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Mating types and mating-inducing factors (gamones) in the ciliate *Euplotes patella* syngen 2

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SUMMARY

Relationships between mating type genes and mating-inducing factors (gamones) were investigated in the ciliate *Euplotes patella* syngen 2. Ten mating types were distinguished, and genetic data indicated that the ten mating types were determined by four codominant alleles in possible combinations of two of them. There were six heterozygous types (*mt1/mt2*, *mt3/mt4*, etc.) and four homozygous types (*mt1/mt1*, *mt2/mt2*, etc.). Conjugation-conditioned fluid (CCF) obtained from a mixture of cells of homozygous types could induce homotypic pair formation in cells of all mating types except for a particular type. Genetic data of cell-CCF combination experiments suggest that each mating type allele controls the production of a specific gamone which induces pair formation in cells which do not produce the same gamone. Gamones and their hypothetical receptors are discussed.

1. INTRODUCTION

Sexual interaction in eukaryotic microbes provides a useful model for analyzing cell-to-cell recognition. Generally, sexual interaction occurs between cells of complementary mating types and is mediated by the mating-type-specific substances (Crandall, 1977). In *Paramecium*, such mating type substances are known as agglutinins bound on cilia and mediate instantaneous agglutination/recognition reaction (Hiwatashi, 1981). Other mating type substances are known as gamones (sex pheromones) which are excreted into the medium and induce intraclonal conjugation in cells of the different mating types (Miyake, 1981).

Gamone excretion has been reported in ciliates such as *Euplotes patella* (American strains, Kimball, 1942), *Blepharisma japonicum* (Miyake, 1968), *Dileptus anser* (Tavrovskaja, 1979), *Euplotes octocarinatus* (Heckmann & Kuhlmann, 1982), *Euplotes raikovi* (Luporini, Miceli & Ortenzi, 1983; Miceli, Concetti & Luporini, 1983), and our *Euplotes patella* syngen 2 (Akada, 1985). Among these ciliates, only *Blepharisma* has been extensively studied for the gamones and their actions (Kubota *et al.* 1973; Miyake & Beyer, 1973, 1974). However, in *Blepharisma*, a genetic approach to gamones seems difficult because the mechanisms of mating type inheritance are poorly understood at present. In the American *E. patella*,

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although Kimball (1939, 1942) found six mating types which were determined by six possible combinations of three alleles and suggested that each of these alleles controlled the production of a specific gamone, the strains have long been lost. In other gamone-excreting ciliates, these relationships between gamones and mating type genes have not been reported in detail at present. Therefore, in the present study, such relationships are investigated using *E. patella* syngen 2 for the purpose of establishing the fundamental genetic background.

2. MATERIALS AND METHODS

(i) *Cells*

The cells used in this study belonged to *Euplotes patella* syngen 2 (Katashima, 1961). One or more strains were used for each mating type. Cells were grown in PS, physiological solution (NaCl: 1·5 mM, KCl: 0·05 mM, $CaCl_2$: 0·2 mM, $MgCl_2$: 0·1 mM, Tris-HCl pH 7·2: 2 mM) at 24 ± 1 °C, and fed with the colourless flagellate *Chilomonas paramecium*, which was cultured in wheat-grain infusion in PS. Under these conditions, spontaneous intraclonal conjugation (selfing) was found occasionally in some strains. If spontaneous selfing pairs were found in a culture dish of a strain, another culture of the same strain or the same mating type was used.

(ii) *Matings*

Conjugation was induced by mixing cells of different mating types in a depression slide or a plastic dish at the cell density of approximately 10^3/ml. When cells of different mating types were mixed, pair formation occurred not only between cells of different mating types but also between those of the same mating type. To distinguish between cross (heterotypic) pairs and selfing (homotypic) pairs, cells of one mating type were fed with congo-red-stained yeasts and those of the other type with unstained white yeasts (Akada, 1985). The resulting red and white cells were mixed and at least 100 pairs were examined to determine the proportions of hetero- and homotypic pairs. Self-fertilized exconjugants were obtained from selfing pairs induced by cell-free fluid of a different type (Akada, 1985). A few days after conjugation, exconjugants were distinguishable from vegetative cells by the presence of a transparent disk (the macronuclear anlage) in the centre of the body, and they were isolated and grown. The survival rate of exconjugant clones was 33–54 % in the cross-fertlizations between different mating types and 7–26 % in the self-fertilizations. Mating types of exconjugant clones were determined by mixing with tester cells of each mating type, usually within 1 or 2 months after the fertilizations.

(iii) *Conjugation-conditioned fluid*

Conjugation-conditioned fluid (CCF) was obtained from a mixture between cells of two complementary types 1 day after mixing. CCF was prepared by removing cells and pairs by filtration and was heated in a water bath at 50–70 °C for 1 min (gamone activity was not lost by the heat treatment). The results obtained in the cell-CCF mixtures were considered valid only when selfing pairs did not appear in the non-mixed control cells.

3. RESULTS

(i) *Mating types*

By mixing possible combinations of the strains in *E. patella* syngen 2, ten mating types were distinguished (Table 1). Each type, when mixed with any of the others, gave a conjugating reaction which never took place in mixtures of strains belonging to the same mating type. The ten mating types are indicated by Roman numerals I–X.

Table 1. *Results of two-by-two mixing of the ten mating types in* Euplotes patella *syngen 2*

(+, Conjugation; −, no conjugation.)

Mating types	I	II	III	IV	V	VI	VII	VIII	IX	X
I	−	+	+	+	+	+	+	+	+	+
II	·	−	+	+	+	+	+	+	+	+
III	·	·	−	+	+	+	+	+	+	+
IV	·	·	·	−	+	+	+	+	+	+
V	·	·	·	·	−	+	+	+	+	+
VI	·	·	·	·	·	−	+	+	+	+
VII	·	·	·	·	·	·	−	+	+	+
VIII	·	·	·	·	·	·	·	−	+	+
IX	·	·	·	·	·	·	·	·	−	+
X	·	·	·	·	·	·	·	·	·	−

(ii) *Homo- and heterotypic pairs in mating mixtures*

I previously showed that when cells of mating types IV and V were mixed, homo- and heterotypic pairs were formed but heterotypic pairs were predominant (Akada, 1985). For cross breeding, this analysis was extended to mixtures of other mating types. In 37 mating combinations between cells of different mating types, percentages of heterotypic pairs ranged from 61 to 97% ($82 \pm 8\%$, mean $\pm$ S.D.). This result and the low viability of selfing offspring (see Materials and Methods) indicated that when cells of different mating types were mixed, most F1-clones were derived from cross pairs.

(iii) *Mating type analysis*

From the results of various matings, it seemed that mating types were controlled by four codominant alleles at a single locus for mating type (*mt*). The genotypes determining each mating type are shown in Table 4. Mating types I–VI are determined by the different heterozygous combinations of four allelic genes (heterozygous types) which in their homozygous state determine types VII–X (homozygous types).

To justify this assumption, I give detailed results of breeding analyses as follows. Table 2 shows the results of various matings, in which probability values (P) calculated by χ-square tests performed on the above hypothesis are presented.

In crosses between cells of different mating types with heterozygous alleles,

Table 2. *Mating types of F1-clones obtained through various matings*

Mating type	Genotype	Mating types of F1-clones I *1/4*	II *2/3*	III *1/3*	IV *1/2*	V *3/4*	VI *2/4*	VII *1/1*	VIII *2/2*	IX *3/3*	X *4/4*	*P**
		(A) Cross between heterozygous types										
I × III	*mt1/mt4 × mt1/mt3*	9	·	10	·	10	·	6	·	·	·	0·7–0·8
II × III	*mt2/mt3 × mt1/mt3*	·	15	16	16	·	·	·	·	12	·	0·8–0·9
II × VI	*mt2/mt3 × mt2/mt4*	·	10	·	·	8	9	·	7	·	·	0·9–0·95
III × VI	*mt1/mt3 × mt2/mt4*	14	11	·	6	10	·	·	·	·	·	0·3–0·5
IV × V	*mt1/mt2 × mt3/mt4*	14	17	13	·	(2)	13	·	·	(1)	·	0·8–0·9
V × VI	*mt3/mt4 × mt2/mt4*	·	5	·	·	12	9	·	·	·	9	0·3–0·5
		(B) Cross between homozygous types										
VII × IX	*mt1/mt1 × mt3/mt3*	·	·	6	·	·	·	·	·	·	·	·
VII × X	*mt1/mt1 × mt4/mt4*	19	·	·	·	·	·	·	·	·	·	·
VIII × IX	*mt2/mt2 × mt3/mt3*	·	31	·	·	·	·	·	(1)	·	·	·
VIII × X	*mt2/mt2 × mt4/mt4*	·	·	·	·	·	19	·	·	·	·	·
		(C) Selfing										
I × I	*mt1/mt4 × mt1/mt4*	6	·	·	·	·	·	3	·	·	1	·
III × III	*mt1/mt3 × mt1/mt3*	·	·	2	·	·	·	3	·	1	·	·
IV × IV	*mt1/mt2 × mt1/mt2*	·	·	·	6	·	·	3	4	·	·	·
V × V	*mt3/mt4 × mt3/mt4*	·	·	·	·	18	·	·	·	6	5	·
VII × VII	*mt1/mt1 × mt1/mt1*	·	·	·	·	·	·	6	·	·	·	·

* χ^2 test for deviation from 1:1:1:1 ratio.

mating types of F1-clones were grouped into four types and their segregation ratio was expected to be 1:1:1:1 for each type (Table 2A). The mating types of the F1-clones were nearly the same as those expected from their hypothetical genotypes. Only in the cross between types IV and V, unexpectedly two clones of type V and one clone of type IX were observed, but these clones might be explained as progenies from homotypic pairs of type V (see selfing of type V).

Crosses between cells of homozygous types produced progenies of only one mating type with heterozygous alleles derived from each parent (Table 2B). Unexpectedly, from the cross VIII × IX one clone was of type VIII, and is considered to be the progeny of a homotypic pair of type VIII cells.

Following selfing of heterozygous types, mating types were segregated into three types: the parental type and two homozygous types (Table 2C). No unexpected clones were observed from these matings. However, P values could not be determined because of the low viabilities. Homotypic pairs of type VII yielded only progeny of the same type.

Even though the tested clones following selfing were few, most results of the matings were consistent with the hypothesis. Although a few unexpected clones indicated by numbers in brackets were obtained, they could be explained by homotypic pair formation in the mixtures. Therefore, the hypothesis for the mating type determination presented here can be accepted.

(iv) *Relationships between gamones and mating type alleles*

In *E. patella* syngen 2, gamones were excreted into the medium and induced homotypic pair formation of a different mating type (Akada, 1985). However, cell-free fluids prepared from cultures did not always contain sufficient gamone activity to induce homotypic pair formation in the different types. This may be due to no or low excretion of gamones – non-autonomous gamone excretion (non-augex form referred to by Miyake & Beyer, 1973). To obtain sufficient gamone activity, conjugation-conditioned fluid (CCF) was prepared from a conjugation mixture of cells of different mating types, since enhancement of gamone excretion occurs during conjugation (Akada, 1985). The effect of this CCF on homotypic pair formation was examined (Table 3). The CCF from the mixture between cells of homozygous types induced pair formation of all type cells except for one heterozygous type which possessed alleles in common with those of the homozygous types from which the CCF was obtained. This result can be interpreted as follows; each of the four alleles of the mating type gene is responsible for the production of a specific gamone which induces conjugation only in cells which do not produce the same gamone. Based on this interpretation, putative gamones excreted by cells of each mating type are indicated in Table 3. For instance, CCF from a mixture of type VII (*mt1/mt1*) and type VIII (*mt2/mt2*) may contain gamones 1 and 2 which are controlled by *mt1* and *mt2*, respectively, and induce pair formation in all cells except those of type IV (*mt1/mt2*), which also excrete both gamones 1 and 2. The effect of CCF from a mixture of heterozygous and homozygous types possessing a common allele was also examined (Table 3). This CCF did not induce pair formation in heterozygous cells sharing alleles with the cells from which the CCF was obtained, but could induce pairing of the other homozygous types. This result confirms the above interpretation.

4. DISCUSSION

The present results concerning the inheritance of mating types in *E. patella* syngen 2 indicate that each of the ten mating types is determined by a possible combination of two of the four codominant alleles at a single *mt* locus (Table 4). Mating type determination like the present system was reported in *E. patella* (American strains) by Kimball (1942) and *Uronychia transfuga* by Reiff (1968). In

Table 3. *Induction of homotypic pairs by conjugation-conditioned fluids from mixtures between cells of various mating types*

(+, Homotypic pair formation; −, no pair formation; ·, not tested.)

Conjugation-conditioned fluid			Cells									
			I	II	III	IV	V	VI	VII	VIII	IX	X
	Genotypes	Expected	*1/4*	*2/3*	*1/3*	*1/2*	*3/4*	*2/4*	*1/1*	*2/2*	*3/3*	*4/4*
Mating types	*mt/mt*	gamones	1,4	2,3	1,3	1,2	3,4	2,4	1	2	3	4
VII × VIII	*1/1 × 2/2*	1,2	+	+	+	−	+	+	+	+	+	+
VII × IX	*1/1 × 3/3*	1,3	+	+	−	+	+	+	+	+	+	+
VII × X	*1/1 × 4/4*	1,4	−	+	+	+	+	+	+	+	+	+
VIII × IX	*2/2 × 3/3*	2,3	+	−	+	+	+	+	+	+	+	+
VIII × X	*2/2 × 4/4*	2,4	+	+	+	+	+	−	+	+	+	+
IX × X	*3/3 × 4/4*	3,4	+	+	+	+	−	+	+	+	+	+
I × VII	*1/4 × 1/1*	1,4	−	·	·	·	·	·	+	·	·	+
I × X	*1/4 × 4/4*	1,4	−	·	·	·	·	·	+	·	·	+
II × VIII	*2/3 × 2/2*	2,3	·	−	·	·	·	·	·	+	+	·
II × IX	*2/3 × 3/3*	2,3	·	−	·	·	·	·	·	+	+	·
III × VII	*1/3 × 1/1*	1,3	·	·	−	·	·	·	+	·	+	·
III × IX	*1/3 × 3/3*	1,3	·	·	−	·	·	·	+	·	+	·
IV × VII	*1/2 × 1/1*	1,2	·	·	·	−	·	·	+	+	·	·
IV × VIII	*1/2 × 2/2*	1,2	·	·	·	−	·	·	+	+	·	·
V × IX	*3/4 × 3/3*	3,4	·	·	·	·	−	·	·	·	+	+
V × X	*3/4 × 4/4*	3,4	·	·	·	·	−	·	·	·	+	+
VI × VIII	*2/4 × 2/2*	2,4	·	·	·	·	·	−	·	+	·	+
VI × X	*2/4 × 4/4*	2,4	·	·	·	·	·	−	·	+	·	+

American *E. patella*, six mating types were determined by three codominant alleles and cells excreted gamone-like factors. On the other hand, mating type determination of *U. transfuga* was controlled by four codominant alleles at a *mt* locus, which was the same system as that of *E. patella* syngen 2, except that the cells of *U. transfuga* did not excrete such gamone-like factors. Additionally, in *Euplotes octocarinatus* and *Euplotes raikovi*, a similar genetic system of mating type determination was suggested (Heckmann & Kuhlmann, 1982; Luporini, Miceli & Pigini, 1984).

Together with the genetic evidence reported here, the results of cell-CCF combinations indicate that each cell probably has its own gamone(s) coded by its own *mt* alleles, and the cell responds to the different gamones produced by other cell types. In the American *E. patella* described above, Kimball (1942) suggested the same relationships between gamones and *mt* alleles. To explain these specific interactions between gamones and cells, Miyake (1981) has applied the gamone-

receptor hypothesis to the system of American *E. patella*. According to this hypothesis, each cell has its own gamone(s) and all kinds of receptor except the receptor for its own gamone(s). This hypothesis can be applied to *E. patella* syngen 2 (Table 4). For instance, heterozygous mating type I (*mt1/mt4*) cells of *E. patella* syngen 2 would bear the G1G4R2R3 formula, where G1 and G4 represent the two

Table 4. *Mating type, genotype, and gamone-receptor hypothesis in* Euplotes patella *syngen 2*

Mating types	Genotypes	Gamone (G)–Receptor (R) formulas
I	*mt1/mt4*	G1 G4 R2 R3
II	*mt2/mt3*	G2 G3 R1 R4
III	*mt1/mt3*	G1 G3 R2 R4
IV	*mt1/mt2*	G1 G2 R3 R4
V	*mt3/mt4*	G3 G4 R1 R2
VI	*mt2/mt4*	G2 G4 R1 R3
VII	*mt1/mt1*	G1 R2 R3 R4
VIII	*mt2/mt2*	G2 R1 R3 R4
IX	*mt3/mt3*	G3 R1 R2 R4
X	*mt4/mt4*	G4 R1 R2 R3

specific gamones produced by the respective genes of *mt1* and *mt4*, and R2 and R3 are individual receptors for G2 and G3. Also in homozygous type VII (*mt1/mt1*), cells would bear the G1R2R3R4, where G1 is the single gamone and R's are the specific receptors for G2, G3, and G4.

According to the scheme in Table 4, it is expected that in particular cell–cell combinations, reciprocal cell–cell activation mediated by gamones would not occur. For example, in the mixture between cells of types I and VII, G1 of type I reacts with R1 of type VII, but type VII has no gamone to react with receptor of type I. Thus only type VII cells are activated for conjugation. Based on the result of Kimball's *E. patella*, it has been supposed that in these one-sided-activated combinations, only homotypic pairs of activated type cells may be formed, but heterotypic pairs cannot be formed (Sonneborn, 1947; Miyake, 1981). Unfortunately, Kimball (1942) did not report these hetero- and homotypic pair formations completely. In our *E. patella* syngen 2, the number of pairs formed in such one-sided activated mating combinations was fewer than in the other mating combinations, which was also indicated in American *E. patella*. However, heterotypic pairs were usually formed even in these one-sided-activated mating combinations, although gamones existed according to the hypothesis (Table 3, lower part). This phenomenon cannot be clearly explained. In a previous paper (Akada, 1985) it was suggested that specificity of pair formation between heterotypic cells was higher than that between homotypic cells. Thus I suggested that if cells of one type were activated and became strongly adhesive, they might form pairs with cells of a different mating type rather than with their own type.

However, further investigation is needed to explain this unexpected pairing. Recently, in *E. raikovi*, cell–cell mating combinations producing mostly homotypic pairs were observed, although a few heterotypic pairs were formed (Luporini *et al.*

1983). In *E. octocarinatus*, which is closely related to *E. patella* syngen 2, the occurrence of only homotypic pair formation of one type in heterotypic cell–cell combinations was briefly reported (Heckmann & Kuhlmann, 1982).

In conclusion, the present results suggest that four gamones in *E. patella* syngen 2 are coded by four *mt* genes and play important roles in the sexual recognition among ten mating types. Therefore, conjugation of *E. patella* syngen 2 will provide a useful model for understanding the general molecular and genetic mechanisms underlying sexual interaction in ciliate multiple mating type systems.

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