtained in a Hp diploid (see Table AIII-1).

The observations listed in Table III-2 and the results of Takano et al. (1977) might be interpreted as indicating that homozygosity at the mating-type locus, i.e., a/a and α/α, occurred in the original a/α

Table III-2. Spontaneous occurrence of cells showing mating potency in heterothallic and homothallic a/α diploids.

Strain	Thallism	Occurrence of cells showing mating potency (prototrophic colonies per 10^4 a/α diploid cells)					
		Exp. 1		Exp. 2		Exp. 3	
		a[a]	α[b]	a	α	a	α
C-435-5B	Homothallism (Hp)	8.10	0.10	3.00	0.02	0.10	0.09
T-1055-16D	Homothallism (Hq)	0.50	2.60	0.03	0.86	0.33	1.70
C-18-2C	Homothallism (Ho; Type II)	0.01	0.04	-	-	0.01	1.40
C-18-16D	Homothallism (Ho; Type I)	0.20	0.77	-	-	0.67	6.80
DR-14	Heterothallism	0.41	0.75	-	-	-	-

[a] Number of switches from a/α to a scored by occurrence of prototrophic colonies with the α haploid cells having complementary auxotrophic markers.

[b] Number of switches from a/α to α scored with the a haploid cells as described above.

diploid cells irrespectively of thallism. If an excess population of haploid a or α cells was present in the culture, as in the experiments listed in Table III-2, the a/a and α/α cells could immediately form aggregates with the haploid cells, and subsequently the zygotes gave rise to prototrophic colonies on SSC media by the complementary combination of the auxotrophic markers. Even if homozygosity at the mating-type locus occurred in a pure culture as in the experiments listed in Table AIII-1, the probability of cell fusion with the opposite mating-type cells in the culture before vegetative growth begins would be low because of the rare occurrence of homozygosity. Thus the conversion of a mating-type allele must occur during vegetative multiplication in the homothallic a/a or α/α cells. It is possible to speculate two alternative types of conversion of the mating-type alleles in a/a and α/α diploid cells. One is from the a/a or α/α homozygous allele to the a/α configuration and the other from a/a to α/α or vice versa. The former conversion will give rise to a non-mater a/α diploid from both a/a and α/α cells. In the later conversion, cell fusion will occur between the converted and unconverted cells and give rise to a/a/α/α tetraploid cells. Since both directions of mating-type conversion, a to α and α to a, are possible in the Ho type of homothallic cells, transiently appearing a/a and α/α cells would be quickly converted to the a/α or a/a/α/α cells as described above. Thus, it is expected that all the colonies in the Ho strain will show no mating reaction with both a and α standards. In the Hp strain, however, α to a conversion is blocked, whereas in the Hp strain a to α conversion is not possible. Hence the α/α cells in the Hp strain and the a/a cells in the Hq strain

could maintain homozygosity at the mating-type locus through vegetative growth.

Occurrence of tetraploid clones from Hp type of diploid hybrid strain: To confirm the above possibility, isolation of a tetraploid was attempted from an Hq type of diploid strain, DR-106, which was constructed by crossing a spore from an Hq type of homothallic diploid strain with an a cell of another Hq haploid by the spore-to-cell mating method. The diploid strain was marked heterozygously with several auxotrophic markers including the thr4 gene. Tetrad analysis showed a 2 homothallic : 2 a segregation for thallism and 2+ : 2- for each genetic marker including thr4. Thus, strain DR-106 was confirmed to be an Hq type diploid.

About 200 cells of this strain were plated on each of 30 nutrient plates and exposed to ultraviolet-light (approximately 90 percent survival) to induce mitotic recombination. The plates were incubated at 30°C. After three days of incubation, the colonies appearing were replicated on minimal plates appropriately supplemented with nutrients required, but lacking threonine (threonine test plate). Of the mitotic crossovers between the centromere and the thr4 locus, only half result in a sectored colony (Fig. III-1). The sectors posses genotypes thr4/thr4 or +/+, and only the +/+ sector grow on threonine test plate. Since the thr4 locus is located at about 30 stranes distal to the mating-type locus, which is situated at 20 stranes from centromere on chromosome III, 40% of those sectored colonies having thr4/thr4 configuration would be expected to be a diploid with an α/α genotype produced by crossing over between the mating-type locus and centromere.

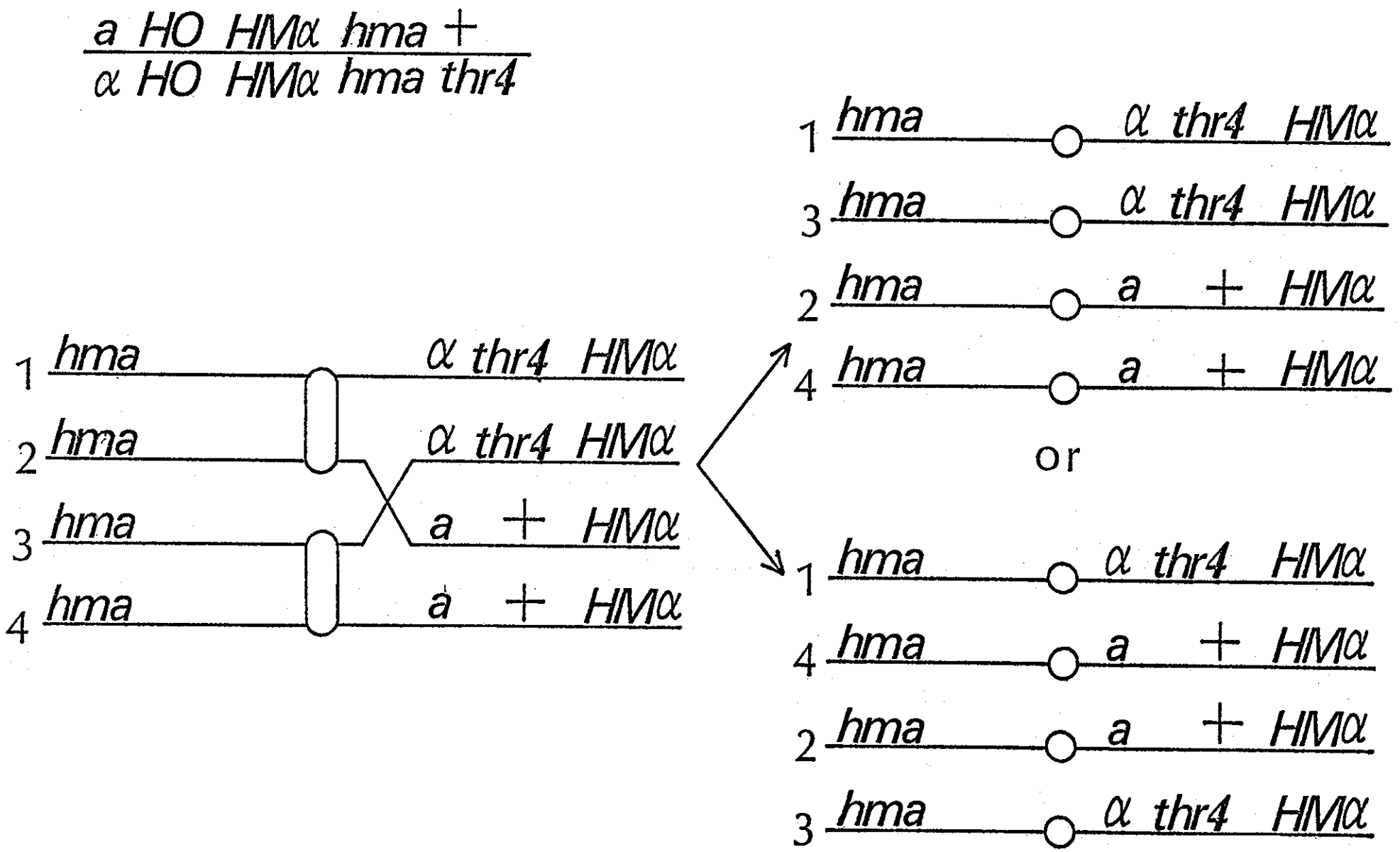

Fig. III-1. Reciprocal mitotic crossing-over in chromosome III in DR-106.

Fig. III-1. Reciprocal mitotic crossing over in chromosome III in DR-106. Mitotic crossing over results in two types of sectored colonies. These occur with equal frequencies and depend on the assortment of the centromeres. Upper (1, 3 : 2, 4): one sector is homozygous for thr4 and α while the other is homozygous for + and a. Lower (1, 4 : 2, 3): both sectors are heterozygous for all markers. Only the 1, 3 : 2, 4 upper pattern would yield a sectored colony on threonine test plate. Cells derived from mating between a/a and α/α cells at the first division after the mitotic crossover event can grow on threonine test plate. Hence they are not analyzed.

Thus, we can isolate α/α diploids from a/α diploids more efficiently by selecting colonies showing sector for threonine requirement than by random isolation of colonies.

Of approximately 6,000 clones tested, 65 sectored colonies were observed and the sectors showing threonine requirement were picked up, purified, and tested for their mating reaction with the standard haploid strain having a or α mating type. All clones tested did not show mating reaction with either a and α of the standard haploid cells. Since strain DR-106 is an Hq strain (HO HMα hma) and has the a-THR4/α-thr4 configuration, the thr4/thr4 sector must have the α/α genotype. Hence these clones should consist of tetraploid cells and/or diploid cells, as described in the preceeding section. Cells of higher ploidy are expected to have larger size (Takano, Yoshizumi and Terashima 1966; Townsend and Lindegren 1954; Gunge and Nakatomi 1972; Takano and Oshima 1973). Thus, clones showing larger cell size than the original diploid cells and having high levels of sporulation were selected by inspection under a microscope, and dissected to ascertain their ploidy by segregation pattern for each heterozygous marker. Six strains showing larger cell size, DR-106-9, DR-106-18, DR-106-25, DR-106-40, DR-106-82, and DR-106-111, were thought to be tetraploid, since those strains generated 4+ : 0-, 3+ : 1- and 2+ : 2- segregation patterns for each marker heterozygously marked in the original diploid DR-106. In contrast to these clones showing larger cell size as the original diploid cells, DR-106-3, DR-106-20, and DR-106-63, were found to be diploid because of the solely 2+ : 2- segregation for the heterozygous markers.

It is possible to expect three types of segregation with respect

to the mating types in asci from a heterothallic an a/a/α/α tetraploid (Table III-3) as described by Roman, Phillips and Sands (1955). In a heterothallic strain, the 4 non-mater (a/α) : 0 mater, 2 non-mater (a/α) : 1 a (a/a) : 1 α (α/α), and 2 a (a/a) : 2 α (α/α) segregations will be expected in asci. If the HO hmα hma and HO HMα HMa genotypes (the Ho strains) are effective for mating-type conversion in both directions in the a/a and α/α diploid cells as well as in the a and α haploid cells, all asci from the Ho type homothallic tetraploid should show a 4 non-mater : 0 mater segregation, while three types of segregation, 4 : 0, 2 : 2, and 0 : 4, would be expected with respect to the diploid : tetraploid ratios in each tetrad culture assuming that the a/a and α/α cells are converted to a/a/α/α tetraploid. Since α to a conversion in the Hp strain and a to α conversion in the Hq strain are not expected, a tetraploid originating from an Hp type homothallic strain will show that 4 non-mater : 0 mater, 3 non-mater : 1 α (α/α) and 2 non-mater : 2 α (α/α) segregation in asci, and that from an Hq type will give the 4 non-mater : 0 mater, 3 non-mater : 1 a (a/a), and 2 non-mater : 2 a (a/a) segregations. All non-mater clones in the asci showing a 4 non-mater : 0 mater segregation should be diploids, as in the Ho strain, and two clones of three non-maters in the asci showing a 3 non-mater : 1 mater segregation should be diploids and the remaining one should be tetraploid. All the non-maters in the asci showing a 2 non-mater : 2 mater segregation should be tetraploids.

To confirm the above possibilities, tetrad analysis of the supposed tetraploids strains derived from the Hq diploid, DR-106, was performed (Table III-4). The clones showing larger cell size, i.e.,

Table III-3. Three principal types of asci expected from three types of homothallic a/a/α/α tetraploid.

Type of homothallism	Types of segregation											
	I				II				III			
	A	B	C	D	A	B	C	D	A	B	C	D
	a/α	a/α	a/α	a/α	a/α	a/α	a/α	α/α	a/a	a/a	α/α	α/α
Ho	a/α[a]	a/α	a/α	a/α	a/α	a/α	a/a/α/α[a]	a/a/α/α	a/a/α/α	a/a/α/α	a/a/α/α	a/a/α/α
Hp	a/α	a/α	a/α	a/α	a/α	a/α	a/a/α/α	α/α[b]	a/a/α/α	a/a/α/α	α/α	α/α
Hq	a/α	a/α	a/α	a/α	a/α	a/α	a/a[b]	a/a/α/α	a/a	a/a	a/a/α/α	a/a/α/α

[a] a/α and a/a/α/α have no mating response.

[b] a/a and α/α have mating response of a and α respectively.

Table III-4. Tetrad segregation in asci from threonine-dependent clones originated from the Hq diploid strain, DR-106.

Strain	Ploidy	segregation of mating type (non[a]:a:α)							Expected genotype of mating type alleles
		4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	0:2:2	
DR-106	original diploid	0	0	0	0	8	0	0	a/α
DR-106-18	tetraploid	8	10	0	0	4	0	0	a/a/α/α
DR-101-82	tetraploid	0	3	0	0	0	0	0	a/a/α/α
DR-106-111	tetraploid	1	2	0	0	1	0	0	a/a/α/α
DR-106-3	diploid	0	0	0	0	7	0	0	a/α
DR-106-20	diploid	0	0	0	0	4	0	0	a/α
DR-106-63	diploid	0	0	0	0	6	0	0	a/α

[a] Non-mater.

supposed tetraploids, sporulated well and the ascospores showed good viability on tetrad dissection. Three types of asci with respect to mating type were observed in the tetrads from the tetraploid having the Hq genotypes for homothallism i.e., the supposed tetraploid segregants gave the 4 non-mater : 0 mater, 3 non-mater : 1 a (a/a), and 2 non-mater : 2 a (a/a) segregation patterns, while the diploid non-mater segregants from them always showed 2 non-mater : 2 a segregation. The three typical types of asci from strain DR-106-18 are shown in Table III-5. Further tetrad analysis of the segregants accorded well with the expectation with respect to Hq type, as described in Table III-3. All these results strongly suggest that the strain DR-106-18 showing large cell size is indeed tetraploid having the a/a/α/α HO/HO/HO/HO HMα/HMα/HMα/HMα hma/hma/hma/hma genotype, and that the homothallic genes are effective in the α/α diploid cells with the same specificity for the mating-type alleles as observed in the haploid cells.

While this work was in progress, I. Takano (personal communication; Takano et al. 1977), reported that similar results were observed using the three kinds of autodiploidized homothallic strains, Ho, Hp and Hq. Consequently, genotypes of these strains are homozygous with respect to all genetic backgrounds except for the mating-type allele. Their results are summarized as follows: From the plates incubated with the ultraviolet-light irradiated a/α diploid cells of the Ho, Hp and Hq type, they isolated several colonial clones showing larger cell size than the original diploids by inspection under a microscope. Three clones, designated T-1269-38C-U1 of Ho, S-14-9C-U3 of Hp and T-1023-23B-U16 of Hq, were thought to be tetraploid by their cell volumes and

Table III-5. Further analysis of three asci showing typical types of segregation from the supposed homothallic (Hq) tetraploid strain, DR-106-18.

Ascus		Mating type	Spore formation	Tetrad analysis				Expected	
				No. of asci tested	4 non:0 mater	3 non:1 *a*	2 non:2 *a*	Ploidy	Genotype of mating-type alleles
7	A	non[a]	+	4	0	0	4	Diploid	*a*/α
	B	non	+	3	0	0	3	Diploid	*a*/α
	C	non	+	4	0	0	4	Diploid	*a*/α
	D	non	+	5	0	0	5	Diploid	*a*/α
12	A	*a*	-	-	-	-	-	Diploid	*a*/*a*
	B	non	+	3	0	0	3	Diploid	*a*/α
	C	non	+	5	1	1	3	Tetraploid	*a*/*a*/α/α
	D	non	+	5	0	0	5	Diploid	*a*/α
1	A	*a*	-	-	-	-	-	Diploid	*a*/*a*
	B	*a*	-	-	-	-	-	Diploid	*a*/*a*
	C	non	+	5	3	1	1	Tetraploid	*a*/*a*/α/α
	D	non	+	6	3	2	1	Tetraploid	*a*/*a*/α/α

[a] Non-mater.

cellular deoxyribonucleic acid (DNA) content, which were both almost double those of their parental diploid cells (Tables AIII-2, AIII-3, and AIII-4). Results of tetrad analysis of these three supposed tetraploid clones accorded with the expectations, as described in Table III-3.

During the course of the above experiment, however, they observed that some asci of the supposed tetraploid clones from Ho gave 1 diploid : 3 tetraploid and 3 diploid : 1 tetraploid segregations according to the estimation of ploidy by cell size. If the mating-type alleles of the a/a or α/α diploids were converted to α/α or a/a, respectively, in a homothallic strain, the above segregations would not be expected (Table III-3). This fact suggests that another possibility, i.e., the a/a or α/α to a/α conversion, might occur in some spore-cultures of those asci. Occurrence of this type of conversion in the tetrad segregants of the supposed tetraploids having the Hp or Hq genotype was also suggested by the tetrad data (Takano; unpublished data).

Tetraploidization by cell fusion during outgrowth of diploid spores: It was strongly suggested by the above genetic studies that diploid cells or diploid ascospores having the a/a or α/α mating-type alleles are changed to either sporogenous non-mater tetraploid or diploid cells by the effect of homothallic genes during their vegetative growth. In a haploid homothallic ascospore carrying a homozygous genotype for HO, HMα and HMa alleles, it has been observed that the diploidization occurs mostly at the four cell stage after the second division of cells immediately following spore germination (Takano and Oshima 1967; Hicks and Herskowitz 1976). To see cell fusion to produce tetraploid cells by the cultivation of a homothallic diploid ascospore, Takano et al.

(1977) observed the manner of tetraploidization in a culture of a homothallic diploid ascospore using the techniques of photomicrographic traces.

Two types of zygote formation were observed: One resulted in twin zygotes and the other formed only one zygote. The former type of zygote formation is quite similar to that observed during the outgrowth of a homothallic haploid spore, and suggests that both the mating-type alleles in the a/a and α/α genotypes were effectively converted to the opposite alleles within a few generations of spore germination, as in haploid cells. Then tetraploidization occurred through the zygote formation between the converted and unconverted cells. The latter cultures produced a mixed population of large and small cells, both of which are non-mater and sporogenous. The large cells were derived from the zygote, and small ones might have originated from unfused cells. These observations, along with the exceptional asci observed in the tetrad analysis of non-maters (Takano et al. 1977), strengthen the possibility that some of the a/a or α/α genotypes would be converted to a/α.

Is there a dominant-recessive relationship with respect to HMα/hmα and HMa/hma alleles?: In Chapter I, it was demonstrated that the strain with the HO hmα hma genotype gives rise to an Ho type homothallic diploid, as in the case of the strain carrying the HO HMα HMa genotype, by the genetic analysis of the homothallic strains. But, as pointed out in Discussion of Chapter I, it is difficult to explain the homothallic switching observed in cells of the HO hmα hma genotype by merely assuming the presence of inactive alleles of the HMα and HMa genes. However, Noumov and Tolstorukov (1973) explained this finding by introducing the

the idea that the hma allele might have the same function as the HMα gene and that the hmα gene might be functionally equivalent to the HMa gene (Model 1). This idea implies that the HMα/hmα or the HMa/hma alleles display co-dominance, in other words, that there is no dominant-recessive relationship with respect to the HMα/hmα and HMa/hma alleles. However it is possible to imagine other molecular models which can explain all the observations described in Chapter I, by assuming the presence of the dominance-recessiveness with respect to the HMα/hmα and HMa/hma alleles. For example, the HO gene product might have activity for both the conversion of mating type a to α, and α to a. The hmα and hma allele might produce an inhibitor molecule against the conversion of α to a and a to α, respectively, while the HMα and HMa alleles do not produce an active molecule. In this model (Model 2), the hmα or hma should be dominant over the HMα or HMa allele. Alternatively, the HMα and HMa alleles might produce an inhibitor molecule against the conversion of a to α and α to a, respectively, while hmα and hma do not produce an active molecule. In this model (Model 3), the HMα or HMa should be dominant over the hmα or hma. In addition, it must be assumed that the gene products of the hmα and hma alleles in Model 2 or those of the HMα and HMa alleles in Model 3 inactivate each other when both are present in a cell.

These three models can be distinguished by testing whether the cells having the a/a HO/HO HMα/HMα hma/HMa or α/α HO/HO hmα/hmα hma/HMa genotype generated from a/α HO/HO HMα/HMα hma/HMa or a/α HO/HO hmα/hmα hma/HMa diploid, respectively, by mitotic recombination between the centromere and the mating-type locus, maintain their mating ability during

vegetative growth. According to Model 1, cells having a/a HO/HO HMα/HMα hma/HMa genotype and α/α HO/HO hmα/hmα hma/HMa genotype should lose mating ability during vegetative growth. According to Model 2, cells having a/a HO/HO HMα/HMα hma/HMa genotype should maintain a mating type while cells carring α/α HO/HO hmα/hmα hma/HMa genotype lose their mating ability during vegetative growth. On the other hand, according to Model 3, cells having a/a HO/HO HMα/HMα hma/HMa genotype should lose their mating potency, whereas cells having α/α HO/HO hmα/hmα hma/HMa should maintain α mating ability during vegetative growth. Thus, the different patterns of switching of the mating-type allele allows differentiation of these three models.

To test above expectations, two kinds of diploid strains, C-415 and C-452, having a/α HO/HO HMα/HMα hma/HMa thr4/+ and a/α HO/HO hmα/hmα hma/HMa +/thr4 genotype, respectively, were constructed by spore-to-cell mating method. Mitotic recombination in chromosome III between the centromere and the mating-type locus will give a/a and α/α sectored colonies. If sectored colonies for thr4 were isolated from strain, C-415 and C-452, 40% of those thr4 dependent sectors would have the a/a thr4/thr4 HO/HO HMα/HMα hma/HMa genotype and α/α thr4/thr4 HO/HO hmα/hmα hma/HMa genotype respectively, as described above, because the centromere distance of the mating-type locus is reported as 20 stranes and that of thr4 is 50 stranes (see Chapter II). In all, 152 threonine-dependent sectors were isolated from strain C-415, and 147 from strain C-452, and the isolates were tested for their mating reaction with standard haploid strains of a and α mating type. All 147 clones isolated from strain C-452, showed no mating reaction with either a or α

standards, while only 3 of 152 clones from strain C-415 showed mating response with standard α haploid. These results clearly exclude the possibility of Model 3. Furthermore, if Model 2 is correct, about 61 ($=152 \times \frac{20 \text{ stranes}}{50 \text{ stranes}}$) of the 152 threonine-dependent sectors from the strain C-415 should maintain mating ability during vegetative growth because they should have a/a HO/HO HMα/HMα hma/HMa genotype, whereas only 3 clones showed mating response. Consequently, the results strongly support Model 1, i.e., the hma allele plays the same role as the HMα allele, and hmα has the same function as HMa for the mating-type interconversion. The appearance of 3 clones showing a mating type under Model 1 will be discussed later.

DISCUSSION

The process for producing the tetraploid clones most probably occurs in two main steps. First, there is a genetical event to produce the a/a and α/α configuration at the mating-type locus from the a/α diploid cells. This might be either mitotic recombination or mutation at the mating-type locus; which event occurred in the present case has not been determined. However, it has been proposed that mitotic recombination, most possibly by mitotic crossing over between the mating-type locus and the centromere on chromosome III, plays a major role in the occurrence of homozygosity of the mating-type allele (Gunge and Nakatomi 1972). This event was detected with a frequency of one in 10^4 to 10^5 cells spontaneously by the modified aggregation mating method (Table

III-2). Frequency of a/α to a/a or α/α switches was significantly increased to approximately 10^{-2} to 10^{-3} of cell population by low dose of ultraviolet-light irradiation (90 percent survival). Furthermore, it might be expected that the frequencies of occurrence of a/a cells and α/α cells in the a/α diploid culture are the same in any a/α diploid culture, irrespective of the thallism of the cells (Table III-2 and Takano et al. 1977). Second, mating-type conversion occurs effectively in some fraction of the a/a and α/α diploid cells by the action of homothallic genes as well as in haploid cells. This is followed by cell fusion between converted and unconverted cells. The resultant zygote produces a new tetraploid bud which presumably has the a/a/α/α configuration. These facts suggest that it is possible to select a/a/α/α tetraploid clones by microscopic inspection of individual colonies, since tetraploid colonies consisting of cells showing larger cell size than diploid cells are expected to appear at frequency of 10^{-2} to 10^{-3}. Furthermore, conversion of the a/a and α/α configuration to a/α was also suggested (Takano et al. 1977).

Though several models have been proposed to explain the mating-type interconversion (Oshima and Takano 1971; see also Chapter I; Holliday and Pugh 1975; J. B. Hicks and I. Herscowitz, personal communication 1976), the exact mechanism of the homothallic genes has not been established. Nor is the dominance-recessiveness between each pair of alleles of the HMα and HMa genes yet understood, although evidence for the dominance of the HO allele over the ho allele has been presented by Hopper and Hall (1975). Regarding this problem, it was observed that all of the supposed α/α HO/HO hmα/hmα hma/HMa and a/a

HO/HO HMα/HMα hma/HMa sectors (except for 3 clones) generated from a strain with genotypes a/α HO/HO hmα/hmα hma/HMa and a/α HO/HO HMα/HMα hma/HMa, lost their mating ability during vegetative growth. This finding strongly supports Naumov and Tolstorukov's idea that the hma allele has the same function as the HMα allele, and the hmα allele plays the same role as the HMa allele for the mating-type interconversion. The 3 clones showing a mating response isolated from strain C-415 might have a/a HO/HO HMα/HMα hma/hma genotype caused by double mitotic recombinations, one recombination occurring between the centromere and the HMa/hma locus on the left arm and the other between the centromere and the mating-type locus on the right arm of chromosome III (see Chapter II). In order to prove conclusively that Naumov and Tolstorukov's idea (Model 1) is correct, further genetic analyses should be carried out to demonstrate tetraploids having a/a/α/α HO/HO/HO/HO HMα/HMα/HMα/HMα hma/HMa/hma/HMa and a/a/α/α HO/HO/HO/HO hmα/hmα/hmα/hmα hma/HMa/hma/HMa genotype among the threonine-dependent sectors showing no mating response.

On the other hand, the technique of ultraviolet-light induced sectoring for the mating-type locus can not be used for diploids carrying the heterozygous genotype for HMα gene. The technical difficulties lie in the fact that the HMα locus is located in the right arm of chromosome III distal to the mating-type locus (see Chapter II). Hence, mitotic recombination between the centromere and the mating-type locus would lead to co-sectoring of the mating-type and the HMα/hmα locus. However, dominance-recessiveness of the HMα and hmα alleles could be determined by testing phenotypic segregation patterns in tetrads of tetraploid

strains marked heterozygously for HMα/hmα.

The observations described in this Chapter indicate a new method for breeding tetraploid strains, though several questions remain to be solved for full understanding of the phenomena described above. The procedure does not require any auxotrophic markers for selection, in contrast to conventional polyploid breeding techniques which require such markers because of the low frequency of zygote formation in forced mating mixture (Gunge and Nakatomi 1972; Pomper, Daniels and Mackee 1954). Most of the tetraploid strains obtained by this procedure sporulate well and segregate a/a/α/α tetraploids, a/a and α/α diploids with a/α diploids depending on the genotype for homothallism. These segregants will be useful for further construction of polyploid cells.

On the other hand, it has been reported that a number of yeast strains used in the brewing industry and bakery are polyploid (Emeis 1961, 1965; Takano, Yoshizumi and Terashima 1966; Gunge 1966). A novel type of semi-homothallic strain, the das (diploid a sporogenous) strain, was described, which is of special interest in its application to the breeding of triploid cells (Oshima and Takano 1972; Takano and Oshima 1972). A mutation, designated dmt, was also reported which allows a/α diploids homozygous for this gene to mate with both a and α diploids giving either a/a/α or a/α/α triploids (Blamire and Melnick 1975). The procedure described in this Chapter is more widely applicable to breeding polyploid strains than that for the das strain or dmt strain, because it does not require such special genetic traits.

ABSTRACT

Mating-type conversion by the homothallic genes in the vegetatively growing diploid cells occurred after the spontaneous or ultraviolet-light induced appearance of homozygosity of the mating type allele, a/a and α/α, from the a/α configuration. When homothallic, semi-homothallic or heterothallic a/α diploid cells were incubated with an excess population of a or α haploid cells having complementary nutritional markers in 0.15M NaCl solution, prototrophic colonies appeared with a frequency of approximately 10^{-4} to 10^{-5} of the diploid cells on subsequent plating of the aggregation mixture on selective medium. However, when a/α cells (irradiated with low dose of ultraviolet-light to accelerate mitotic recombination) were directly plated on nutrient agar, and each colony appearing on the plate was tested for its mating response with the standard haploid cells, striking differences were observed depending on the genotypes for homothallism of the deploids cells. None of the 1,000 colonies of a perfect homothallic strain (the Ho type) so far tested showed mating reaction, while some isolated colonies of a heterothallic strain showed either a or α mating type activity. In the isolates showing mating potency from an Hp type semi-homothallic strain, solely α mating-type was observed, whereas in those from an Hq type semi-homothallic diploid showed a mating-type. These findings suggest that switching of the a/α heterozygous configuration to a/a and α/α occurs in both heterothallic and homothallic cells. And the conversion of the a/a alleles to α/α or vice versa, or from the a/a and α/α to a/α depends on the genotype for homothallism of the cell.

The former conversion is followed by cell fusion between the converted and the unconverted cells to produce tetraploid cells. This inference was supported by tetrad analyses of the supposed tetraploid clones. It was also demonstrated that a/a and α/α sectors generated from strains with the a/α HO/HO HMα/HMα hma/HMa and a/α HO/HO hmα/hmα hma/HMa genotypes lost their mating ability during vegetative growth. This finding and results obtained in Chapter I support Naumov and Tolstorukov's idea that HMα and hmα, and HMa and hma are co-dominant and the hma allele has the same function as the HMα allele, and hmα is functionally equivalent to HMa.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSION

GENERAL DISCUSSION AND CONCLUSION

The mating type in Saccharomyces yeasts is controlled by a single pair of alleles, a and α. These two alleles are mutually interchageable by the action of the homothallic genes. In Chapter I, three kinds of homothallic genes were described, each consisting of a single pair of alleles, HO/ho, HMα/hmα, or HMa/hma. A spore having the HO hmα hma genotype gives rise to the Ho type of homothallism, as in the case of the D strain which has the HO HMα HMa genotype. A spore having the a HO hmα HMa or α HO HMα hma genotype will produce an Hp (showing a 2 homothallic diploid:2 heterothallic α haploid segregation) or Hq (showing a 2 homothallic diploid:2 heterothallic a haploid segregation) type of homothallic diploid culture, respectively. The other genotypes, a HO HMα hma and α HO hmα HMa, and the genotypes combined with the ho allele give rise to a heterothallic spore culture. The discovery of these three kinds of homothallic genes resulted from the isolation of the Hp and Hq strains from various strains of Saccharomyces species (Takano and Oshima 1976; Santa Maria and Vidal 1970), rather than from mutagenic studies. This fact is true especially for two pairs of alleles, HMα/hmα and HMa/hma.

In addition to these three homothallic genes, another homothallic gene has been introduced into the system by the isolation of a recessive mutation, cmt (change of mating type), which showed the same pattern of mating-type conversion as HO except in that the HO gene is dominant (Hopper and Hall 1975). The cmt mutation is thought to occur in the nucleus, as it showed a 2 CMT:2 cmt segregation in all the asci tested.

It is not linked to mating-type locus, and is not allelic to the HO gene. The authors have suggested several models to account for the cmt function in connection with the system consisting of HO, HMα, and HMa genes: 1) sequential negative control, i.e., HO negatively controls the cmt gene, cmt negatively controls HMα and HMa, and these in turn act to change mating type; 2) the separate control of HO and cmt over the HMα and HMa genes, or 3) direct action of cmt upon mating-type locus independent of the HO, HMα, and HMa gene system. Though these possibilities have not been tested yet, further isolation and characterization of this type of mutants will be one of the main approaches to the elucidation of mating-type differentiation.

On the other hand, how does the homothallic genes stimulate a switch between a and α? The answer to this question would also require more detailed understanding of the structure of the mating-type locus. The mating-type alleles, a and α, determine the mating type of cells and direct the mating reaction. They are also involved in the control of other cellular process, including meiosis and ascospore formation, meiotic and mitotic recombination, and recovery from the X-ray damage. Heterozygosity for the mating-type alleles (a/α) is a necessary condition for the meiosis and ascospore formation of diploid or polyploid cells. Diploids homozygous for mating type, a/a or α/α, do not sporulate, but instead exhibit a mating response consistent with the mating-type allele present (Roman and Sands 1953). This fact suggests that gene products of both a and α alleles are necessary for meiosis and ascospore formation and also block mutually the expression of a and α phenotypes. The activity of the homothallic genes is also blocked by the establish-

ment of heterozygosity at the mating-type locus (Takano and Oshima 1967). For more precise understanding of the mating-type conversion, it is worthwhile to discuss and summarize present knowledge of mutations occurring in the mating-type locus or its vicinity. It is of interest to test whether a mating-type allele having a mutation is converted to another or not, because the gene product of the homothallic genes might act directly upon the mating-type locus itself.

Several mutations or deletions occurring in or near the mating-type locus are reported. Hawthorne (1963) isolated a lethal mutation of chromosome III in which the deletion might be extended from the mating-type locus to the thr4 locus. The deletion causes allelic conversion of α to a. Genes essential for the mating process have been identified by the isolation of 12 different classes of non-mating (ste) mutants (MacKay and Manney 1974a). Of these mutations, some of those unlinked to the mating-type locus are non-specific for the mating type while others are specific for the mating type and act only on a or α. Of the linked mutants, two classes of α ste mutants from α strains carry mutations inseparable from the mating-type locus; ste mutations linked to the a mating type allele have not been isolated. Similar temperature sensitive non-mating mutants (mts) were isolated by Takahashi and Sakai (1974). Their mutants were classified into three groups, all of which are unlinked to the mating-type locus and these non-mating mutations were found to be recessive. Another type of defective α allele, α-inc (an α allele inconvertible to a) was reported by Takano, Kusumi and Oshima (1973). The α-inc shows normal behavior as the α mating-type allele for mating potency and for meiosis. However, the α-inc allele

showed low frequency of α to a conversion in the presence of effective homothallic genes. Nevertheless, all the a clones derived from the α-inc clone behaved normally and the a allele could be converted to a normal α allele. Hicks and Herskowitz (personal communication 1976) reported a similar observation with respect to the defective α allele (the stel allele described by MacKay and Manney 1974a, b): it can be converted to a functional a allele and subsequently to a functional α allele by the action of the homothallic gene system. In contrast, the action of the homothallic gene system does not restore mating ability to strains having the other ste mutations which are not at the mating-type locus. These observations indicated that a yeast cell contains an additional copy (or copies) of the α information.

From these observations two kinds of models have been proposed for differentiation of mating-type. These involve either modification or recombination of the DNA at the mating type locus. According to the observations described above, all yeast cells should contain information for both mating-type alleles, of which only one is expressed. In the modification model, expression of either the a or α information is determined by a promoter (or regulatory site) of the mating-type locus which has been specifically methylaed or modified in DNA sequence (Hicks and Herskowitz, personal communication 1976; MacKay 1972; see also Holliday and Pugh 1975). The enzyme responsible for modification of the DNA and, hence, for control of transcription would thus be under the control of the homothallic genes.

By contrast, in the recombination model, intramolecular recombination within the mating-type locus flanking the promoter inverts the

promoter and changes the direction of transcription of the whole locus. The recombination event in the locus is presumed to be mediated by the homothallic gene, which perhaps codes for a site-specific recombination enzyme like that found in bacteriophage λ (Signer and Weil 1968). This model predicts that homothallic mating-type switching might be blocked in a recombination negative (rec) genetic background. Strains containing the rad52 (= rec2) mutation were capable of diploidization by homothallic genes. However, whether other rec mutant alleles have any effect on homothallism remains to be investigated (Klar and Fogel, personal communication 1976). These two kinds of model easily account for the observation that the a information is not affected by the α stel mutation, but not sufficient to account for the recovery of α function which occurred at high frequency in α stel homothallic strain. The same difficulty will arise in explaining the observation that the normal α allele was derived from α-inc via conversion through the normal a allele.

These difficulties can be easily explained by the idea originally proposed by Oshima and Takano (1971; Chapter I). According to their model, the elementary structure of the mating-type locus for both the a and α alleles is essentially the same. The association of some kind of controlling element with this locus would cause the differentiation of two mating-type alleles. The HMα, hmα, HMa, and hma alleles produce the specific controlling elements. The association of an HMα (or hma) element with the mating-type locus would form the a mating-type allele and the association of an HMa (or hmα) element with the mating-type locus would give rise to the α mating-type allele. The HO gene controls insertion or removal of the controlling element into the mating-type locus,

this type of modification probably being analogous to the bacterial IS (insertion sequence) (Hirsch, Starlinger, and Brachet 1972; Fiandt, Szybalski, and Malamy 1972; Malamy, Fiandt, and Szybalski 1972) or to the controlling elements in maize (McClintock 1956). According to this model, the α-inc allele could be explained as an α mating-type allele in which a controlling element from the HMa (or hmα) gene was attached abnormally to its affinity site. The abnormal association of the HMa (or hmα) element could cause the insensitiveness to the action of the HO gene. If the abnormally attached element is removed in low freqnency, and replaced by a newly produced element from the HMα (or hma) gene by the action of the HO gene, a normal a mating-type allele will arise. With the same model, the observation that the stel mutation was completely restored by converting it to a mating type and further to a normal α by the action of the HO gene, can also be explained by assuming that the stel mutation occurred in the controlling element. A similar explanation (cassette model) of recovery of the normal α allele from the defective α stel strain has been proposed by Hicks and Herskowitz (personal communication 1976): additional α information exists as a complete copy (or copies) which is ordinarily not expressed. One of these DNA copies can be substituted for the resident information at the mating-type locus by the action of the homothallic genes. They suggested that the HMa and hmα alleles are silent copies of the α information, and HMα and hma alleles are silent copies of the a information and that these copies are moved around by the action of the HO gene. Thus, the model described Chapter I and that proposed by Hicks and Herskowitz are essentially the same mechanism. Further elucidation of the mechanisms of homothallic

genes will require molecular investigation of the active entities of the HO, HMα, HMa and other genes concerned with homothallism.

In addition to the genes described above, there are several reports on other mutations concerned with the ability to sporulate and with mating. The das (diploid a sporogenous) strain isolated by Takano and Oshima (1973) is a diploid having significant potency of a mating-type, and shows 2 das:2 a segregation in each ascus upon self-sporulation because of its HO/HO HMα/HMα hma/hma genotype. Gerlach (1974) obtained a recessive mutation, sca (sporulation capable), that is unlinked to the mating-type locus and able to sporulate the a/a or α/α diploids. Hopper and Hall (1975) also detected a similar mutation, designated CSP (control of sporulation), which is dominant to the wild-type allele and unlinked to the mating-type locus. Some of the α/α diploids carrying the sca or CSP mutation produce asci containing four α spores upon sporulation. Kassir and Simchen (1976) have shown a locus for the regulator of meiosis (rem) which, if homozygous for the rem/rem mutation, bypasses the control of sporulation by the mating-type locus and enables several diploids, even if the a/a and α/α genotypes, to go through meiosis, probably by starting the premeiotic DNA replication without the commitment of the a/α locus. The rem locus is linked to the mating-type locus but not within it. In the same report, Kassir and Simchen described an a mutant allele (a*) characterized by defectiveness of sporulation. The mutation is dominant and occurs in the mating-type locus. It may produce an abnormal gene-product or an abnormal configuration of the mating-type locus. For the elucidation of the structure and function of the mating-type locus, further genetic study

with respect to the mutual interaction of these genes and the homothallic genes is of particular interest.

Recently, a striking observation on the switching of mating type has been described by Hicks and Herskowitz (1976): the switch from a to α and vice versa is detectable after a minimum of two cell divisions. 50% of the clones tested showed switching by the four-cell stage. Of the four cells descended from a single cell, only the oldest cell and its immediate daughter were observed to change mating-type. This pattern suggests that one event in the switching process occurs in the first cell division cycle. Restriction of the switched mating type to two particular cells may reflect the action of the homothallism system followed by non-random segregation of DNA strands in mitosis.

On the other hand, it has been reported that industrial yeasts are commonly polyploid: most brewer's yeasts are tetraploid, some are triploid (Emeis 1961; 1965; Takano, Yoshizumi, and Terashima 1966) and tetraploid strains of baker's yeasts have also been reported (Gunge 1966). These observations suggest polyploid cells may have some advantage in industrial uses. In fact, Oshima et al. (unpublished data) tried to breed a yeast strain suitable for alcoholic fermentation of a mash prepared by hydrolysis of starch, but the resultant diploid strains never gave more potent fermentation than the authentic polyploid strains. In this respect, it is noteworthy that homothallic genes can function in vegetatively growing cells as well as soon after spore germination. Consequently, cell fusion could double the ploidy subsequent to the mating-type conversion by the action of homothallic genes. This finding suggests the idea that the action of homothallic genes is involved

in the appearance of polyploid in industrial yeasts, and indicates the possibility of breeding hexaploid from triploid, and octaploid from tetraploid cells. Since polyploids resulting from this process must be isogenic, except in the mating-type locus, it will be possible to test the effect of ploidy on cell activity and function. Through these studies, it will be possible to elucidate why polyploid yeasts are advantageous in industrial uses and, consequently, what kinds of yeasts should be breed for practical use. The concepts and techniques established in the studies on yeasts will be applicable to improvement of other industrial eukaryotic microorganisms for further development of fermentation technology.

APPENDIX I

FREQUENCIES OF TWELVE ASCUS-TYPES AND ARRANGEMENT OF THREE GENES FROM TETRAD DATA

INTRODUCTION

It is not always possible to map a gene with the genetic data obtained by direct inspection of a phenotype due to the complex correspondence of genotypes to the phenotype. In the course of mapping of homothallic genes, HMα and HMa, in Saccharomyces cerevisiae and its related species, we have encountered this difficulty because the determination of the allelic 2 : 2 segregation in tetrads concerning those genes is not always possible. To solve this difficulty, I have derived equations for three factor analysis of the tetrad data in the case of three linked genes (see Chapter II). This procedure is applicable to the mapping of genes in other situations, for example, when two genes, X and Y, showing same phenotype are marked heterozygously in a diploid and the map position of X is not known while that of Y is known. To map X on the chromosome by conventional tetrad analysis, allelic segregations of X/x and of several heterozygously marked standard genes should be determined in each ascus. Hence it is necessary to test allelism to determine the 2X : 2x segregation for most of the tetrads in the conventional procedure. But the new procedure proposed here does not require the allelism test. The tetrad distribution for the X - Y gene pair can be easily obtained by scoring three types of asci, the 0+ : 4-, 1+ : 3-, and 2+ : 2- tetrads, if the X and Y genes are complementary for the X (and Y) phenotype in a xY × Xy cross. Since the map position of Y is already known, it is possible to determine the X locus on chromosome with the aid of another appropriate standard gene, Z, by three factor analysis.

This appendix describes theoretical equations to express frequencies of the ascus types for three heterozygous genes on two or three chromosomes. The reliability of these equations was confirmed with the authentic auxotrophic genetic markers. Application of these equations for gene mapping is described.

FREQUENCIES OF TWELVE ASCUS-TYPES

Twelve ascus-types abbreviated as X1 to X12 are expected from a diploid of triply heterozygous cross, XYZ/xyz, as illustrated in Table AI-1. Their frequencies of occurrence, denoted as x_1 to x_{12}, are obviously related to the linkage among the three genes and to the gene arrangement on chromosome(s). Possible arrangements of three genes on chromosome(s) can be summarized by the four cases illustrated in Fig. AI-1. In Case I, all genes are located on the same chromosome. In Case II, three genes are distributed on three different chromosomes. In Case III, two of three loci are on the same arm of a chromosome and the other on another chromosome, whereas in Case IV, two genes are located on opposite arms of the same chromosome. Different mathematical equations for calculation of theoretical frequencies of 12 ascus-types should be applied for these different arrangements of three genes.

a) Equations for Case I (three genes on the same chromosome): For the mapping of two homothallic genes, HMα and HMa, I have already described the equations to estimate the frequencies of 12 ascus-types in the case of three linked genes (see Chapter II). These equations are:

Table AI-1. Twelve possible segregations in asci from triply heterozygous (*XYZ*/*xyz*) diploid.

Ascus type	Spore			
	A	B	C	D
X1	*XYZ*	*XYZ*	*xyz*	*xyz*
X2	*XYZ*	*XYz*	*xyZ*	*xyz*
X3	*XYz*	*XYz*	*xyZ*	*xyZ*
X4	*XYZ*	*Xyz*	*xYZ*	*xyz*
X5	*XYZ*	*XyZ*	*xYz*	*xyz*
X6	*XYz*	*XyZ*	*xYZ*	*xyz*
X7	*XYz*	*Xyz*	*xYZ*	*xyZ*
X8	*XYZ*	*Xyz*	*xYz*	*xyZ*
X9	*XYz*	*XyZ*	*xYz*	*xyZ*
X10	*Xyz*	*Xyz*	*xYZ*	*xYZ*
X11	*Xyz*	*XyZ*	*xYz*	*xYZ*
X12	*XyZ*	*XyZ*	*xYz*	*xYz*

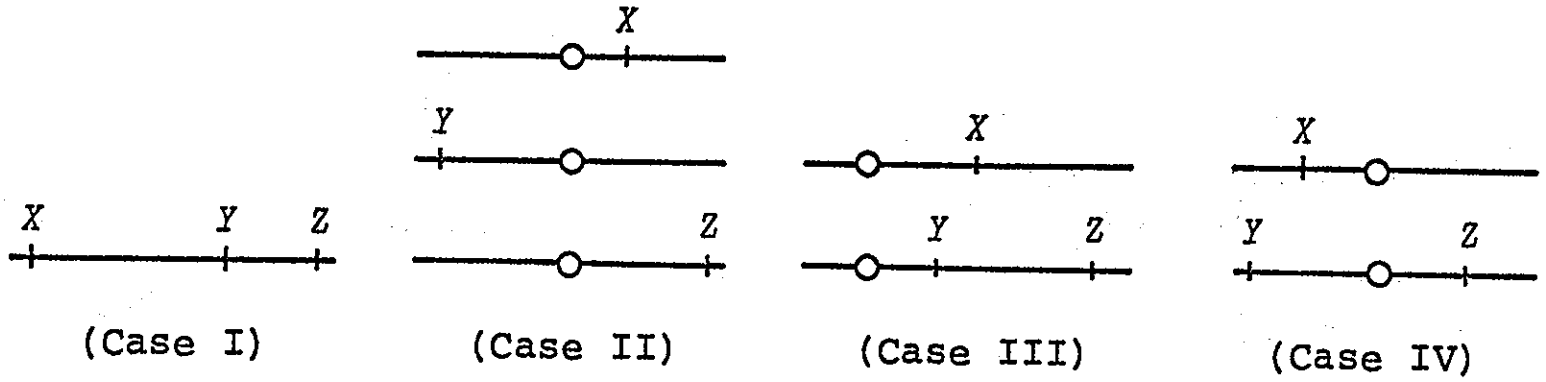

Fig. AI-1. Four possible arrangements of three genes on chromosome(s).

Fig. AI-1. Four possible arrangements of three genes on chromosome(s).

$$x_1 = a_1 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.1)$$

$$x_2 = a_1 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.2)$$

$$x_3 = a_1 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.3)$$

$$x_4 = a_3 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.4)$$

$$x_5 = a_3 \cdot b_3/4 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.5)$$

$$x_6 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.6)$$

$$x_7 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.7)$$

$$x_8 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.8)$$

$$x_9 = a_3 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.9)$$

$$x_{10} = a_2 \cdot b_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.10)$$

$$x_{11} = a_2 \cdot b_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.11)$$

$$x_{12} = a_2 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.12)$$

where the tetrad distribution, i.e., ratio of the parental ditype (PD) : nonparental ditype (NPD) : tetratype (T) asci, for X - Y and Y - Z gene-pairs in the segregants from the XYZ/xyz diploid in the Case I arrangement of the gene order of X - Y - Z (Fig. AI-1) were denoted as $a_1 : a_2 : a_3$ and $b_1 : b_2 : b_3$ ($a_1 + a_2 + a_3 = 1$ and $b_1 + b_2 + b_3 = 1$), respectively. A given distance between two genes, e.g. X to Y distance (D_{XY}), can be converted to the expected values of tetrad distribution by the equations:

$$a_3 = \frac{2}{3}[1 - \exp(-D_{XY}/33.3)] \quad \ldots\ldots\ldots \quad (1.13)$$

$$a_2 = \frac{1}{2}[1 - a_3 - \exp(-0.02D_{XY})] \quad \ldots\ldots \quad (1.14)$$

$$a_1 = 1 - (a_2 + a_3) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1.15)$$

The reliability of these equations was tested by comparison of expected and observed frequencies of tetrad data (1,380 asci) obtained

from the triply heterozygous diploids for his4, leu2, and thr4. It was observed that both values showed good fit (70% > P > 50%) (see Chapter II).

Since these equations were derived assuming that all crossing over occur in a Poisson manner, the units of map distance may be calculated with the values of tetrad distribution either $D_1 = -50 \ln(a_1 - a_2)$ or $D_2 = -33.3 \ln(1 - 3a_3/2)$ (Shult and Lindegren 1956a, 1956b; Desborough and Lindegren 1959). The unit of map distance has been termed "strane" which is equal to 50× the average number of crossovers per meiosis. It corresponds closely to centimorgans (cM) over short distances but deviates markedly, becoming greater, as the distances increase. The considerable shrinkage distortion characteristic of maps based on centimorgans is avoided by the use of stranes (Lindegren, Lindegren, Shult and Desborough 1959).

b) Equations for Case II (three genes located on different chromosomes): General solutions for calculation of the frequencies of second division segregation from the given frequencies of tetratype tetrads for three genes on three different chromosomes or more genes on two or more different chromosomes have been proposed by Whitehouse (1950, 1957). However, his procedure cannot be directly applied to calculate the frequencies of 12 ascus-types from the given map distances of three genes or vice versa.

In the Case II arrangement (Fig. AI-1) of three genes, frequencies of occurrence of 12 ascus-types, i.e., x_1 to x_{12}, are obtained using the frequencies of the second division segregations, χ, υ, and ζ, of the genes, X, Y, and Z, respectively. It is assumed that the χ, υ,

and ζ values are not greater than 2/3. The frequency of X1 ascus will be given in the following manner: the frequencies of first division segregation of X and Y are $1 - \chi$ and $1 - \upsilon$, respectively, and $(1 - \chi)(1 - \upsilon)$ equals the frequency of occurrence of the PD and NPD asci for the X - Y gene-pair. Thus, the frequency of occurrence of the PD ascus between the X and Y genes will be $(1 - \chi)(1 - \upsilon)/2$. In addition, Z should be segregated as PD with X to give the X1 ascus. Thus $(1 - \chi)(1 - \upsilon)(1 - \zeta)/4$ contributes to the occurrence of the X1 asci (the second term of equation 2.1). When both X and Y genes show second division segregation, 50% of the asci will show PD and NPD segregations with equal frequencies (25% each) and the remaining 50% will show T segregation. When the Z gene also shows second division segregation, 25% of the asci will show the PD segregation with X as described above. Thus, 1/16 of the asci in which X, Y, and Z all show second division segregation will contribute the frequency of the X1 ascus (the first term of equation 2.1). If one gene shows first division segregation and another gene shows second division segregation, all of the asci will show the T segregation. Equations for frequencies of the other ascus types were derived in the same manner using the frequencies of second division segregation of three genes. Thus, the equations for the Case II arrangement are:

$$x_1 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{4}(1 - \chi)(1 - \upsilon)(1 - \zeta) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

$$x_2 = \frac{1}{8}\chi\upsilon\zeta + \frac{1}{4}\chi\upsilon(1 - \zeta) + \frac{1}{2}(1 - \chi)(1 - \upsilon)\zeta \quad \ldots\ldots\ldots\ldots \quad (2.2)$$

$$x_3 = x_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.3)$$

$$x_4 = \frac{1}{8}\chi\upsilon\zeta + \frac{1}{2}\chi(1 - \upsilon)(1 - \zeta) + \frac{1}{4}(1 - \chi)\upsilon\zeta \quad \ldots\ldots\ldots\ldots \quad (2.4)$$

$$x_5 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{4}\chi(1 - \upsilon)\zeta + \frac{1}{2}(1 - \chi)\upsilon(1 - \zeta) \quad \ldots\ldots\ldots\ldots \quad (2.5)$$

$$x_6 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{4}\chi(1 - \upsilon)\zeta + \frac{1}{4}(1 - \chi)\upsilon\zeta + \frac{1}{4}\chi\upsilon(1 - \zeta) \quad \ldots\ (2.6)$$

$$x_7 = x_5 \quad \ldots\ (2.7)$$

$$x_8 = x_6 \quad \ldots\ (2.8)$$

$$x_9 = x_4 \quad \ldots\ (2.9)$$

$$x_{10} = x_1 \quad \ldots\ (2.10)$$

$$x_{11} = x_2 \quad \ldots\ (2.11)$$

$$x_{12} = x_1 \quad \ldots\ (2.12)$$

A given centromere distance for a certain gene can be converted to the expected frequency of second division segregation by the equations:

$$\chi = \frac{2}{3}[1 - \exp(-D_{\underline{OX}}/33.3)] \quad \ldots\ (2.13)$$

where $D_{\underline{OX}}$ is the map distance of the $\underline{X}$ locus to its centromere. Expected frequencies of second division segregations, υ and ζ, are calculated by $D_{\underline{OY}}$ or $D_{\underline{OZ}}$ in the same equation. By using the frequencies of second division segregation, e.g. χ and υ for the $\underline{X}$ and $\underline{Y}$ genes, respectively, the expected tetrad distribution for the $\underline{X}$ - $\underline{Y}$ gene-pair is calculated by the equations:

$$a_1 = \frac{1}{2}(1 - \chi)(1 - \upsilon) + \frac{1}{4}\chi\upsilon \quad \ldots\ (2.14)$$

$$a_2 = a_1 \quad \ldots\ (2.15)$$

$$a_3 = (1 - \chi)\upsilon + \chi(1 - \upsilon) + \frac{1}{2}\chi\upsilon \quad \ldots\ (2.16)$$

c) Equations for Case III (two of three loci on the same arm of a chromosome and the other on another chromosome): We may consider this case as a variation of Case I, though $a_1 = a_2$ in Case III, while $a_1 > a_2$ in Case I. In this case, the expected values of the tetrad distributions for $\underline{X}$ - $\underline{Y}$ gene-pair, i.e., a_1, a_2 (= a_1), and a_3, were

calculated from given centromere distances of the X and Y loci via the frequencies of their second division segregation (equations 3.1, 3.2, 3.3, 3.7, and 3.8), while b_1, b_2, and b_3 were calculated by the same procedure as in Case I (equations 3.4, 3.5, and 3.6). Hence the frequency of 12 ascus-types are expressed by equations 1.1 to 1.12 where

$$a_1 = \frac{1}{2}(1 - \chi)(1 - \upsilon) + \frac{1}{4}\chi\upsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

$$a_2 = a_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

$$a_3 = \chi(1 - \upsilon) + (1 - \chi)\upsilon + \frac{1}{2}\chi\upsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.3)$$

$$b_3 = \frac{2}{3}[1 - \exp(-D_{\underline{YZ}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.4)$$

$$b_2 = \frac{1}{2}[1 - b_3 - \exp(-0.02D_{\underline{YZ}})] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.5)$$

$$b_1 = 1 - (b_2 + b_3) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.6)$$

$$\chi = \frac{2}{3}[1 - \exp(-D_{\underline{OX}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.7)$$

$$\upsilon = \frac{2}{3}[1 - \exp(-D_{\underline{OY}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.8)$$

$$\zeta = \frac{2}{3}[1 - \exp(-D_{\underline{OZ}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.9)$$

d) Equations for Case IV (two loci on opposite arms of a chromosome and the other on another chormosome): This case will be regarded as a variation of Case II though these two cases could be distinguished. Since Y and Z genes are located on different chromosomes, $b_1 = b_2$ will be expected in Case II. In Case IV, however, the Y and Z genes are directly linked to each other and should show $b_1 > b_2$ in the tetrad distribution. Thus, equations 2.1, 2.3, 2.4, 2.9, 2.10, and 2.12 in Case II should be modified for Case IV as follows: If the centromere is regarded as a kind of gene, O/o, and the frequencies of asci in which gene-pairs of Y or Z to the respective centromere, Y - O and Z - O, segregated as PD and NPD may be expressed as υ_{pd} and υ_{npd}, and ζ_{pd} and

ζ_{npd}, respectively,and the term $(1 - \upsilon)(1 - \zeta)$ in Case II becomes $(\upsilon_{pd} + \upsilon_{npd})(\zeta_{pd} + \zeta_{npd})$, i.e., $\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd}$. In Case IV, however, it would be expected that the terms $\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd}$ contribute to the x_1, x_4, x_{10} values and the terms $\upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd}$ contribute to x_3, x_9, and x_{12}. Thus, we obtain equations:

$$x_1 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{2}(1 - \chi)(\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd}) \quad \ldots\ldots\ldots \quad (4.1)$$

$$x_2 = \frac{1}{8}\chi\upsilon\zeta + \frac{1}{4}\chi\upsilon(1 - \zeta) + \frac{1}{2}(1 - \chi)(1 - \upsilon)\zeta \quad \ldots\ldots\ldots \quad (4.2)$$

$$x_3 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{2}(1 - \chi)(\upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd}) \quad \ldots\ldots\ldots \quad (4.3)$$

$$x_4 = \frac{1}{8}\chi\upsilon\zeta + \chi(\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd}) + \frac{1}{4}(1 - \chi)\upsilon\zeta \quad \ldots\ldots \quad (4.4)$$

$$x_5 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{4}\chi(1 - \upsilon)\zeta + \frac{1}{2}(1 - \chi)\upsilon(1 - \zeta) \quad \ldots\ldots\ldots \quad (4.5)$$

$$x_6 = \frac{1}{16}\chi\upsilon\zeta + \frac{1}{4}\chi(1 - \upsilon)\zeta + \frac{1}{4}(1 - \chi)\upsilon\zeta + \frac{1}{4}\chi\upsilon(1 - \chi) \quad \ldots. \quad (4.6)$$

$$x_7 = x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.7)$$

$$x_8 = x_6 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.8)$$

$$x_9 = \frac{1}{8}\chi\upsilon\zeta + \chi(\upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd}) + \frac{1}{4}(1 - \chi)\upsilon\zeta \quad \ldots\ldots \quad (4.9)$$

$$x_{10} = x_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.10)$$

$$x_{11} = x_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.11)$$

$$x_{12} = x_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.12)$$

where

$$\upsilon_{pd} + \upsilon_{npd} + \upsilon = 1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.13)$$

$$\zeta_{pd} + \zeta_{npd} + \zeta = 1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.14)$$

$$\chi = \frac{2}{3}[1 - \exp(-D_{\underline{OX}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.15)$$

$$\upsilon = \frac{2}{3}[1 - \exp(-D_{\underline{OY}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.16)$$

$$\zeta = \frac{2}{3}[1 - \exp(-D_{\underline{OZ}}/33.3)] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.17)$$

$$a_1 = \frac{1}{2}(1 - \chi)(1 - \upsilon) + \frac{1}{4}\chi\upsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.18)$$

$$a_2 = a_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.19)$$

$$a_3 = (1 - \chi)\upsilon + \chi(1 - \upsilon) + \frac{1}{2}\chi\upsilon \quad \ldots\ldots (4.20)$$

$$b_1 = \upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd} + \frac{1}{4}\upsilon\zeta \quad \ldots\ldots (4.21)$$

$$b_2 = \upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd} + \frac{1}{4}\upsilon\zeta \quad \ldots\ldots (4.22)$$

$$b_3 = (1 - \upsilon)\zeta + \upsilon(1 - \zeta) + \frac{1}{2}\upsilon\zeta \quad \ldots\ldots (4.23)$$

or $$b_1 = 1 - (b_2 + b_3) \quad \ldots\ldots (4.24)$$

$$b_2 = \frac{1}{2}[1 - b_3 - \exp(-0.02D_{\underline{YZ}})] \quad \ldots\ldots (4.25)$$

$$b_3 = \frac{2}{3}[1 - \exp(-D_{\underline{YZ}}/33.3)] \quad \ldots\ldots (4.26)$$

It is also possible to calculate b_1 and b_2 by equations:

$$b_1 = \frac{1}{4}[2 - 2\upsilon - 2\zeta + 3\upsilon\zeta + 2(1 - 3\upsilon/2)^{\frac{2}{3}}(1 - 3\zeta/2)^{\frac{2}{3}}] \ldots (4.27)$$

$$b_2 = \frac{1}{4}[2 - 2\upsilon - 2\zeta + 3\upsilon\zeta - 2(1 - 3\upsilon/2)^{\frac{2}{3}}(1 - 3\zeta/2)^{\frac{2}{3}}] \ldots (4.28)$$

To confirm the above equations experimentally, tetrad data from a diploid triply heterozygous for three different auxotrophic markers, in which frequencies of occurrence of the 12 ascus-types and the tetrad distributions corresponding to both the X - Y and Y - Z gene pairs can be determined directly, were collected using triplets of genes among ade1 (chromosome I), lys2 (II), his4 (III), leu2 (III), thr4 (III), and urs3 (V). The collected data were compared with expected frequencies of ascus types calculated by equations for a particular type of gene arrangement using two or three of the tetrad distribution data in each triple of genes (Table AI-2). (The confirmation of the equations for Case I has been described in Chapter II.) In all cases, good fit ($P > 30\%$) was obtained between calculated and observed frequencies of each ascus-type. Thus, it will be possible to use these equations for analysis of tetrad data with three point crossing.

Observed and theoretical ratios of ascus types from triply heterozygous hybrids for various auxotrophic markers.

Ascus type	Case II		Case III		Case IV	
	ade1 leu2 ura3		lys2 leu2 his4		ade1 leu2 thr4	
	Observed[a]	Expected	Observed	Expected	Observed	Expected
X1	81	85.2	92	88.2	63	60.9
X2	45	35.9	27	30.3	113	116.2
X3	81	85.2	3	3.4	11	15.8
X4	20	22.7	383	387.7	21	22.1
X5	31	34.6	32	33.3	15	15.6
X6	2	7.1	37	33.3	14	14.1
X7	35	34.6	33	33.3	19	15.6
X8	7	7.1	36	33.3	13	14.1
X9	28	22.7	15	15.1	12	10.4
X10	73	85.2	92	91.1	60	60.9
X11	40	35.9	30	31.3	120	116.2
X12	98	85.2	4	3.5	19	15.8
Total	541	541.4	784	783.8	478	477.7
χ^2	12.45		1.32		3.98	
d.f.[b]	11		9		11	
Probability (%)	50>P>30		P>99		98>P>95	

Legend for Table AI-2

Observed data were collected according to the ascus type as described in Table AI-1. Each of three auxotrophic markers corresponds to $\underline{X}/\underline{x}$, $\underline{Y}/\underline{y}$, and $\underline{Z}/\underline{z}$ of Fig. AI-1 and Table AI-1 in that order as described in this table.

Expected frequencies of the ascus types, x_1 to x_{12}, were calculated by the following procedure: The tetrad distributions for the $\underline{X}$ - $\underline{Y}$ (a_1, a_2, and a_3), $\underline{Y}$ - $\underline{Z}$ (b_1, b_2, and b_3), and $\underline{X}$ - $\underline{Z}$ (c_1, c_2, and c_3) gene-pairs were scored from the entire collected data. Expected frequencies of the ascus types were then calculated by substituting the a_1, a_2, a_3, b_1, b_2, and b_3 values into equations 1.1 to 1.12 in Case III. In Case II and IV, frequencies of second division segregation of the $\underline{X}$, $\underline{Y}$, and $\underline{Z}$ genes, χ, υ, and ζ, were calculated by the following three simultaneous equations (Whitehouse 1950):

$$a_3 = \chi + \upsilon - 3\chi\upsilon/2$$

$$b_3 = \upsilon + \zeta - 3\upsilon\zeta/2$$

$$c_3 = \chi + \zeta - 3\chi\zeta/2$$

Expected frequencies of the ascus types were then calculated by substituting the values of χ, υ, and ζ into equations 2.1 to 2.12 in Case II, and 4.1 to 4.12 in Case IV. In Case IV, $(\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd})$ and $(\upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd})$ values were calculated from equations 4.21 and 4.22, i.e., $\upsilon_{pd}\cdot\zeta_{pd} + \upsilon_{npd}\cdot\zeta_{npd} = b_1 - \upsilon\zeta/4$, and $\upsilon_{pd}\cdot\zeta_{npd} + \upsilon_{npd}\cdot\zeta_{pd} = b_2 - \upsilon\zeta/4$.

[a] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

[b] Each term of the ascus type in which expected occurrence of asci is less than five was not included in the calculation of χ^2 statistics.

PROCEDURE FOR MAPPING

When the condition was set up as that a gene to be mapped is X and another gene, Y, of the same phenotype of the X gene is known map position, X will be mapped with an additional standard gene, Z, whose allelic segregation in asci can be determined independently of the X and Y phenotype. Under these conditions, the tetrad distribution of the X - Y gene pair can be determined by scoring three ascus types, i.e., 0+ : 4- (PD), 1+ : 3- (T), and 2+ : 2- (NPD) from a xY × Xy cross assuming that X and Y are complementary. When the X and Y genes are unlinked and segregate independently, PD : NPD : T ratio is 1 : 1 : 4 (N distribution) (Shult and Lindegren 1956a). When X - Y shows L distribution, i.e., the frequency of occurrence of PD exceeds that of NPD, and that of T asci is less than 2/3 of total asci tested, it is possible to conclude that X and Y are linked. When the tetrad distribution shows F distribution, i.e., equal frequencies of PD and NPD asci occur while T frequency is less than 2/3 of the total asci tested, both genes are linked to their respective centromeres.

a) X and Y on the same chromosome: Determination of whether the X gene is located to the left or right side of the Y gene can be made using an appropriate standard marker, Z, on the same linkage group as in the case of the mapping of the HMα and HMa genes (see Chapter II). This procedure might be summarized as follow: The theoretical frequencies of 12 ascus-types were calculated assuming two alternative positions of X on the chromosome, i.e., arrangements A and B (Fig. AI-2), by application of the equations 1.1 to 1.12. In calculation of the map

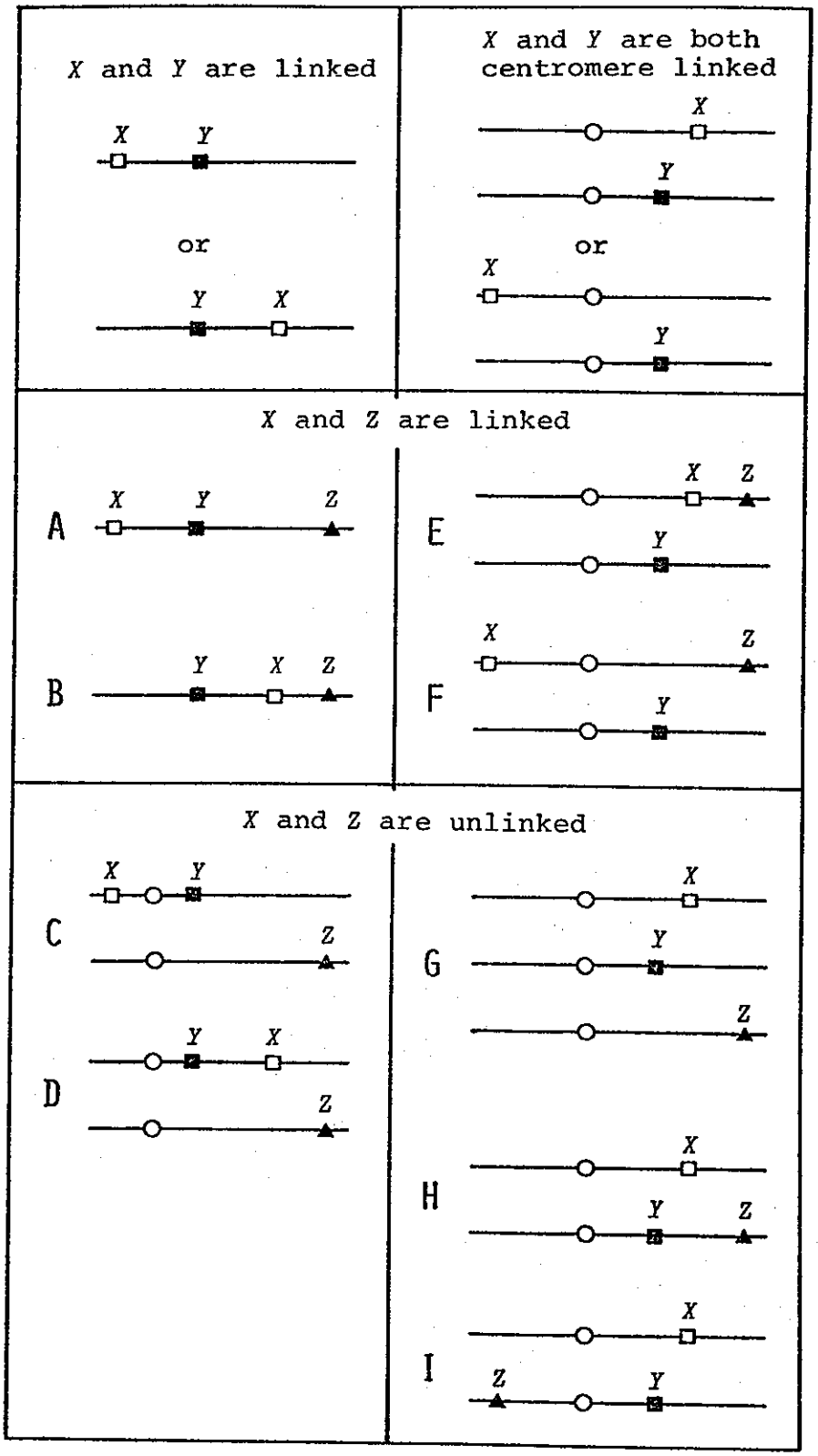

Fig. AI-2. Several possible arrangements of a gene, X, with the standard genes Y and Z.

Fig. AI-2. Several possible arrangements of a gene, X, with the standard genes Y and Z. The gene, X (□), to be mapped has the same phenotype as the standard gene, Y (■), while the other standard, Z (▲), has an independent phenotype. Those gene symbols, X, Y, and Z, were assigned independently from those in Table AI-1 and Fig. AI-1.

position under arrangement A, the values of tetrad distribution for the X - Y gene pair (a_1, a_2, and a_3) can be determined directly by scoring three ascus-types with respect to the X (and Y) phenotype, and those for Y - Z (b_1, b_2, and b_3) are obtained by substituting a given distance, D_{YZ}, into equations 1.13 to 1.15. When the X and Y are complementary for a certain phenotype and the parental combination has the XYZ/xyz configuration, twelve theoretical ascus-types (X1 to X12) can be compiled into six ascus-types (Y1 to Y6) as follows:

Ascus type	Phenotype of spore in asci			
	A	B	C	D
Y1 = X1	+Z	+Z	-z	-z
Y2 = X2	+Z	-Z	+z	-z
Y3 = X3	-Z	-Z	+z	+z
Y4 = X4 + X5 + X8	+Z	-Z	-z	-z
Y5 = X6 + X7 + X9	-Z	-Z	+z	-z
Y6 = X10 + X11 + X12	-Z	-Z	-z	-z

where + and - indicate the wild type and mutational phenotypes of the X and Y genes. Z and z indicate the phenotype of the Z and z alleles, respectively. In the calculation under arrangement B, tetrad distribution for X - Z will be given by substituting the map distance of D_{XZ} (= D_{YZ} - D_{XY}) into equations 1.13, 1.14, and 1.15. Then the expected frequencies of ascus types under arrangements A and B are compared with the observed frequencies by the calculation of χ^2 statistics. It is also possible to compare the theoretical values with the observed ones

with respect to the frequencies (z_1 to z_4) of four categories of spore types, Z1 (+Z), Z2 (-Z), Z3 (+z), and Z4 (-z) in random spore culture using the data for analysis with respect to the ascus types. Typical examples of three factor analysis of this situation were given in Chapter II.

Mapping of X with the aid of Z located on another chromosome is also possible, if Z is sufficiently linked to the centromere (Fig. AI-2 arrangements C and D). If X is located on the left side of Y (arrangement C), it should show a different segregation pattern to that from arrangement D in which X is located on the right side of Y, even though the X - Y distance is the same in both the arrangements.

Example: An X gene (the leu2 marker was adopted) has a direct linkage with the thr4 locus (corresponding to the Y gene in the above description) on chromosome III in S. cerevisiae as they showed a PD : NPD : T ratio of 144 : 42 : 292. This data is classified as the L distribution and the distance between X to thr4 (Y) is calculated as 83 stranes (57 cM) by the D_2 formula. If the X gene were located on the left side of thr4, it should be mapped on left arm of the chromosome III at 33 stranes from the centromere, because the centromere distance of thr4 is 50 stranes (44 cM) on the right arm of the chromosome (Takano and Oshima 1970; and the unpublished data) (Fig. AI-2; arrangement C). This value is sufficient to detect its centromere linkage with an appropriate centromere marker. On the other hand, if X were on the right side of thr4 (Fig. AI-2; arrangement D), its centromere distance (50 + 83 = 133 stranes) will be too great to detect linkage. This was tested by the χ^2 minimization procedure with the adel marker on chromosome

I as the standard (corresponding to the Z gene). The centromere distance of adel was deduced to be 5 stranes (5 cM) from the data of Mortimer and Hawthorne (1966). Expected frequencies of six ascus-types were calculated assuming that X is located at various map distances from the thr4 locus, on the left side by equation 4.1 to 4.28 and on right side by equations 1.1 to 1.12 and 3.1 to 3.9. In Table AI-3, some typical calculations of expected frequencies of six ascus-types and four spore-types and their fit with the observed data derived from 378 asci are listed. The results clearly indicate that the X locus is located on the left arm of the chromosome as it showed much lower values of χ^2. The optimum fit with the observed frequencies in comparison of ascus types was obtained with X at a position of 71 stranes to the left of thr4 (Fig. AI-3). Thus, X should be located on left arm of chromosome III at approximately 21 (= 71 - 50) stranes to the centromere. Although the map distance between X and Y was calculated as 83 stranes, this value showed lower fit ($2\% > P > 1\%$) in the χ^2 minimization procedure. The generally accepted centromere distance of leu2 is 7 stranes (Takano and Oshima 1970; and the unpublished data), while 57 (= 50 + 7) stranes to the left of thr4 gave poor fit ($\chi^2 = 16.89$, $P < 1$). This discrepancy might be due to the fluctuation of recombination frequencies in the present tetrad sample.

If the comparison were made with the frequencies of spore-type, the results will be less meaningful due to the F distributions for X - Z and Y - Z gene-pairs; the frequency of NPD ascus is the same as that of PD ascus and the T ascus also contains equal frequencies of parental and recombinant spores for both the gene-pairs. Hence both arrangements

Observed and theoretical frequencies of the ascus types or spore types from the triply heterozygous diploid for X (leu2), thr4 (Y), and ade1 (Z) assuming that X has the same phenotype as Y, and Z is phenotypically independent of X and Y.

		Phenotype of spore[a] A	B	C	D	Obsd.[b]	Centromere distance of X: 7 stranes on left arm	21 stranes on left arm	21 stranes on right arm	33 stranes on left arm	33 stranes on right arm
	Y1	+Z	+Z	-z	-z	63	80.5	62.6	36.1	51.6	31.9
	Y2	+Z	-Z	+z	-z	21	23.9	31.2	84.2	35.2	74.5
Ascus	Y3	-Z	-Z	+z	+z	60	80.5	62.6	36.1	51.6	31.9
type	Y4	+Z	-Z	-z	-z	139	130.6	140.4	140.4	146.2	146.2
	Y5	-Z	-Z	+z	-z	153	130.6	140.4	140.4	146.2	146.2
	Y6	-Z	-Z	-z	-z	42	32.0	40.8	40.8	47.4	47.4
Total						478	478.1	478.0	478.0	478.2	478.2
χ^2							16.89	4.59	84.72	10.94	94.87
Probability (%), d.f. = 5							P<1	50>P>30	P<1	2>P>1	P<1
	Z1	+Z				294	315.4	296.8	296.8	284.4	284.4
Spore	Z2	+z				286	315.4	296.8	296.8	284.4	284.4
type	Z3	-Z				662	640.6	659.2	659.2	671.6	671.6
	Z4	-z				670	640.6	659.2	659.2	671.6	671.6
Total						1912	1912.0	1912.0	1912.0	1912.0	1912.0
χ^2							6.27	4.59	4.59	0.47	0.47
Probability (%), d.f. = 3							10>P>5	20>P>10	20>P>10	95>P>90	95>P>90

Legend for Table AI-3

[a] + and - indicate wild-type and mutant phenotypes for the complementary situation for the X and Y genes. The other standard marker, Z/z (ADE1/ade1), could be scored for 2+ : 2- segregation independently of the X and Y genes.

[b] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

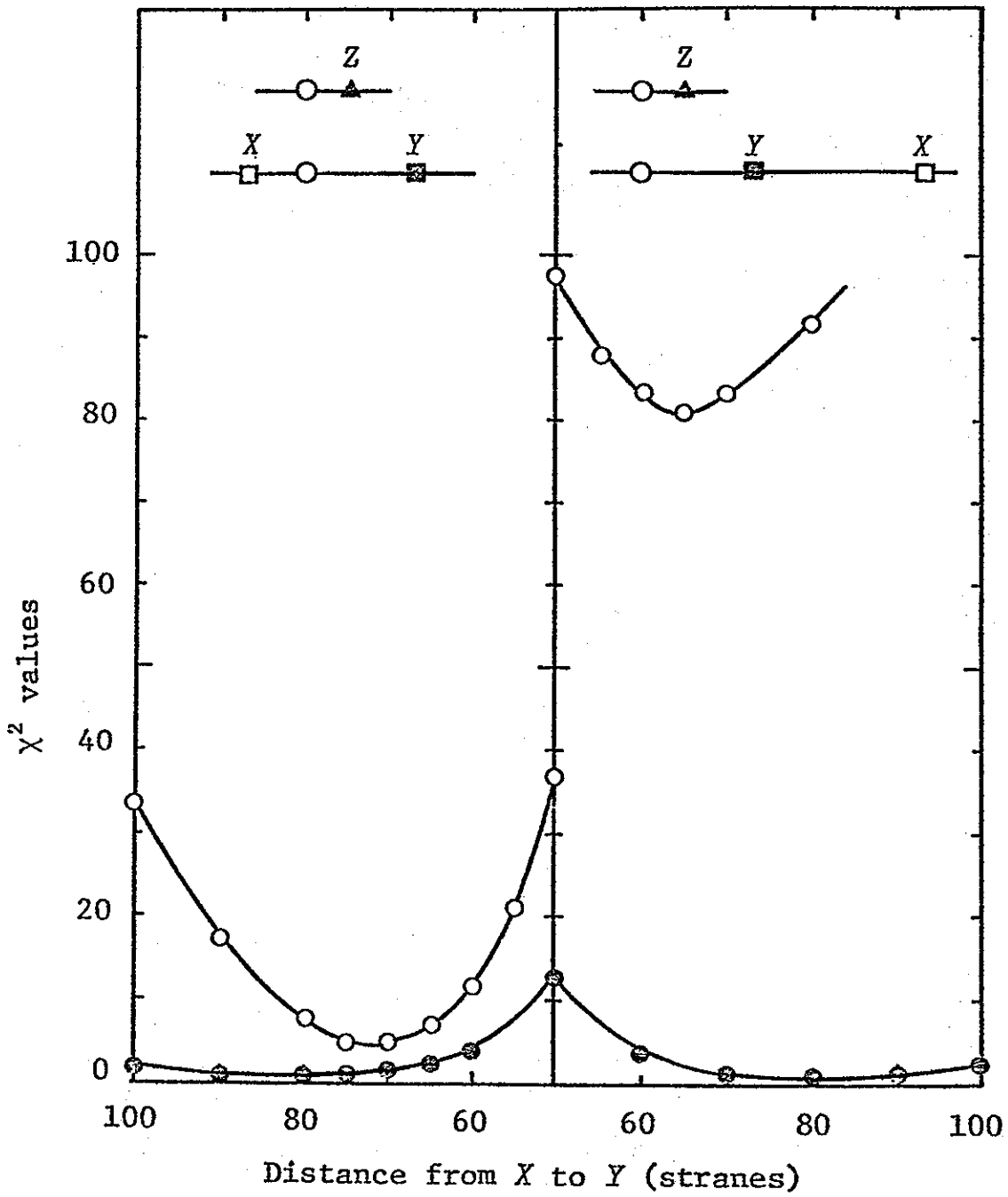

Fig. AI-3. Mapping of a gene, X (leu2), with thr4 (Y) of the same phenotype as X, and adel (Z) of independent phenotypes, by the χ^2 minimization procedure.

Fig. AI-3. Mapping of a gene, X (leu2), with thr4 (Y) of the same phenotype as X, and ade1 (Z) of independent phenotypes, by the χ^2 minimization procedure. Open and closed symbols indicate the values calculated from the data with respect to ascus types and spore types, respectively.

always showed equal frequencies for Z1 and Z2 and for Z3 and Z4, while the frequencies of occurrence of the XY and xy spores depend strictly on the recombination frequency between X and Y genes. This gives the same X^2 values in comparison of spore types in both arrangements (Fig. AI-3).

b) X and Y on different chromosomes: When the tetrad distribution of the X and Y genes shows F distribution, the map position of X will be determined with another appropriate genetic marker Z, in arrangements E and F (Fig. AI-2). To determine which chromosome bears X, and whether X is located on the left or right arm of the chromosome, several standard markers (Z) should be selected for which the 2 : 2 segregation in each ascus can be scored independently of the phenotypic segregation of the X and Y genes. In addition, since a Z marker having direct linkage to Y is obviously worthless (Fig. AI-2; arrangements H and I), Z should be selected from the linkage groups other than that to which Y belongs. Then several XYZ/xyz crosses are made using various markers as independent standards. Three different types of gene arrangement on chromosomes (Fig. AI-2; arrangements E, F, and G) will be expected using various Z genes. Of these, the G arrangement, i.e., three genes on three different chromosomes, is meaningless, as are the H and I arrangements. Thus, we should eliminate the crosses showing the G arrangement by the inspection of tetrad data.

It is obviously expected that the frequencies of Y1 and Y3 ascus-types, and also the Y4 and Y5 ascus-types should show same values, respectively, in the G arrangement because the Z gene is segregated independently of the X and Y genes, while the frequencies of each ascus-type will be expected to show different values in arrangements E and F

as Z is linked to X. Similary in the frequencies of spore types, it will be expected that z_1 is the same as z_2 and that z_3 is the same as z_4 in the G arrangement. On the other hand, in the E and F arrangements showed these frequencies different values. This was clearly indicated, for example, with the three genes, leu2 (X; on chromosome III), ade1 (Y; on chromosome I), and ura3 (Z; on chromosome V) as shown in Table AI-4. Here the observed frequency of Y1 was the same as Y3 and that of Y4 was almost equal to Y5. This was the same for the frequencies of Z1 and Z2, and also Z3 and Z4. On the other hand, when Z was linked with X, all the categories of ascus type and spore type showed different values as shown in Table AI-5. Thus, it will be possible to disinguish the G arrangement of genes from the E and F arrangements by the inspection of the segregational patterns of ascus types and spore types.

Tetrad data showing different frequencies of occurrence for six ascus-types will strongly suggest direct linkage between X and Z. Since the map position of Z is already known, it will be possible to determine the position of the X locus, whether its position relative to Z is as arrangement E or F, by calculation of expected frequencies of each ascus-type or spore-type based on these alternative assumptions using given values of D_{OY} and D_{OZ} in equations 1.1 to 1.12, 3.1 to 3.9, and 4.1 to 4.12. Then the expected frequencies are compared with the observed data. More critical estimation of map position will be given by the χ^2 minimization procedure.

Example: Taking ade1 as Y and thr4 as Z, the position of an X

Table AI-4

Observed and theoretical frequencies of the ascus types or spore types from the triply heterozygous diploid for X (leu2), ade1 (Y), and ura3 (Z) on different chromosomes assuming that X has the same phenotype as Y, and Z is phenotypically independent of X and Y.

		Phenotype of spore[a]				Observed[b]	Centromere distance of Z (ura3)[c]	
		A	B	C	D		10 stranes	6 stranes
Ascus type	Y1	+Z	+Z	-z	-z	81	85.2	95.4
	Y2	+Z	-Z	+z	-z	45	35.9	25.1
	Y3	-Z	-Z	+z	+z	81	85.2	95.4
	Y4	+Z	-Z	-z	-z	58	64.4	54.6
	Y5	-Z	-Z	+z	-z	65	64.4	54.6
	Y6	-Z	-Z	-z	-z	211	206.3	215.9
Total						541	541.4	541.0
χ^2							2.83	22.55
Probability (%), d.f. = 5							80>P>70	P<1
Spore type	Z1	+Z				272	270.7	270.5
	Z2	-Z				265	270.7	270.5
	Z3	+z				810	812.1	811.5
	Z4	-z				817	812.1	811.5
Total						2164	2165.6	2164.0
χ^2							0.19	0.16
Probability (%), d.f. = 3							98>P>95	99>P>98

Legend for Table AI-4

[a] + and - indicate wild-type and mutant phenotypes for the complementary gene system with the X and Y genes. The other standard marker, Z/z (URA3/ura3), could be scored for 2+ : 2- segregation independently of the X and Y genes.

[b] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

[c] The centromere distances for X and Y were adopted as 7 (7 cM) and 5 (5 cM) stranes, respectively. That of ura3 was reported as 6 stranes (6 cM) (Mortimer and Hawthorne 1966), but this value did not fit with the observed one ($P < 1$), while the optimal fit was obtained with 10 stranes (9 cM).

Table AI-5

Observed and expected frequencies of the ascus types or spore types from the triply heterozygous diploid for X (leu2), ade1 (Y), and thr4 (Z) assuming that X has the same phenotype as Y, and Z is phenotypically independent of X and Y.

		Phenotype of spore[a]				Observed[b]	X on[c]	
		A	B	C	D		left arm	right arm
Ascus type	Y1	+Z	+Z	-z	-z	63	82.0	89.9
	Y2	+Z	-Z	+z	-z	113	100.9	94.9
	Y3	-Z	-Z	+z	+z	11	11.3	9.4
	Y4	+Z	-Z	-z	-z	47	52.1	63.4
	Y5	-Z	-Z	+z	-z	45	37.6	26.2
	Y6	-Z	-Z	-z	-z	199	194.2	194.2
Total						478	478.1	478.0
χ^2							7.95	29.58
Probability (%), d.f. = 5							20>P>10	P<1
Spore type	Z1	+Z				180	161.0	139.9
	Z2	-Z				286	317.0	338.1
	Z3	+z				776	795.0	816.1
	Z4	-z				670	639.0	617.9
Total						1912	1912.0	1912.0
χ^2							7.22	25.92
Probability (%), d.f. = 3							10>P>5	P<1

Legend for Table AI-5

[a] + and - indicate wild-type and mutant phenotypes for the complementary situation for the X and Y genes. The other standard marker, Z/z (THR4/thr4), could be scored for 2+ : 2- segregation independently of the X and Y genes.

[b] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

[c] Expected frequencies of ascus types and spore types were calculated assuming that X is located on left or right arm of the chromosome with the centromere distance of 6 stranes (6 cM).

gene (leu2) can be determined with the same sample as in the previous example. The tetrad distribution for the X - Y (leu2 - ade1) gene pair showed a PD : NPD : T ratio of 189 : 199 : 92 for the sample consisting of 478 asci. Since this data shows F distribution, the false linkage between X and Y is 11 stranes (10 cM) and the centromere distance of ade1 (Y) is given as 5 stranes (5 cM), X should be located at 6 stranes (= 11 - 5) from the centromere. Whether the X gene is located on the left or right arm of the chromosome was determined by calculation of the expected frequencies of occurrence for six ascus-types and four spore-types assuming that the X - Z distance is 56 (= 50 + 6) (arrangement F) or 44 (= 50 - 6) (arrangement E) stranes, taking the centromere distance of Z (thr4) as 50 stranes. Results listed in Table AI-5 clearly indicate that the X gene should be located on left arm of chromosome, the assumption that X is on the right arm showing much lower probability ($P < 1$). More critically, minimum χ^2 value was obtained when X was placed at 7 stranes from the centromere on the left arm with respect to the frequencies of six ascus-types (Fig. AI-4). This value agrees well with the value calculated from false map distance between ade1 and leu2 and is equal to the generally accepted centromere distance of leu2. Significance of the spore-type frequencies for the determination of the X gene might be much less than that of the ascus-types as the χ^2 values showed lower values of for wide range of X positions.

c) X and Y showing N distribution: In general, when X and Y segregate independently and show N distribution (the PD : NPD : T ratio is 1 : 1 : 4), data will not offer information for gene mapping. However, when the segregation pattern of a triply heterozygous hybrid shows

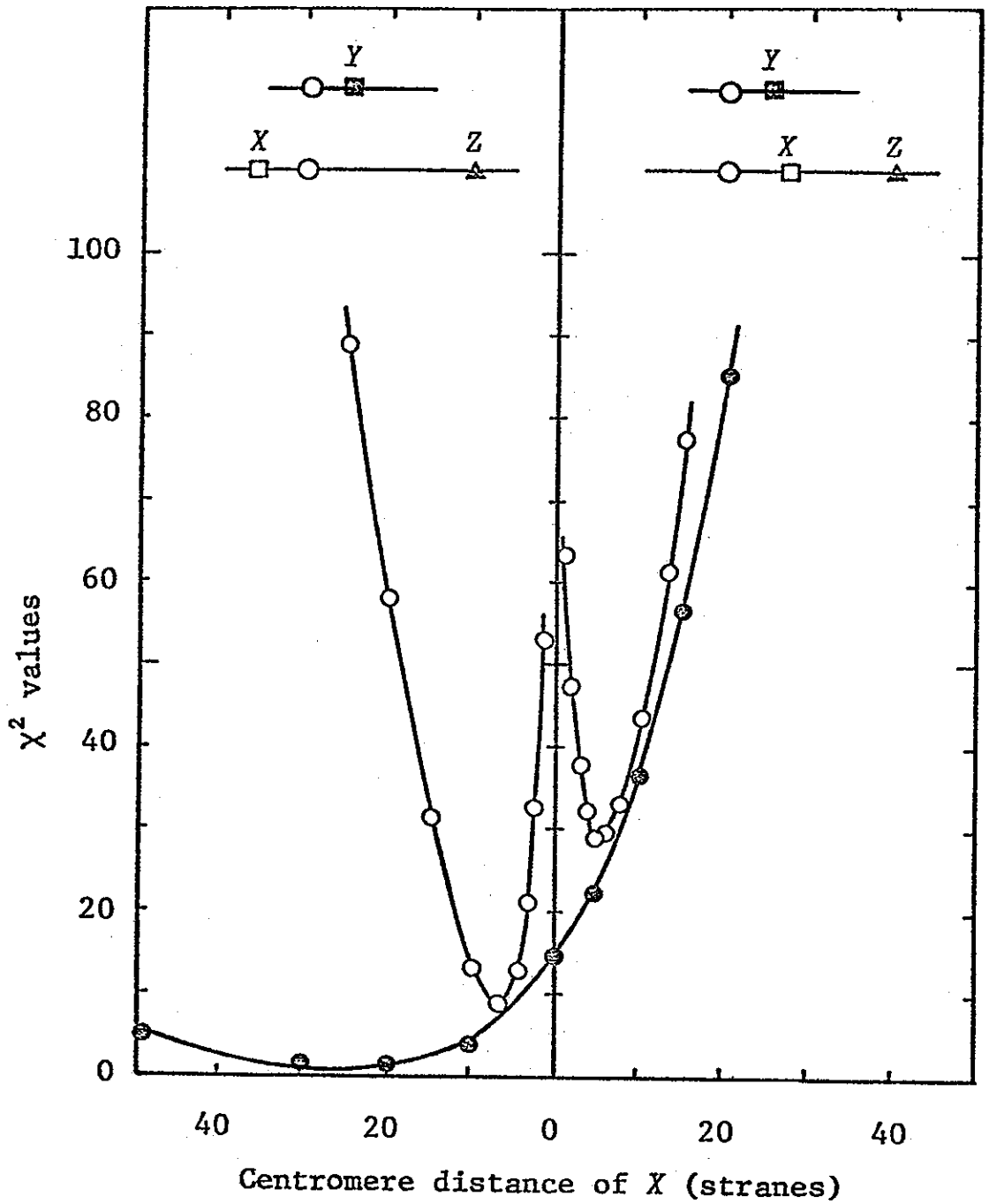

Fig. AI-4. Mapping of a gene, X (leu2), with the standard gene ade1 (Y) of the same phenotype as X, and thr4 (Z) of independent phenotype, by the χ^2 minimization procedure.

Fig. AI-4. Mapping of a gene, X (leu2), with the standard gene ade1 (Y) of the same phenotype as X, and thr4 (Z) of independent phenotype, by the χ^2 minimization procedure. Open and closed symbols indicate the values calculated from the data with respect to ascus types and spore types, respectively.

significant deviation from the ratio of ascus-type frequencies for three independently segregating genes, linkage between X and Z or linkage of X and Z to their respective centromeres on different chromosmes will be expected. For example, if X and Y were complementary for a certain phenotype, three unlinked genes, X, Y, and Z, will give a $y_1 : y_2 : y_3 : y_4 : y_5 : y_6$ ratio of 1 : 4 : 1 : 12 : 12 : 6 or a $z_1 : z_2 : z_3 : z_4$ ratio of 1 : 1 : 3 : 3. In this case, further study is required to determine whether the X gene is located on the left or right side of the Z gene or centromere.

Example: Taking leu2 as the X gene and lys2 as the Y gene in S. cerevisiae, the tetrad distribution of X - Y (leu2 - lys2) showed a PD : NPD : T ratio of 122 : 126 : 536. When the same cross was scored for his4 as the Z marker in addition to the X - Y phenotype, the observations listed in Table AI-6 clearly indicated that the observed frequencies of ascus types and of spore types deviated significantly from those expected for three independent genes. The observed segregations were most adequately simulated by supposing that X is linked to Z with the map distance of 18 stranes (17 cM) (Fig. AI-5), while the location of X to the left or right side of his4 is obscure.

DISCUSSION

Comparison of the results based on the frequencies of ascus types and on those of spore types showed that those based on spore types were generally less dependable, especially in triplet genes with the standard

Table AI-6

Observed and theoretical frequencies of the ascus types or spore types from the triply heterozygous diploid for X (leu2), lys2 (Y), and his4 (Z) assuming that X has the same phenotype as Y, and Z is phenotypically independent to that of X and Y.

		Phenotype of spore[a] A	B	C	D	Observed[b]	Expected[c] D_{XZ} is 18 stranes	Expected[c] 3 unlinked genes
	Y1	+Z	+Z	-z	-z	92	92.7	21.8
	Y2	+Z	-Z	+z	-z	27	36.4	87.1
Ascus	Y3	-Z	-Z	+z	+z	3	1.6	21.8
type	Y4	+Z	-Z	-z	-z	451	443.7	261.4
	Y5	-Z	-Z	+z	-z	85	79.0	261.4
	Y6	-Z	-Z	-z	-z	126	130.7	130.7
	Total					784	784.1	784.2
	χ^2						3.16	540.5
	Probability (%)						80>P>70 (d.f. = 4)[d]	P<1 (d.f. = 5)
	Z1	+Z				118	118.5	392.0
Spore	Z2	-Z				662	665.5	392.0
type	Z3	+z				1450	1449.5	1176.0
	Z4	-z				906	902.5	1176.0
	Total					3136	3136.0	3136.0
	χ^2						0.03	374.8
	Probability (%), d.f. = 3						P>99	P<1

Legend for Table AI-6

[a] + and - indicate wild type and mutant phenotypes for the complementary situation for the X and Y genes. The other standard marker, Z/z (HIS4/his4), could be scored for 2+ : 2- segregation independently of the X and Y genes.

[b] Tetrad data from several different crosses were normalized with respect to the genotypes and compiled into each category of segregation.

[c] Expected frequencies of ascus types and spore types were calculated assuming that the X - Y distance is 18 stranes (17 cM) to give the optimal fit or that X and Y are unlinked to each other and to the respective centromeres.

[d] Each term of the ascus type in which expected occurrence of asci is less than five was not included in the calculation of χ^2 statistics.

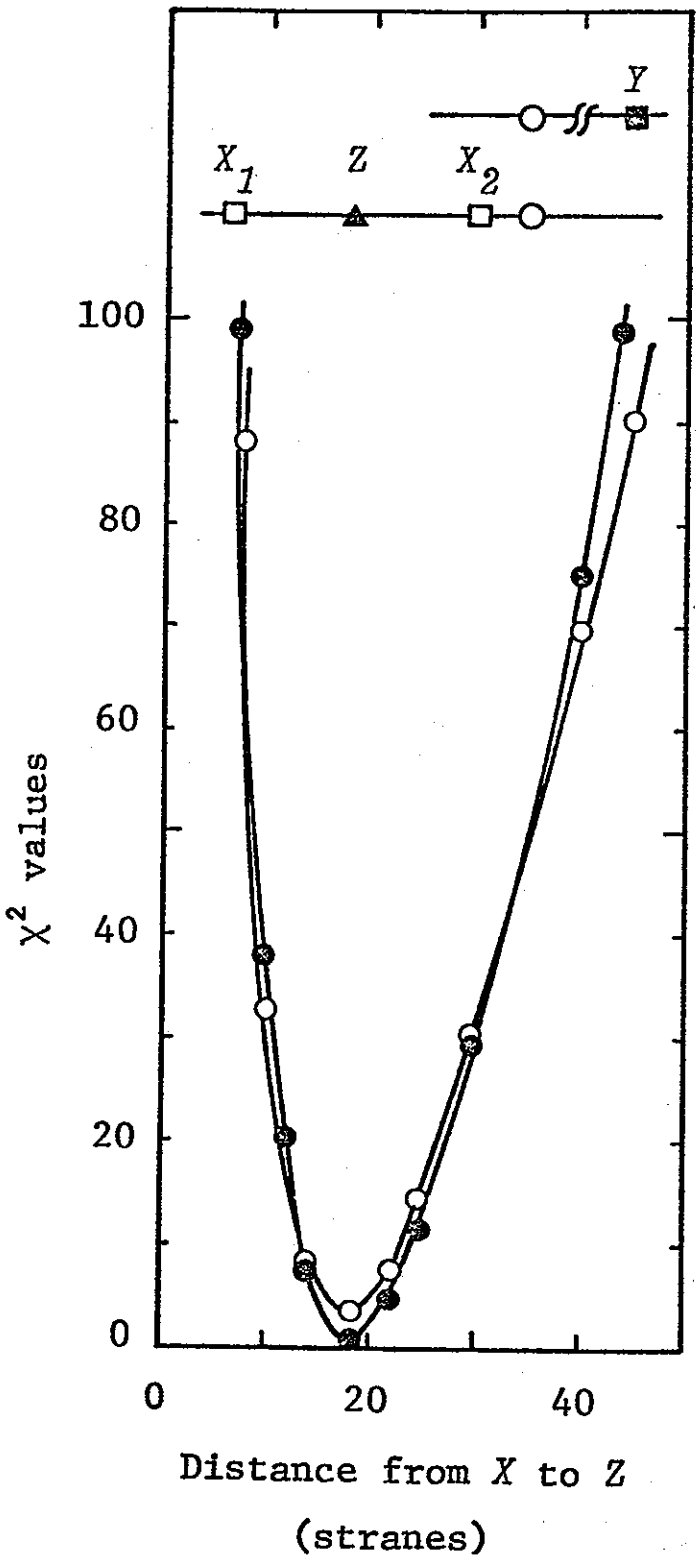

Fig. AI-5. Mapping of a gene, X (leu2), with the unlinked standard gene, lys2 (Y), of the same phenotype, as X, and the other standard gene, his4 (Z), of independent phenotype, by the χ^2 minimization procedure.

Fig. AI-5. Mapping of a gene, X (leu2), with the unlinked standard gene, lys2 (Y), of the same phenotype as X, and the other standard gene, his4 (Z), of independent phenotype, by the χ^2 minimization procedure. Open and closed symbols indicate the values calculated from the data with respect to ascus types and spore types, respectively. The X gene is placed at either side of his4 (X_1 or X_2).

markers, Y and/or Z, on different chromosomes to X. This is because centromere linkage could be detected in tetrad analysis showing F distribution, while no linkage could be detected with random spore analysis of the same cross. On the other hand, if the Y and Z genes are both on the same chromosome as X, or X and Y are unlinked while X and Z are directly linked, data based on scoring of spore-type frequencies is also effective (see Chapter II; and Fig. AI-5).

In mapping using either type of frequencies, the χ^2 minimization procedure requires a great amount of numerical calculation. However, this is easily performed by the aid of a digital computer with a well organized program. For example, data presented in Fig. AI-3 were obtained within a few seconds operation of a computer (NEAC series 2200, model 700-2).

The mapping procedure by three factor analysis does not require the laborious allelism test of the segregants to determine the genotype, which is indispensable for mapping by the conventional procedure. Thus, method will be helpful, for example, in the mapping of a gene of macromolecular synthesis such as the cdc mutants (Hartwell et al. 1973) or a combination of suppressor and suppressible mutation using one of them as the standard.

ABSTRACT

Equations expressing the theoretical frequencies of twelve ascus-types in the tetrad analysis of a triply heterozygous diploid were described. Using these equations, a mapping procedure for a gene, X, is proposed. The procedure requires that two genes, X and Y, of the same phenotype are marked heterozygously in a diploid and the map position of Y is known, and that another standard gene, Z, shows independent phenotype from X and Y. This procedure does not require the laborious allelism test of the segregants to determine the allelic 2 : 2 segregation in tetrads for the X and Y genes, which is indispensable for mapping by the conventional procedure. The exact placement of the X gene on a chromosome is possible by the χ^2 minimization procedure in comparison with the expected frequencies of six ascus-types or four spore-types deduced from the twelve expected ascus-types to give the optimal fit with the observed data.

APPENDIX II

REFERENCE TABLE FOR CHAPTER I

Table AII-1. The expected ratios of ascus-type of the segregants derived from the diploids prepared by crosses between the Hp strain of S. norbensis SBY 2535 to various strains having different genotype for the homothallic genes.

Segregation in asci	α Hp × a ho_α hm[a]			α Hp × a ho_α HM			α Hp × a HO_α hm		s Hp × s HO_α HM		
	I		II	I		II	I	II	I		II
	α X y_1 y_2 Z_1 Z_2	α X y_1 y_2 Z_1 Z_2	α X y Z	α X y_1 y_2 Z_1 Z_2	α X y_1 y_2 Z_1 Z_2	α X y Z	α X y_1 y_2 Z_1 Z_2	α X y Z	s X y_1 y_2 Z_1 Z_2	s X y_1 y_2 Z_1 Z_2	s X yZ
	α x Y_1 Y_2 z_1 z_2	α x y_1 Y_2 z_1 z_2[b]	α x Y z	α x Y_1 Y_2 z_1 z_2	α x y_1 Y_2 z_1 Z_2[c]	α x Y Z	α X Y_1 Y_2 z_1 z_2[d]	α X Y z	s X Y_1 Y_2 z_1 Z_2	s X y_1 Y_2 z_1 Z_2	s X YZ
Ho Ho Ho Ho							36	6	6	0	0
Ho Ho Ho Hp							72	0	12	0	0
Ho Ho Ho Hq							72	0	0	0	0
Ho Ho Hp Hq							144	8	0	0	0
Ho Ho Hp Hp							6	0	1	1	1
Ho Ho Hq Hq							6	0	0	0	0
Ho Hp Hp Hq							12	0	0	0	0
Ho Hp Hq Hq							12	0	0	0	0
Hp Hp Hq Hq							1	1	0	0	0
Ho Ho Ho *a*							144	0	0	0	0
Ho Ho Hp *a*							84	4	0	0	0
Ho Ho Hq *a*							48	0	0	0	0
Ho Hp Hq *a*							28	0	0	0	0
Ho Ho Hq α							144	0	8	0	0
Ho Ho Hp α							48	0	8	4	4
Ho Ho Hq α							84	4	0	0	0
Ho Hp Hq α							28	0	0	0	0
Ho Ho *a* α	1444	208	40	76	4	4	196	8	0	0	0
Ho Hp *a* α	608	184	16	32	8	8	28	0	0	0	0
Ho Hq *a* α	608	40	16	0	0	0	28	0	0	0	0
Hp Hq *a* α	256	64	16	0	0	0	4	4	0	0	0
Ho Ho *a* *a*	361	52	10	19	1	1	19	0	0	0	0
Ho Hq *a* *a*	304	20	8	0	0	0	0	0	0	0	0
Hq Hq *a* *a*	19	1	1	0	0	0	0	0	0	0	0
Ho Ho α α	361	52	10	19	1	1	19	0	1	1	1
Ho Hp α α	304	92	8	16	4	4	0	0	0	0	0
Hp Hp α α	19	19	1	1	1	1	0	0	0	0	0
Ho *a* *a* α	1296	132	24	16	4	4	16	0	0	0	0
Hq *a* *a* α	252	36	12	0	0	0	0	0	0	0	0
Ho *a* α α	1296	204	24	32	8	8	16	0	0	0	0
Hp *a* α α	252	108	12	4	4	4	0	0	0	0	0
a *a* α α	396	84	18	1	1	1	1	1	0	0	0
	7776	1296	216	216	36	36	1296	36	36	6	6

Legend for Table AII-1

[a] Symboles for the standard strains were followed previous system (Takano and Oshima 1970a)

[b] According to hypothesis I, duplicated genes for HMα were suggested. Before interbreeding, the active HMα gene(s) must homogeneously be distributed in our materials and lacked in the S. norbensis SEY 2535. However, it is possible to speculate that the distribution of both the HMα1 and HMα2 genes in our materials is not necessary to explain the data. In other words, the HMα2 should be homogeneously distributed in our yeast, because the D strain has the HO hmα1 HMα2 hma1 HMa2 genotype as indicated in Table I-3, while HMα1 may be marked in some strains and some have not. Two types of segregation patterns were calculated according to the genotype of HMα in the standard strain.

[c] Two HMa genes were suggested in S. norbensis SBY 2535 from hypothesis I. However, all the segregation data described in previous publications indicated that single pair of alleles, i.e., HMa2/hma2, was recognized in their yeast strain (Takano and Oshima, 1967; 1970a; 1970b; and Oshima and Takano 1971; 1972).

[d] In this combination, a particular strain, T-1023-9C, should have the a HO HMα1 HMα2 hma1 hma2 genotype from hypothesis I due to the segregation data described previously (Oshima and Takano 1972).

Symboles X/x, Y1/y1, Y2/y2, Z1/z1, and Z2/z2 should be read HO/ho HMα1/hmα1, HMα2/hmα2, HMa1/hma1, and HMa2/hma2, respectively.

APPENDIX III

REFERENCE TABLES FOR CHAPTER III

Table AIII-1. Occurrence of colonies showing mating potency from a/α diploids after irradiation of low dose of ultraviolet-light.

Strain	Thallism	Number of colonies examined	Number of colonies showing mating potency	
			a	α
T-1068	Heterothallism	425	16	5
T-1071-8	Heterothallism	1,000	3	4
T-1269-38C	Homothallism (Ho)	1,000	0	0
S-14-9C	Homothallism (Hp)	1,550	0	25
T-1023-23B	Homothallism (Hq)	780	15	0
T-891-13B	Homothallism (Hq)	1,100	27	0

Table AIII-2. Further analysis of three asci showing typical types of segregation from the supposed homothallic (Ho) tetraploid strain, T-1269-38C-U1.

Ascus		Mating type	Spore formation	Cell volume (μm^3)	DNA contents ($mg/10^{11}$ cells)	Tetrad analysis		Expected	
						No. of asci tested	4 non : 0 mater	Ploidy	Genotype of mating-type alleles
1	A	non[a]	+	93.4	4.55	20	20	Diploid	*a*/α
	B	non	+	107.9	4.92	12	12	Diploid	*a*/α
	C	non	+	103.7	5.48	18	18	Diploid	*a*/α
	D	non	+	97.6	5.08	20	20	Diploid	*a*/α
3	A	non	+	219.8	8.16	16	16	Tetraploid	*a*/*a*/α/α
	B	non	+	201.5	7.43	12	12	Tetraploid	*a*/*a*/α/α
	C	non	+	107.6	5.09	15	15	Diploid	*a*/α
	D	non	+	101.5	5.41	15	15	Diploid	*a*/α
14	A	non	+	209.2	7.82	17	17	Tetraploid	*a*/*a*/α/α
	B	non	+	190.1	7.20	14	14	Tetraploid	*a*/*a*/α/α
	C	non	+	192.3	7.99	17	17	Tetraploid	*a*/*a*/α/α
	D	non	+	196.2	8.23	15	15	Tetraploid	*a*/*a*/α/α

[a] Non-mater.

Table AIII-3. Further analysis of three asci showing typical types of segregation from the supposed homothallic (Hp) tetraploid strain, S-14-9C-U37.

Ascus		Mating type	Spore formation	Cell volume (μm^3)	DNA contents ($mg/10^{11}$ cells)	Tetrad analysis				Expected	
						No. of asci tested	4 non : 0 mater	3 non : 1 α	2 non : 2 α	Ploidy	Genotype of mating-type alleles
1	A	non[a]	+	87.5	4.30	12	0	0	12	Diploid	*a*/α
	B	non	+	92.7	4.67	12	0	0	12	Diploid	*a*/α
	C	non	+	99.2	4.36	12	0	0	12	Diploid	*a*/α
	D	non	+	88.7	4.78	19	0	0	19	Diploid	*a*/α
7	A	non	+	87.2	4.29	20	0	0	20	Diploid	*a*/α
	B	non	+	161.5	8.33	15	4	6	5	Tetraploid	*a*/*a*/α/α
	C	non	+	86.1	4.27	17	0	0	17	Diploid	*a*/α
	D	α	-	93.7	4.91	-	-	-	-	Diploid	*a*/α
6	A	α	-	94.7	5.10	-	-	-	-	Diploid	*a*/α
	B	non	+	182.2	8.38	16	7	3	6	Tetraploid	*a*/*a*/α/α
	C	α	-	91.3	4.95	-	-	-	-	Diploid	*a*/α
	D	non	+	168.6	8.17	14	5	7	2	Tetraploid	*a*/*a*/α/α

[a] Non-mater.

Table AIII-4. Further analysis of three asci showing typical types of segregation from the supposed homothallic (Hq) tetraploid strain, T-1023-23B-U16.

Ascus		Mating type	Spore formation	Cell volume (μm^3)	DNA contents ($mg/10^{11}$ cells)	Tetrad analysis				Expected	
						No. of asci tested	4 non : 0 mater	3 non : 1*a*	2 non : 2*a*	Ploidy	Genotype of mating-type alleles
5	A	non[a]	+	100.7	6.06	19	0	0	19	Diploid	*a*/α
	B	non	+	118.2	5.37	18	0	0	18	Diploid	*a*/α
	C	non	+	122.3	6.94	20	0	0	20	Diploid	*a*/α
	D	non	+	110.1	6.19	19	0	0	19	Diploid	*a*/α
2	A	*a*	-	110.9	6.21	-	-	-	-	Diploid	*a*/*a*
	B	non	+	230.8	11.42	11	6	5	0	Tetraploid	*a*/*a*/α/α
	C	non	+	103.6	6.42	18	0	0	18	Diploid	*a*/α
	D	non	+	120.8	6.02	19	0	0	19	Diploid	*a*/α
8	A	non	+	188.6	10.62	10	7	1	2	Tetraploid	*a*/*a*/α/α
	B	non	+	236.6	10.11	14	5	6	2	Tetraploid	*a*/*a*/α/α
	C	*a*	-	130.7	5.56	-	-	-	-	Diploid	*a*/*a*
	D	*a*	-	130.5	5.97	-	-	-	-	Diploid	*a*/*a*

[a] Non-mater.

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