

Reports

Sexual Agglutination of Heterothallic Yeasts in Diverse Taxonomic Areas

Abstract. Species in four genera of yeasts produce mating types that clump when brought together in liquid or on solid media. All species of a phylogenetic line of *Saccharomyces* are sexually agglutinative. Unisexuals of the latter species are believed to have from one to four sets of chromosomes, whereas bisexuals are believed to have from two to eight sets.

In 1956 I reported that cells of opposite mating types of *Hansenula wingei* Wickerham agglutinate immediately when mixed (1). This clumping reaction led to the fastest production of zygotes and diploid vegetative cells that I had yet observed in any yeast. Ascosporic isolates of other strains of *H. wingei* from the same geographic area yielded mating types which were not agglutinative and which were exceedingly weak in their mating reaction. Opposite sexes, consisting of agglutinative and nonagglutinative haploid cultures, were crossed to yield a diploid hybrid, and ascosporic isolates were obtained from this hybrid by a heat-treatment procedure (2) which kills vegetative cells but does not kill all the spores. When these ascosporic cultures were studied, 96 percent were agglutinative; of these, 70 percent agglutinated immediately when they were mixed with a tester of the opposite sex and 26 percent agglutinated within a few minutes. Apparently in nature the agglutinative form gradually eliminates the nonagglutinative form by mating with it. Since the nonagglutinative haploids react sexually so very little,

their existence is hardly endangered by the agglutinative type.

As far as can be surmised from knowledge of phylogenetic lines, the four species in which agglutinative strains have now been found are recently evolved. The weakest sexual agglutination is shown by a new haploid species (NRRL Y-2408) which is yet to be described. The cells become agglutinative many hours after the opposite sexes are mixed—just before or when asci are formed.

Citeromyces matritensis Santa Maria (3) yields ascospores that are sexually agglutinative. The reaction of agar-grown haploid cells is immediate but not as dramatic as it is with *Hansenula wingei*, where the cells immediately adsorb or bind water and the mixture of opposite mating type cells becomes dry and viscous. The mixture of *Citeromyces matritensis* does not bind water appreciably and consequently retains the creamy consistency of each individual mating type. When the mixed cells are put in water, however, they are seen to be agglutinated. Reciprocally, liquid cultures of *C. matritensis* agglutinate in a much more striking manner than do those of *Hansenula wingei*. When shaken cultures are mixed, the cells agglutinate instantly and settle to the bottom. This impressive reaction is probably due in part to the fact that *Citeromyces matritensis* cells tend to grow in large colonies in liquid media.

The haploid form of *C. matritensis* is known by the name of *Torulopsis globosa* (Olsen et Hammer) Lodder et Kreger-van Rij (4). The single strain in our collection agglutinates and sporulates with one of the mating types derived from Santa Maria's diploid.

Not one of the three species already discussed equals in interest *Saccharomyces kluyveri* Phaff, Miller, and Shifrine (5). This species is sufficiently plastic genetically to yield many interesting forms, yet rigid enough so that all forms mentioned in this report may be kept pure. In common with the other more recently evolved species of its genus, *S. kluyveri* produces unisexual diploids. Unisexuals which are believed to be triploid and tetraploid are also produced. Each ploidy level is morphologically distinct, and all are sexually agglutinative. The unisexual diploids agglutinate and

mate abundantly with haploids or with unisexual diploids of the opposite sex. Four to six hours after opposite sexes are mixed the cells are streaked on agar. Two days later the largest colonies, which consist of triploid and tetraploid cells, depending on the ploidy of the cells that were mixed, are selected. Bisexuals are not agglutinative and hence are readily differentiated from their agglutinative parents. Each ploidy level by itself produces four-spored asci, whether unisexual or bisexual. The haploids produce conjugated asci. Higher unisexual ploidy levels originate through conjugation of cells of various degrees of ploidy. The zygotes may produce buds rather than ascospores and thus may give rise to vegetative forms. When unisexuals having more than one set of chromosomes sporulate, the asci are not conjugated, but consist of a single cell.

Saccharomyces kluyveri grows rapidly and is a rather strong fermenter. These characteristics, plus the agglutinative reaction, give the species industrial possibilities. For example, growth rates of its unisexual diploids compare favorably with those of *Candida* (*Torulopsis*) *utilis*, the common food yeast. The two sexes of *Saccharomyces kluyveri* may be grown separately and then combined in a tank. Following agglutination and settling, the supernatant could be run off; hence, the amount of centrifugation required for harvesting the crop would be greatly reduced. Naturally, such utilization depends upon the cells' being of desirable nutritional composition. The composition has not been determined. *Saccharomyces kluyveri* exhibits exceedingly strong sexual reactions, has the ability to produce polyploids more abundantly than any other yeast known, and holds a potential for commercial uses. Because of these factors, *S. kluyveri* and closely related species possessing the property of sexual agglutination may prove superior to all other known species for genetic, and possibly also for industrial, studies.

The heat-treatment procedure devised for the isolation of mating types from diploid species of *Hansenula* (2) is equally effective for *Saccharomyces*, and no modification is required. The more strongly heterothallic strains of *S. cerevisiae*, *S. carlsbergensis*, and *S. diastaticus*, which geneticists generally use, readily yield colonies of opposite mating types on heat treatment of sporulated cultures at 60° or 63°C. Plates are streaked at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15 minutes, and thereafter at 5-minute intervals through 60 minutes. Fifteen minutes at 57°C is adequate for *S. kluyveri*. Most of the colonies on the terminal plates consist of one or the other mating type.

At present four species of yeasts, occurring in four genera, have been found to

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should not repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to one 2-column figure (that is, a figure whose width equals two columns of text) or to one 2-column table or to two 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

possess the sexual agglutination reaction. This mating mechanism is fairly common among yeasts, especially among species evolved in terms of recent geological time.

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6. The assistance of Mr. K. A. Burton and Mrs. Jane Roberson is gratefully acknowledged.

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Hormonal Control of Onset of Corneal Reflex in the Frog

In a previous study (1) it was reported that the corneal (wink) reflex is unelicitable in the tadpole until just before the period of metamorphic climax. In *Rana pipiens* the onset of the reflex normally precedes forelimb emergence from the branchial chamber by an average of 4 days (range, 0 to 10 days). In *R. catesbeiana* this is also true, except for rare instances of earlier onset. It was further established (1, 2) that the onset of the reflex was strictly related to metamorphosis; it never appeared in the non-metamorphosing hypophysectomized tadpole, and the time of onset could be moved forward by treatment of the whole normal tadpole with thyroxine, but the extent of acceleration was less than for other metamorphic changes.

However, local stimulation of the reflex center in the medulla oblongata of large midlarval tadpoles with pellets containing thyroxine produced a premature maturation of the center, as well as an unusually early onset of the reflex; maturation occurred as much as 18 days before forelimb emergence. These results demonstrated the dependence of the reflex center upon thyroid hormone for its final maturation.

It has been shown that the later stages of induced metamorphosis in thyroidless or hypophysectomized tadpoles are passed through progressively more slowly than the early stages, at a constant thyroxine dosage level (3), and that successive events or stages of metamorphosis tend to have a higher thyroxine concentration requirement or threshold (4). Hence it may be assumed that the later metamorphic event, forelimb emergence, displays a higher threshold than does the onset of the corneal reflex, the earlier metamorphic event.

To test this assumption, concentrations of thyroxine were sought which would in fact permit the establishment of the corneal reflex without concomitantly stimulating the rupture of the skin windows through which the forelimbs emerge. In tests of over 150 *R. pipiens* and a few *R. catesbeiana*, over a large range of thyroxine concentrations in the surrounding water (from 0.002 to 2 µg/lit., water and food being changed daily, with thyroxine added immediately thereafter), the validity of the assumption has been demonstrated in two instances (see data in Table 1 for 108- and 177-day animals). In six other instances a related and very significant lengthening of the interval between the onset of the reflex and forelimb emergence has been recorded. In general, it has been found that, at 25°C, concentrations of dl-thyroxine of 0.6 µg/lit. invariably bring about forelimb emergence, if permitted

to act for a long enough time. Concentrations of 0.4 µg/lit. rarely produce forelimb emergence, although incipient thinning of the skin-window area is usually obtained. A concentration of 0.2 µg/lit. is insufficient to initiate the corneal reflex or forelimb emergence. At 15°C, even 1.0 µg/lit. is ineffective in producing rupture of the skin window.

The study discussed in this report provides further evidence in support of the belief that most metamorphic changes in the frog tadpole are separable events, capable of being brought about individually by local hormone treatment (2, 5), or capable of being separated from succeeding metamorphic events by careful manipulation of hormone concentration and temperature (6).

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Factors Affecting the Relative Deposition of Strontium and Calcium in the Rat

Abstract. Varying the calcium, phosphorus, carbonate, and lactate content of the diet was shown to affect the deposition in bone of Sr⁹⁰ to a degree quite different from concurrent effects on Ca⁴⁵ deposition. The influence of these findings on the evaluation of the Sr⁹⁰ fallout hazard is discussed.

In a recent report in *Science* we presented evidence against the commonly accepted concept that the deposition and retention of Sr⁹⁰ in bone is simply related to the concomitant deposition and retention of calcium (1). This concept has been widely employed in the evaluation of the hazard to human beings of Sr⁹⁰ from fallout. We now wish to report further experiments, of an admittedly preliminary nature, which support our earlier position and which suggest an explanation for the varied results which have been reported by investigators in this field (see 2).

Six groups of four rats each were maintained for 8 days on diets which varied in one or more of the following constituents: calcium, phosphorus, car-

Table 1. Record of treatment of hypophysectomized tadpoles, demonstrating separability of the onset of the corneal reflex from the emergence of the forelimbs. Thyroxine was added to the water in which the animals were raised.

Species (<i>Rana</i>)	Form of thyroxine	Thyroxine concn. (µg/lit.)	Tempera- ture (°C)	Treatment time in days	
				Before reflex onset	Between reflex onset and forelimb emergence
<i>R. pipiens</i>	dl	1.0	25	73	41
<i>R. pipiens</i>	dl	1.0	15	161	37*
<i>R. pipiens</i>	dl	1.0	25	71	45
<i>R. pipiens</i>	dl	1.0	25	47	44*
<i>R. pipiens</i>	l	0.2	25	71	47
<i>R. pipiens</i>	l	0.4	15	170	108*
<i>R. pipiens</i>	l	0.4	25	87	43
<i>R. catesbeiana</i>	dl	0.4	15	70	177†

* Tadpole died prior to emergence of forelimbs.

† The metamorphic progress came to a complete standstill at 15°C; skin windows thinned only slightly. The tadpole was transferred to a 25°C bath after 164 days; forelimb emergence occurred 13 days later.