nificant F-ratio of 15.61 ($p < .01$) for the means of the five combinations of associative values studied. The greatest part of the between-variance (11.87) was caused by a linear component (10.18).

To find the relationship between visual recognition and associative strength, only two lists of nonsense trigrams were used. The pairs of the third list could not be ranked reliably according to associative strength. A first list of 40 trigrams of 47–47 associative values yielded a nonsignificant rank-order correlation between the mean number of correct recognitions and the mean number of trials to criterion ($r = .21$). A second list of 40 pairs (10 pairs for each of the following combinations: 100–100, 0–100, 100–0, and 0–0), ranked according to the mean number of correct responses per pair within ten learning and ten transfer trials (*4*), yielded a positive and significant rank-order correlation with the mean number of correct recognitions ($r = .41$, $p < .05$).

Another factor determining the accuracy of recognition was found to be retinal locus. The mean number of correct recognitions was greater to the left than to the right of the fixation point. These results are similar to those reported by Heron (*3*). He found a more accurate recognition in the left visual field than in the right field, when three English letters were exposed simultaneously rather than successively on either side of the fixation point. Such a "differential recognition" was attributed to "the dominant tendency to move the eyes to the beginning of the line" (*3*, p. 47). In addition to associative value and associative strength as determinants of visual recognition of nonsense trigrams, retinal locus should also be taken into consideration in studying visual recognition of nonsensical and of meaningful materials (*5*).

LUCIANO L'ABATE
Department of Psychiatry and Neurology, Washington University School of Medicine, St. Louis, Missouri

References and Notes

1. M. R. Rosenzweig and L. Postman, *Science* **127**, 263 (1958).
2. L. L'Abate, *Am. Psychologist* **13**, 386 (1958).
3. W. Heron, *Am. J. Psychol.* **70**, 38 (1957); D. O. Hebb, *The Organization of Behavior* (Wiley, New York, 1949).
4. This measure is related inversely to the number of trials or of correct responses to a learning criterion (the mean coefficients of correlation with these measures are –.74 and –.62 respectively).
5. This experiment was conducted during my tenure as U.S. Public Health Service postdoctoral fellow at the Institute for Psychosomatic and Psychiatric Research and Training of Michael Reese Hospital, Chicago, Ill.

21 January 1960

Extracellular Invertase Production by Sexually Agglutinative Mating Types of Saccharomyces kluyveri

Abstract. Unisexual strains of both mating types of *Saccharomyces kluyveri* produce exceedingly high yields of extracellular invertase. Yields generally increase with an increase in the number of sets of chromosomes possessed by the unisexuals. The bisexual forms give small amounts in comparison.

In 1956 Wickerham reported the agglutination of opposite sexes in the yeast *Hansenula wingei* Wickerham (*1*). He coined the term "sexual agglutination" (*2*) to differentiate agglutination by opposite mating types from mutual agglutination. The latter process is a much weaker reaction discovered by Eddy (*3*) in strains of brewery yeasts. Both strains of the mutually agglutinative pair (NCYC 74 and 1109) which we obtained from Eddy proved to be bisexual. Recently, Brock (*4*) presented evidence that sexual agglutination in *H. wingei* is due largely to the presence of a specific protein on the cell wall of one of the sexes that reacts with a specific polysaccharide on the cell wall of the opposite sex. He believes the reaction is comparable to that of antigen with its specific antibody. Sexual agglutination is also known to occur in sea urchins and in a few species of algae and protozoa. In these, likewise, there is evidence of stereospecific reactions.

Wickerham (*2*) has reported the occurrence of sexual agglutination in species of four genera of yeasts. The most highly developed species are in *Saccharomyces*, as judged by the ease with which unisexual diploids and presumably unisexual polyploids are produced, as well as by the copious formation of bisexual tetraploids and presumably bisexual hexaploids and octaploids.

Contrary to the generally accepted belief that yeasts do not produce extracellular invertase, we have shown that certain yeasts actually do produce significant amounts (*5, 6*). The maximum yields of extracellular invertase from *Saccharomyces uvarum* NRRL Y-972 were 126 units per milliliter in aerobic (shaken) culture and 50 units per milliliter in anaerobic (still) culture. Enzyme activity was measured by the procedure of Sumner and Howell (*7*), with slight modification (*6*).

In the present study, bisexual forms of *Saccharomyces kluyveri* Phaff, Miller, and Shifrine (*8*) were found to produce low yields of extracellular invertase commensurate with yields by unisexuals and bisexuals of industrially important species of *Saccharomyces* (*S. cerevisiae, S. carlsbergensis,* and *S. diastaticus*). The unisexuals of *S. kluyveri,* however, gave astonishingly high yields (Table 1). Unisexuals 7H1 and 7H2 are two haploids of one sex, and 13H1 and 13H2 are two haploids of the opposite sex. Unisexual diploids 7D1 and 13D1 were derived from 7H1 and 13H1, respectively. Unisexuals designated by code numbers also were largely derived from ascosporic isolates 7 and 13, and are presumed to be triploids and tetraploids. There seems to be a general increase in yields of invertase with an increase in number of sets of chromosomes possessed by the unisexuals. With increasing ploidy the unisexuals also increase in intensity of sexual agglutination; so much so, that presumably unisexual polyploids often show some agglutination by themselves in liquid culture. The ratio of invertase

Table 1. Production of extracellular invertase in aerobic and anaerobic cultures by bisexual and unisexual forms of the sexually agglutinative species *Saccharomyces kluyveri* NRRL Y-4288.

Sex and ploidy	Extracellular invertase	
	Aerobic (unit/ml)	Anaerobic (unit/ml)
Parent bisexual	26	
Bisexual		
Diploid		
7H1 × 13H1	15	
7H1 × 13H2	13	
7H2 × 13H1	13	
7H2 × 13H2	17	
Triploid		
7H1 × 13D1	13	
7H2 × 13D2	14	
7D1 × 13H1	20	
7D2 × 13H2	24	
Tetraploid		
7D1 × 13D1	13	
7D1 × 13D2	24	
7D2 × 13D1	22	
7D2 × 13D2	25	
Unisexual		
Haploid		
7H1	298	
7H2	297	
13H1	366	
13H2	253	
Diploid		
7D1	313	
7D2	337	
13D1	351	
13D2	361	
Presumed unisexual polyploid		
Code 3	792	163
Code 4	491	267
Code 7	295	182
Code 12	340	214
Code 15	380	224
Code 17	340	232
Code 18	279	434
Code 20	580	201
Code 24	325	419
Code 25	385	465
Code 26	492	173
Code 27	230	248
Code 3, transferred serially 2 months	800	
Code 26, transferred serially 3 months	456	

produced aerobically and anaerobically by mating types varies with the individual presumed unisexual polyploid. Both sexes 7 and 13 gave high yields, indicating that invertase is not the specific protein reported by Brock to take part in the sexual agglutination reaction of *Hansenula wingei*. It is to be expected that in addition to the extracellular enzyme, much invertase is bound to the surface of the cells (*6, 9*).

The isolates designated as code 3 and code 26, of opposite sex, are available from this laboratory. They may be used to demonstrate sexual agglutination and consequent easy harvesting of yeast cells from liquid media. The mating types are grown separately for a few days on YM slants, being transferred daily, and then grown for 48 to 96 hours in shaken flasks at about 28°C in 3 percent glucose or sucrose YM medium, or other liquid media suitable for yeast. The cultures are mixed and sexual agglutination immediately occurs, with rapid settling of the large clumps of agglutinated cells. Codes 3 and 26 are stable. They were transferred daily on YM slants, except for weekends, for 2 and 3 months, respectively, without monitoring, and at the end of this time they gave high yields of invertase (Table 1).

LYNFERD J. WICKERHAM
ROBERT G. DWORSCHACK
Fermentation Laboratory,
U.S. Agricultural Research Service,
Peoria, Illinois

References and Notes

1. L. J. Wickerham, *Compt. rend. trav. lab. Carlsberg. Sér. physiol.* **26**, 423 (1956).
2. ———, *Science* **128**, 1504 (1958).
3. A. A. Eddy, *J. Inst. Brewing* **64**, 143 (1958).
4. T. D. Brock, *J. Bacteriol.* **78**, 59 (1959).
5. L. J. Wickerham, *Arch. Biochem. Biophys.* **76**, 439 (1958).
6. R. G. Dworschack and L. J. Wickerham, ibid. **76**, 449 (1958).
7. J. B. Sumner and S. F. Howell, *J. Biol. Chem.* **108**, 51 (1935).
8. H. J. Phaff, M. W. Miller, M. Shifrine, *Antonie van Leeuwenhoek. J. Microbiol. Serol.* **22**, 145 (1956).
9. D. T. Demis, A. Rothstein, R. Meier, *Arch. Biochem. Biophys.* **48**, 55 (1954).
10. The assistance of K. A. Burton and Jane Roberson is gratefully acknowledged.

16 November 1959

Mayaro Virus Isolated from a Trinidadian Mosquito, Mansonia venezuelensis

Abstract. A strain of Mayaro virus has been isolated in Trinidad from the mosquito *Mansonia venezuelensis*. This is the first record of isolation of this agent from naturally infected mosquitoes, caught in the wild.

Mayaro virus was first isolated in 1954 from the blood of a human being working in a forested area of southeastern Trinidad (*1*). The original report recorded the finding of this new agent during August and September in five persons widely scattered over the island. With the exception of the isolation reported here, there had been no further recoveries of this virus in Trinidad through September 1959. However, a survey conducted in 23 representative localities throughout the island has shown that 11 percent of a human population of 615 possess neutralizing antibodies for Mayaro, with localization in southeastern Trinidad, where rates as high as 48 percent were encountered (*2*).

Table 1. Biological properties of two strains of Mayaro virus and of Semliki Forest virus.

Pathogenicity for adult mice	Hemagglutinating antigen from mouse brain	Growth in chick embryo tissue culture
	Semliki Forest	
Highly pathogenic by intracerebral route	Yes	Multiplication and cytopathogenic changes
	Mayaro TRVL 4675	
Not pathogenic by intracerebral route	No*	No multiplication or cytopathogenic changes
	Mayaro TRVL 15537	
Moderately pathogenic by intracerebral route	Yes	Multiplication but no cytopathogenic change

* No hemagglutinating antigen results from acetone-ether extraction of baby mouse brain, but an antigen can be prepared by the sucrose-acetone method.

Mayaro virus is also found in the Amazon valley of Brazil, where it is associated with human illness (*3*), and immunity surveys indicate its presence in the Rupununi savannah and Mazaruni River regions of British Guiana (*2*). We present in this report the first record of the occurrence of this agent in a naturally infected arthropod.

Limited laboratory evidence indicates that mosquitoes are capable of harboring the virus for at least 12 days, and that on one occasion virus was transmitted by the bite of *Aëdes scapularis* (*4*).

Mayaro virus was not recovered from arthropods in this laboratory during 1955 and 1956, although well over 200,000 specimens were ground and inoculated into baby mice. Prior to 1955 this agent would have escaped attention in the entomological work, since only adult mice were then used to receive the original arthropod inocula. Not until March 1957 were we successful in isolating this virus from naturally infected forest mosquitoes. The isolation reported here is the sole Mayaro isolation from mosquitoes, despite the fact that 401,578 mosquitoes were examined in the interval from March 1957 to October 1959.

Mayaro virus was isolated in baby mice inoculated on 28 March 1957 with a suspension from a pool of 49 *Mansonia venezuelensis* (TRVL 15537). These mosquitoes had been taken over a period of 12 working days, between 11 and 27 March, and stored daily as whole insects in sealed ampules at −60°C. They were collected while attempting to bite human beings on the forest floor at our Rio Grande Forest tree station about 7 miles north of Sangre Grande in northeastern Trinidad.

The virus was isolated from the brains of baby mice inoculated intracerebrally with a suspension of this *Mansonia* pool; the agent was established in baby mice and was shown to be filtrable. The virus was reisolated from the original mosquito suspension, both in baby mice and in hamster-kidney tissue culture.

This virus from *Mansonia* is indistinguishable from Mayaro virus (TRVL 4675) by complement-fixation, hemagglutination-inhibition, and neutralization test techniques. Several interesting biological differences among TRVL 4675, TRVL 15537, and Semliki Forest viruses are presented in Table 1 (*6*).

T. H. G. AITKEN, W. G. DOWNS,
C. R. ANDERSON, L. SPENCE
Trinidad Regional Virus Laboratory,
Port-of-Spain, Trinidad, West Indies
J. CASALS
Rockefeller Foundation Virus
Laboratories, New York, New York

References and Notes

1. C. R. Anderson, W. G. Downs, G. H. Wattley, N. W. Ahin, A. A. Reece, *Am. J. Trop. Med. Hyg.* **6**, 1012 (1957); J. Casals and L. Whitman, *ibid.* **6**, 1004 (1957).
2. W. G. Downs and C. R. Anderson, *West Indian Med. J.* **7**, 190 (1958).
3. O. R. Causey and O. M. Maroja, *Am J. Trop. Med. Hyg.* **6**, 1017 (1957).
4. T. H. G. Aitken, *West Indian Med. J.* **6**, 229 (1957).
5. S. M. Buckley, *Ann. N.Y. Acad. Sci.* **81**, 172 (1959).
6. The studies and observations on which this report is based were conducted by the Trinidad Regional Virus Laboratory with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme, and the Rockefeller Foundation.

18 November 1959

Effect of Kinetin on Paramecium caudatum under Varying Culture Conditions

Abstract. When kinetin (1 mg/liter) is added to hay infusion medium, the generation time of *Paramecium caudatum* is shortened immediately upon transfer of the protozoa from stock to isolation culture. Kinetin is particularly effective when culture conditions are suboptimal, perhaps because it substitutes for or supplies some factor which becomes limiting after transfer.

In a previous report, increased rates of cell division in *Paramecium caudatum* were reported after addition of low doses of kinetin (6-furfuryl amino purine) to the culture medium (*1*). In subsequent tests with a new clone of *Paramecium* and new preparations of