SEXUAL RECOMBINATION IN ROTIFERS*

CHARLES E. KING† and TERRY W. SNELL

Department of Biology, University of South Florida, Tampa, Florida 33620

Received 18.iii.77

SUMMARY

Data exist suggesting both the presence and absence of recombination in monogonont rotifers. It was therefore previously unknown whether members of this group have true sexual reproduction. We have used acrylamide gel electrophoresis to identify clones of *Asplanchna girodi* homozygous for two different alleles at a single malic enzyme locus. Crosses made among these clones produced heterozygotes, thus demonstrating that classical sexual recombination occurs in this group of rotifers.

WHETHER or not recombination occurs during bisexual reproduction in monogonont rotifers has been a topic of considerable debate among workers with this group of aquatic invertebrates. The monogonont life cycle (fig. 1) typically involves a series of parthenogenetic generations in which diploid amictic females produce diploid eggs by mitosis. These eggs develop into either amictic (parthenogenetic) or mictic (sexual) females. Mictic females form haploid eggs by meiosis. If these eggs are not fertilised, they develop into haploid males; if fertilised they become thick-shelled resting eggs which hatch into a new generation of amictic females.

This general outline of the life history has recently been supported by cytological evidence (Robotti, 1975; Jones and Gilbert, 1976) and is accepted by most students of monogonont rotifers. The controversy has centred on whether there is actually a syngametic fusion of male and female pronuclei. Until recently the strongest evidence for recombination was provided by Birky (1965) using clones of *Asplanchna brightwelli* that produced sterile males. A number of crosses were made and the pattern of sterility followed in males derived from the offspring. Birky concluded that his results were consistent with an interpretation of the male-sterile effect as being caused by a single sex-limited allele which underwent recombination. However, maternal inheritance and incomplete penetrance were required to explain some of his results. Although both of these phenomena are of course well-known, they complicate the analysis and other interpretations of Birky's data are possible (Ruttner-Kolisko, 1969).

In a study involving hybridisation of two congeneric species, Ruttner-Kolisko (1969) was able to show that only maternal characteristics appeared through five generations of inbreeding. These data led to the suggestion that inheritance in monogonont rotifers is matroclinal and that sperm function only to stimulate production of resting eggs. Under this view, bisexual reproduction is in reality "cryptoparthenogenesis". Additional observations supporting the genetic insignificance of males (Ruttner-Kolisko, 1969, 1972) include the occurrence in one species of "pseudosexual" eggs

* This research was supported by grant No. BMS 73-00996 from the National Science Foundation.

† Present address: Department of Zoology, Oregon State University, Corvallis, Oregon 97331.

(thick-shelled unfertilised eggs produced by amictic females of *Keratella quadrata*—see fig. 1), and the total absence of males in bdelloid rotifers.

During the past 2 years we have developed techniques for acrylamide gel electrophoresis of rotifer isozymes. In the spring of 1975, a population

FIG. 1.—Generalised life cycle of monogonont rotifers.

of *Asplanchna girodi* in Golf Course Pond, near Tampa, Florida was found to be composed solely of heterozygotes at a malic enzyme locus. Two of the clones established from this population were selfed to obtain resting eggs. Progeny hatched from these eggs were cloned and scored by electrophoresis.

Presuming segregation and independent assortment of the two alleles, designated S (for slow migration) and F (for fast), a 1 : 2 : 1 ratio of phenotypes is expected. As reported in King (1977), two groups of resting eggs were obtained from these heterozygotes. Group I resting eggs produced seven clones, all of which were homozygous for the slow allele ($\chi^2 = 21.00$, $P < 0.05$). Group II had 44 clones and was composed of 13 slow homozygotes, 24 heterozygotes, and seven fast homozygotes ($\chi^2 = 1.99$, $P > 0.05$). The results from group II suggest classical Mendelian inheritance, however, those of group I do not. The two groups therefore lead to different conclusions. Moreover, if results from the two groups are combined the chi-square is 6.80 leading to rejection of the hypothesis of simple Mendelian segregation and random recombination. These data are discussed in more detail by King (1977).

The obvious way to demonstrate true sexual reproduction is to cross individuals homozygous for the slow allele to individuals homozygous for the fast allele. We have recently done this for two sets of clones. One of these sets employed F_1 clones derived from the intraclonal crosses described above. The second cross was made between fast and slow homozygotes obtained from different lakes. All progeny from these crosses were found to be heterozygous for the slow and fast alleles (fig. 2).

FIG. 2.—Malic enzyme genotypes: (from left to right) slow homozygote, heterozygote, and fast homozygote.

These data indicate that the failure of group I to meet expected Mendelian ratios is due either to chance events (the probability of obtaining seven slow homozygotes in a sample of seven offspring is $0.27^5 = 6.10 \times 10^{-5}$), or to undetected selective differences between the environments of groups I and II. In spite of the unusual ratio in group I, the production of heterozygotes from crosses between alternative homozygotes clearly indicates Mendelian inheritance. The production of heterozygotes further indicates that males do make a genetic contribution to resting eggs, thereby requiring rejection of the view that resting eggs are produced by cryptoparthenogenesis.

We conclude that recombination, and therefore true sexual reproduction occurs in monogonont rotifers.

REFERENCES

- BIRKY, C. W., JR. 1965. Studies on the physiology and genetics of the rotifer, *Asplanchna*. II. The genic basis of a case of male sterility. *J. Exp. Zool.*, 158, 349-356.

- JONES, P. A., AND GILBERT, J. J. 1976. Male haploidy in rotifers: relative DNA content of nuclei from male and female *Asplanchna*. *J. Exp. Zool.*, 198, 281-285.
- KING, C. E. 1977. Genetics of reproduction, variation, and adaptation in rotifers. In *Proceedings 1. International Rotifer Symposium*, ed. C. E. King. Schweizerbart-Verlag, Stuttgart. In press.
- ROBOTTI, C. 1975. Chromosome complement and male haploidy of *Asplanchna priodonta* Gosse 1850 (Rotatoria). *Experientia*, 31, 1270-1272.
- RUTTNER-KOLISKO, A. 1969. Kreuzungsexperimente zwischen *Brachionus urceolaris* und *Brachionus quadridentatus*, ein Beitrag zur Fortpflanzunbiologie der heterogonen Rotatoria. *Arch. Hydrobiol.*, 65, 397-412.
- RUTTNER-KOLISKO, A. 1972. Rotatoria. *Die Binnengewasser* (suppl), 26, 99-233.