# A PRELIMINARY GENETIC INVESTIGATION OF *VOLVOX CARTERI*

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ABSTRACT

A preliminary genetic analysis of a number of genetic variants of *Volvox carteri* f. *nagariensis* is presented. Techniques are outlined for mutagenesis of Volvox, isolation of mutants and routine genetic analysis. All of the mutants show simple Mendelian segregation patterns and have been tentatively placed in 14 linkage groups.

THE colonial green alga Volvox has a number of characteristics that make it an excellent model system for studying basic questions of development (STARR 1970): the embryo divides by synchronous cleavage; most of the cells differentiate to express the various functions of vegetative cells; a few cells retain the capacity to produce new individuals; the mature embryo undergoes morphogenetic movements to produce the adult organisms; and reproduction occurs by either asexual or inducible, sexual means.

The normal development of *Volvox carteri* has been investigated extensively at both the light (STARR 1969) and electron microscope level (KOCHERT and OLSON 1970; OLSON and KOCHERT 1970; SESSOMS 1974). The process of inversion has been studied in great detail, and the cell shape changes involved in this process are particularly striking when compared to similar events in higher eukaryotes (VIAMONTES and KIRK 1977). Investigations at the moleculer level have been reported on the chemical nature of the sexual hormone (STARR and JAENICKE 1974; KOCHERT and YATES 1974); the synthesis of nucleic acids (KOCHERT and SANSING 1971; MARGOLIS-KAZAN and BLAMIRE 1976, 1977); and the synthesis of proteins (KIRK and KIRK 1976). All of these reports lead to the conclusion that Volvox is amenable to the use of a variety of techniques for studying developmental phenomena.

Since Volvox is haploid and the sexual cycle is easily controlled, the use of genetic techniques to study development in Volvox should be as productive as such studies on other organisms of varying complexity. Several genetic variants of Volvox have been reported (STARR 1970; SESSOMS and HUSKEY 1973; PALL 1975), but a systematic genetic analysis has not been previously reported. We now report a study of the isolation, characterization and genetic analysis of a number of mutants of *Volvox carteri*. These mutants affect processes at various

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levels of cellular and organismal organization, and they provide the basis for a number of approaches to the study of basic developmental processes.

## MATERIALS AND METHODS

*Strains:* All strains used are listed in Table 1, along with their source. The female HK-10 strain and the male 69-1b strain of *Volox carteri* f. *nagariensis* were obtained from the Algal Culture Collection through the courtesy of R. C. STARR (STARR 1969, 1970). All of the mutants were derived from these strains.

TABLE 1

*Mutant strains*

| Linkage group | Name | Mutant description | Mutagen* | Source or reference† |
|---|---|---|---|---|
| I | *mtf* | Mating-type female; spheroids develop as sexual females in the presence of inducer. (HK-10 of STARR). | | c |
| | *mtm* | Mating-type male; spheroids develop as sexual males in the presence of inducer. (69–1b of STARR). | | c |
| | *sex$^c$C* | Sexual constitutive; spheroids always in the sexual pathway in the absence of inducer. (1) | SP | a |
| | *eyeB* | Eyespot; randomly oriented eyespots (2) | UV | d |
| | *expD* | Nonexpander; adult spheroids remain smaller than normal due to an apparent defect in matrix synthesis or assembly. (3) | NG | b |
| | *invA* | Inversionless; embryo does not invert. (1) | NG | b |
| | *mulC* | Multiple gonidia; spheroids have fewer cells and variable number of gonidia (same as *s-reg* of b†). (3) | NG | b |
| II | *regA* | Regenerator; somatic cells act as reproductive cells. (40) | NG, SP, EMS | a,b,c,d,e |
| III | *mesA* | Methionine sulfoximine resistant. (3) | NG | a |
| | *d-disA* | Delayed-dissolver; spheroids disassociate two to four days after inversion. (1) | NG | b |
| | *lts$^s$B* | Lethal(ts); grows at 24°, lethal at 35°. (1) | NG | a |
| | *flgA* | Flagella; no flagella, eyespots randomly oriented. (1) | NG | a |
| IV | *expA* | Nonexpander; spheroids small and dark with little extra-cellular matrix. (1) | NG | b |
| | *expB* | Nonexpander; spheroids have less matrix than wild type but phenotype is variable. (1) | NG | b |
| | *relA* | Release; daughter spheroids fail to release at normal time. (1) | NG | a |
| | *lts$^s$A* | Lethal(ts); grows at 24°; lethal at 35°. (1) | NG | a |
| V | *lts$^s$D* | Lethal(ts); grows at 24°; lethal at 35°. (1) | NG | a |
| | *megA* | Multiple egg; sexual female strains have increased number of eggs. (5) | NG | a |
| | *u-expA* | Unequal expander; anterior of spheroid expands but posterior does not. (1) | NG | b |
| VI | *doA* | Doughnut; spheroids have increased number of gonidia and embryos invert from both ends. (1) | NG | a |
| | *mulD* | Multiple gonidia; spheroids have fewer cells and variable number of gonidia (same as *s-reg* of b†). (3) | NG | b |

TABLE 1—Continued

| Linkage group | Name | Mutant description | Mutagen* | Source or reference† |
|---|---|---|---|---|
| VII | *expC* | Nonexpander; similar to *expB*. (1) | NG | a |
| | *flgC* | Flagella; no flagella when grown at 35°, flagella present or regrow at 24°. (1) | NG | a |
| VIII | *mulA* | Multiple gonidia; clusters of two to four gonidia in wild-type pattern (similar to *multi* of STARR). (1) | NG | b |
| | *mulB* | Multiple gonidia; increased number of gonidia in a random pattern over most of the spheroid. (1) | SP | d |
| IX | *nitA* | Ammonia-requiring; does not grow in presence of nitrate but will grow in presence of ammonia. (15) | NG | e |
| | *nitB* | Ammonia-requiring; similar to *nitA*. (1) | NG | a |
| X | *eyeA* | Eyespot; randomly oriented eyespots. (1) | NG | a |
| | *frA* | Fruity; variable, abnormal spheroid morphology due to abnormal cleavage. (1) | NG | b |
| XI | *nitC* | Ammonia-requiring; similar to *nitA*. (5) | NG | e |
| XII | *rotA* | Rotator; spheroids rotate in opposite direction to wild type. (1) | UV | d |
| XIII | $l^{ts}C$ | Lethal(ts); grows at 24°, lethal at 35°. (1) | NG | a |
| XIV | $sex^cA$ | Sexual constitutive; spheroids always in the sexual pathway in absence of inducer. (1) | NG | a |
| ? | $sex^cB$ | Sexual constitutive; similar to $sex^cA$. (1) | NG | a |
| ? | *sicA* | Sickle; gonidia have "sickled" appearance in presence of inducer and do not cleave. (1) | NG | a |

Independent mutant loci of similar phenotype are designated by a letter following the mutant abbreviation. The number of isolates at each locus is given in parenthesis.

* Mutagens are abbreviated as follows: ethyl methanesulfonate (EMS); nitrosoguanidine (NG); spontaneous, no mutagen used (SP); ultraviolet light (UV).

† The source of a mutant strain or a reference to the mutant is as follows: (a) this work; (b) SESSOMS and HUSKEY (1973); (c) STARR (1970); (d) P. O'FARRELL (personal communication); (e) D. L. KIRK (personal commmunication).

*Maintenance of cultures:* All strains were grown axenically in minimal Volvox medium (Vm) (STARR 1969), which had been supplemented with sodium acetate, 150 mg/l, (Va) and buffered to pH7 (Va-7) or pH8 (Va-8) (STARR and JAENICKE 1974). The medium for ammonia-requiring mutants was supplemented with 1.5 mM $NH_4Cl$. Stock cultures were maintained in 16 × 125mm screw-cap tubes and were transferred every two to three weeks. Progeny from crosses were grown in 13 × 100 mm metal-capped tubes containing 5 ml of media. All cultures were illuminated on a 16 hr/8 hr light-dark cycle by 40-watt cool-white fluorescent lamps with an intensity of 800 to 1600 foot candles as measured by a Goosen Pan Lux light meter. Tube cultures and flask cultures were illuminated from one side by a horizontal lamp. Cultures in three-spot depression plates or in petri dishes were illuminated from above, using suspended lamps or from below by placing the petri dish or dish containing the spot plate on a glass shelf over a lamp. Illumination from below was particularly important in crosses involving nonmotile female strains in order to bring the positively phototactic, motile sperm packets into close proximity with the female spheroids on the bottom of the dish. Most cultures were maintained at room temperature, 21° to 31°, although some temperature-sensitive mutants were grown in controlled-temperature, illuminated incubators. In order to inactivate the sexual inducer, all glassware was baked in an oven at 175° for six hr. This procedure was also used to sterilize pipettes, capillary tubes contained in cannisters and spot plates contained in petri dishes. Volvox medium and the various supplements were sterilized by autoclaving. Penicillin (Sigma) solutions were sterilized by Millipore filtration and were stored at —4°.

*Mutagenesis:* Only female strains were mutagenized because male cultures tend to go sexual due either to a naturally occurring sexual male that produces inducer or to the occurrence of a mutation to spontaneous sexuality. A single wild-type female spheroid was transferred to a flask with Va-8 medium and grown with aeration for several generations. The spheroids were transferred to a flask containing Va-8 medium and adjusted to a concentration of five to ten spheroids/ml. N-methyl-N-nitroso-N′-guanidine (Aldrich) was dissolved in warm sterile, distilled water and added to the culture at a final concentration of 2 to 3 μg/ml. Mutagenesis was found to be most efficient during early cleavage, which begins eight to ten hrs into the light part of the cycle. The combination of pH8 and aeration decomposes the mutagenic by-products of nitrosoguanidine so that no decontamination procedures were necessary (LAWLEY 1968). Mutagenesis and enrichment for ammonia-requiring mutants were done by D. L. KIRK (personal communication) and differed slightly from the above procedure. Details will appear in a subsequent publication. Some of the mutants reported here were isolated by P. O.'FARRELL (personal communication) using UV mutagenesis (methods not available), while others appeared spontaneously. Because a number of mosaic spheroids were observed in the generation immediately following mutagenesis, mutants were not isolated until the next generation. This allowed the full expression of a mutation in a whole spheroid, but it also added complications. Since a spheroid may contain up to 16 gonidia, a nonmosaic mutation in the first generation would produce as many as 16 identical spheroids in the next generation. Thus, only one isolate of any particular phenotype was retained from a particular mutagenesis in order to avoid re-isolating the same mutation. This mini-jackpot problem also confounds any calculation of mutation rate.

*Enrichment and selection schemes for the isolation of mutants*

*Morphological mutants:* Mutagenized cultures were incubated at 33° to 35° following mutagenesis to allow the expression of temperature-sensitive mutations, as well as any temperature-independent mutations. Each culture was transferred to a sterile petri dish and examined for variant spheroids. Putative mutants were subcloned and incubated to verify the stability of the phenotype. Samples of stable variants were transferred to stock tubes and were tested for temperature sensitivity by subculturing a sample at 24°.

*Enrichment by phototactic selection:* A number of the morphological mutants were found to be inefficient at phototactic movement; therefore, a phototactic selection scheme was used for the isolation of some morphological mutants as well as for mutants with affected flagella, eyespots or general movement. A foil-wrapped sterile chromatography trough (4 × 59 cm) was partly filled with sterile distilled water in a UV-sterilized transfer hood. A mutagenized culture was concentrated by partial filtration, and a sample of the concentrated culture was placed at one end of the trough. The unfiltered beam of a microscope lamp was directed at the opposite end of the trough. After 30 min, the spheroids remaining at the original site of deposition were removed, placed in a petri dish and examined for mutant forms.

*Isolation of temperature sensitive lethals:* After one generation of growth at 24° following mutagenesis, single spheroids of apparent wild-type were isolated and placed in screw-cap vials with Va-8 medium and cultured for two generations. A sample was transferred to another vial and grown at 33° to 35°. Lethality was scored in the succeeding generation. Possible mutants were retested prior to genetic analysis.

*Selection for resistance to methionine sulfoximine:* The minimum lethal concentration of methionine sulfoximine (Sigma), MES, was determined by growing wild-type spheroids in serial ten-fold dilutions of a 0.1M MES stock solution. Wild-type Volvox exhibits normal growth in $10^{-5}$M MES, but becomes colorless and dies in $10^{-4}$M MES. Mutants resistant to MES were selected by adding MES to a mutagenized culture at a final concentration of $5.0 \times 10^{-4}$M. MES-resistant spheroids were isolated from the survivors of the inhibitor treatment. Two explanations for MES toxicity have been postulated: (1) MES may function as a methionine analogue, or (2) MES may inhibit the glutamine synthetase enzyme (CARLSON 1973).

*Sexual inducer:* Preparation of sexual inducer was similar to that of STARR (1969; STARR and JAENICKE 1974). A male strain carrying the *megA1* mutation was used for inducer production because of the high yield. This is due to the 100% sexual response of this strain even in poor

growth conditions. Inducer was titered as described by STARR (1969) and stored in small samples at —4°.

*Mating procedures:* The mating procedures varied somewhat depending on the growth characteristics of the mutants involved. A cross was most successful in terms of zygote production when the parental female sexual spheroids were ready to be released and the parental male sexual spheroids had already been released. If the mutant strains had similar growth rates, 10 to 20 asexual spheroids of each parental type were placed in a 15 × 100mm petri dish containing 30 to 40 ml of Va-8 medium plus 1 ml of sexual inducer that titered at $10^6$. Zygotes with the characteristic red color appeared as early as eight days after mating, but best germination results were obtained by waiting three to four weeks before isolating zygotes. At this time, zygotes in the original cross medium or in sterile water can be stored for several months if left in the dark, but care must be taken to insure that they do not dry out or else the germination frequency is reduced. If the mutant strains had different growth rates, it was necessary to induce the cultures at different times or to reduce the growth rate of the faster growing strain relative to the slower growing strain. In this case each parental strain was grown and induced separately. When the parental cultures had reached the proper stage, as indicated above, 10 to 15 ml of each culture was placed in the same petri dish and incubated. One variation of this technique was to grow each parental strain in 5 ml of Va-8 medium containing inducer and, when the proper sexual stages had been reached, mix the cultures in a petri dish containing 15 to 20 ml of sterile distilled water. This dilution of the culture medium appeared to prolong the fertile period of the sexual female strain.

*Zygote germination*: Drawn-out capillary tubes that were inserted into an adaptor and attached by rubber tubing to a mouthpiece adaptor were used to manipulate zygotes. In order to remove residual inducer and to reduce contamination, zygotes were rinsed by serial transfer through 1 ml aliquots of sterile distilled water or buffer contained in the wells of a nine-spot depression plate. The wash sequence was as follows: serial transfer through four wells with water, transfer to 0.3 M glycylglycine buffer adjusted to pH 10.0, transfer to water, transfer to glycylglycine buffer adjusted to pH 4.0, and transfer to water. Ten to 30 zygotes were then transferred to a well of water containing penicillin (two drops per ml of a 3 μg/ml solution) to reduce bacterial contamination, and incubated beneath the lights for two to three days. This last step increases the synchrony of germination. Each group of zygotes was then transferred to Vm-7 medium containing penicillin in the well of a spot plate. Zygotes germinated after two to four days of continued incubation. The germling colonies were individually transferred to metal-capped tubes containing Va-8 medium plus appropriate nutrients for subsequent growth in preparation for progeny testing. Routinely, 60 to 80% of the zygotes germinated to give germling spheroids, and in most crosses at least 100 germlings were isolated for progeny testing.

*Phenotype determination of progeny:* Morphological mutants were scored in the original germling isolation tubes by microscopic examination of spheroids after one to two generations of growth. Sexual type was determined by placing five to ten spheroids in Va-8 medium containing inducer and scoring for sexual mating type after three to seven days of incubation. MES resistance was determined by placing five to 20 spheroids in a tube with Va-8 medium containing methionine sulfoximine at a concentration of $10^{-4}$M and culturing for four to six days. Growth was scored as resistance; the presence of colorless or dying colonies was scored as sensitivity. Crosses involving ammonia-requiring mutants were tested by placing five to 10 spheroids from the germling tube in Va-8 medium without $NH_4Cl$ supplement and scored for growth or no growth four to six days later. Temperature-conditional mutants were tested by transferring three to five spheroids to a tube containing Va-8 medium, incubating at the appropriate restrictive temperature in an illuminated incubator, and scoring for lethality or mutant phenotype after three to seven days.

## RESULTS

All phases of the Volvox life cycle are haploid except for the zygote, which results from the fusion of egg and sperm. The zygote, which represents an

TABLE 2

*Two-factor crosses among linked genes*

| Group | Parents (Female × Male) | Number of Progeny Parental | Recombinant | Total | Recombination (%) | *P* (<) |
|---|---|---|---|---|---|---|
| I | *eyeB18* × *mtm* | 70 | 1 | 71 | 1.4 | 0.0005 |
| | *q-inv-3* × *mtm* | 69 | 1 | 70 | 1.4 | 0.0005 |
| | *q-invA2* × *mtm* | 81 | 4 | 85 | 4.7 | 0.0005 |
| | *expD6* × *mtm* | 36 | 6 | 42 | 14.3 | 0.0005 |
| | *expD6* × *mtm* | 52 | 5 | 57 | 8.8 | 0.0005 |
| | *expD9* × *mtm* | 31 | 2 | 33 | 6.1 | 0.0005 |
| | *expD9* × *mtm* | 46 | 4 | 50 | 8.0 | 0.0005 |
| | *mtf* × *expD9* | 64 | 6 | 70 | 8.6 | 0.0005 |
| | *mtf* × *expD9* | 462 | 69 | 531 | 13.0 | 0.0005 |
| | *mtf* × *expD9* | 42 | 10 | 52 | 19.2 | 0.0005 |
| | *expD13* × *mtm* | 130 | 10 | 140 | 7.0 | 0.0005 |
| | *invA4* × *mtm* | 45 | 3 | 48 | 12.5* | 0.005 |
| | *mulC302* × *mtm* | 65 | 32 | 97 | 33.0 | 0.001 |
| III | *flgA3* × *mesA1* | 23 | 7 | 97 | 23.0 | 0.001 |
| | *d-disA1* × *mesA1* | 152 | 4 | 156 | 3.0 | 0.0005 |
| | $l^{ts}B3$ × *mesA1* | 269 | 69 | 338 | 20.0 | 0.0005 |
| IV | $l^{ts}A1$ × *expA4* | 241 | 11 | 252 | 8.7* | 0.0005 |
| | *expB3*, *relA1* × *mtm* | 58 | 38 | 92 | 39.6 | 0.05 |
| V | *megA1* × *u-expA2* | 57 | 34 | 91 | 37.4 | 0.025 |
| | *megA1*, *u-expA2* × *mtm* | 30 | 13 | 43 | 30.2 | 0.01 |
| | *megA1* × $l^{ts}D5$ | 45 | 52 | 97 | 53.6 | 0.50 |
| | *u-expA2* × $l^{ts}D5$ | 71* | 72 | 143 | 50.3 | 0.95 |
| VI | *doA2* × *mulD307* | 87 | 17 | 104 | 16.3 | 0.0005 |
| VII | *expC14* × *flgC11* | 79 | 28 | 107 | 26.2 | 0.0005 |
| VIII | *mulB3* × *mulA1* | 56 | 22 | 78 | 28.2 | 0.0005 |
| IX | *nitB3* × *nitA1* | 252 | 13 | 265 | 9.8* | 0.0005 |
| X | *eyeA1* × *frA1* | 70 | 20 | 90 | 22.2 | 0.0005 |

The level of significance, *P*, is based on a $\chi^2$ test assuming equal frequencies of the two classes of progeny (parental and recombinant).

* One of the recombinant classes in these crosses cannot be distinguished from one or both parental classes. The recombinants listed are the + + recovered in the progeny, and the percent recombination was figured by doubling the number of recombinants and dividing by the total. The significance level in these crosses was determined by comparing the results to an expected 3:1 ratio.

encystment-like stage, is more resistant to drying and other harsh environmental conditions. The zygote germinates by meiosis to produce one haploid zoospore and three nonproductive polar bodies (STARR 1975). The zoospore undergoes a series of cleavage divisions to produce a small germling spheroid containing eight gonidia. Thus, unlike Chlamydomonas in which all meiotic products are viable, Volvox crosses are not amenable to tetrad analysis, but cross results can be subjected to random spore analysis.

Each mutant strain was crossed to either a wild-type male or a male strain with known genetic markers. This initial cross was scored for the segregation ratio of the original mutant phenotype in order to determine whether or not the phenotype was due to a single gene mutation. All of the mutants reported here

behave as single gene mutations in crosses; that is, they show a 1:1 segregation with the wild-type phenotype. Some of the mutant strains reported earlier (SESSOMS and HUSKEY 1973) and included in this study contained more than one mutation. In all of these cases, the single gene mutations have been recovered from crosses of the original strain to wild type. Examples of this include *relA1*, which segregated from the original *expB3* isolate, and *flgA3*, which segregated from the *regA106* isolate.

TABLE 3

*Three-factor crosses*

| Linkage group | Parents (Female × Male) | Progeny Classes | | | Number in cross | | $P_T$* (<) |
|---|---|---|---|---|---|---|---|
| I | *mulC302, mtf* × *expD13, mtm* (cross A) | *mul*† | *exp* | *mt* | *A* | *B* | |
| | *expD9, mtf* × *mulC302, mtm* (cross B) | — | + | f | 37 | 9 | |
| | | + | — | m | 35 | 3 | |
| | | + | + | f | 12 | 0 | |
| | | — | — | m | 6 | 0 | |
| | *mulC* —— *expD* —— *mt* | + | — | f | 3 | 31 | |
| | | — | + | m | 4 | 26 | |
| | 21.0 10.0 | | | | | | |
| | | — | — | f | 3 | 13 | |
| | 29.6 12.2 | | | | | | |
| | | + | + | m | 0 | 15 | |
| | | Total | | | 100 | 98 | 0.0005(A) |
| | | | | | | | 0.0005(B) |
| IV | *expA4* × *expB3, relA1* | *expA* | *expB* | *rel* | | | |
| | | — | + | + | 47 | | |
| | | + | — | — | 53 | | |
| | | — | — | — | 55 | | |
| | *expA* —— *expB* —— *relA* | + | + | + | 44 | | |
| | | — | + | — | 54 | | |
| | 43.0 43.0 | | | | | | |
| | | + | — | + | 45 | | |
| | | — | — | + | 21 | | |
| | | + | + | — | 30 | | |
| | | Total | | | 349 | | 0.005 |
| V | $l^{ts}D5$ × *megA1, u-expA2* | *l* | *meg* | *u-exp* | | | |
| | | — | + | + | 6 | | |
| | | + | — | — | 11 | | |
| | | — | — | — | 12 | | |
| | *lD* —— *megA* —— *u-expA* | | | | | | |
| | | + | + | + | 15 | | |
| | 45.7 37.1 | | | | | | |
| | | — | + | — | 13 | | |
| | | + | — | + | 8 | | |
| | | — | — | + | 3 | | |
| | | + | + | — | 2 | | |
| | | Total | | | 70 | | 0.025 |

* Level of significance was determined based on all eight progeny classes (P7).
† (—) = Mutant phenotype; (+) = wild-type phenotype; (f) = female; (m) = male.

The results of 186 crosses are presented in Tables 2, 3 and 4. These crosses allow the tentative designation of 14 linkage groups involving 31 loci. Linkage or nonlinkage of two genetic loci was determined by the level of significance of the deviation among the progeny from the expected segregation pattern of parental and recombinant types as compared to the $\chi^2$ distribution. A deviation with a significant level of 0.05 or less was defined as an indication of linkage (Table 2); a deviation with a significance level of 0.10 or greater was defined as nonlinkage (Table 4).

Based on the two-factor crosses, it is possible to designated nine linkage groups with two or more mutant loci (I and III to X) and five linkage groups consisting of one mutant locus each (II and XI to XIV) (Table 1). The only inconsistencies in linkage assignment using two-factor crosses are in groups IV and V, and these linkage assignments were verified by three-factor crosses.

The three-factor cross involving mutant loci in group I demonstrates the order of the *mulC*, *expD* and *mt loci* (Table 3) and shows recombination frequencies that are consistent with the two-factor data. Attempts were made to order the other three loci in group I, but were not successful due to either low numbers of progeny or an inability to score some of the recombinant classes. It should be noted that *expD13* is temperature sensitive and at 37° has an inversionless phenotype. Since *invA4* has a recombination frequency with the *mt* locus that is similar to the *expD* mutants, these four mutants could be at the same locus, with *invA4* having the most extreme phenotype for a mutation at the locus.

The three-factor cross of mutant loci in group IV shows that *expA4* is linked to the *expB3*, *relA1* groups. Enough progeny were recovered in this cross to calculate an interference index number of 0.78, indicating a small amount of positive interference. *l*$^{ts}$*A1* has not been mapped relative to these three loci.

The three-factor cross of group V loci confirms the linkage of *megA1* and *u-expA2*, with a recombination value similar to that of the two factor crosses. In addition, *l*$^{ts}$*D5* is apparently linked to the other two markers, even though the frequency of recombination is essentially 50%. This loose linkage is best seen by comparing the ratio of mutant to wild-type alleles for *l*$^{ts}$*D5* among *megA*$^+$, *u-expA*$^-$ recombinants (6.50) to that ratio among *mega*$^-$, *u-expA*$^+$ recombinants (0.38). If *l*$^{ts}$*D5* were not linked to the other markers, both ratios would be equal to unity.

Due to the fact that the only diploid stage is the zygote, it is not possible, as yet, to perform complementation tests on mutants with similar phenotypes or to determine dominance relationships of any of the mutants. For this reason, it has been impossible to define mutations as allelic or not by a functional test. Instead, independently isolated mutations with identical phenotypes have been operationally designated as allelic if no wild-type recombinants were recovered in a cross of the mutant strains in which there were at least 100 progeny. In Table 5 are the results of crosses demonstrating allelism at seven loci. Partial corroboration of the allelic nature of the three *expD* mutants is demonstrated by the fact that they show similar recombination frequencies with the *mt* locus (Table 2), and two of the alleles occupy similar positions in the linkage group (Table 3).

TABLE 4

*Two-factor crosses among unlinked genes*

| Linkage groups | Parental configuration (Female × Male) | Progeny classes A | B | C | D | Total | $P<$ |
|---|---|---|---|---|---|---|---|
| I, II | *regA* × *mtm* | 528 | 538 | 552 | 530 | 2148 | 0.90 |
| I, III | *mesA1* × *mtm* | 30 | 24 | 26 | 39 | 119 | 0.30 |
| I, IV | *expA4* × *mtm* | 43 | 43 | 47 | 42 | 175 | 0.95 |
| I, IV | *relA1* × *megA1* | 39 | 38 | 31 | 40 | 148 | 0.80 |
| I, V | *megA1* × *mtm* | 22 | 48 | 21 | | 91 | 0.90† |
| I, V | *u-expA2* × *mtm* | 37 | 40 | 43 | 34 | 154 | 0.90 |
| I, V | *megA1, flgC11* × *l$^{ts}$D5** | 52 | 39 | 45 | 45 | 181 | 0.70 |
| I, VI | *mulD307* × *mtm* | 21 | 24 | 26 | 19 | 90 | 0.80 |
| I, VI | *doA2* × *mesA1* | 13 | 16 | 13 | 11 | 53 | 0.90 |
| I, VII | *megA1* × *flgC11** | 22 | 33 | 27 | 29 | 111 | 0.60 |
| I, VII | *expC11* × *mtm* | 29 | 23 | 31 | 22 | 106 | 0.60 |
| I, VIII | *mulA1* × *mtm* | 30 | 23 | 27 | 36 | 116 | 0.40 |
| I, VIII | *mulB3* × *mtm* | 18 | 28 | 14 | 14 | 74 | 0.80 |
| I, IX | *nitA1* × *mtm* | 42 | 34 | 42 | 35 | 153 | 0.70 |
| I, IX | *nitB3* × *mtm* | 21 | 24 | 31 | 31 | 107 | 0.50 |
| I, X | *megA1* × *frA1** | 39 | 38 | 39 | 30 | 146 | 0.70 |
| I, X | *eyeA1* × *mtm* | 21 | 30 | 17 | 26 | 94 | 0.30 |
| I, XI | *nitC19* × *mtm* | 45 | 45 | 43 | 32 | 165 | 0.50 |
| I, XII | *rotA1* × *mtm* | 72 | 87 | 65 | 76 | 300 | 0.40 |
| I, XIII | *l$^{ts}$C4** × *mesA1* | 30 | 22 | 34 | 30 | 116 | 0.50 |
| I, XIV | *sex$^{c}$A*, sicA* × *mtm* | 95 | | | 103 | 198 | 0.60‡ |
| II, III | *regA* × *mesA1* | 298 | 267 | 319 | 270 | 1154 | 0.10 |
| II, IV | *regA101* × *expA4* | 127 | 68 | | 53 | 248 | 0.40† |
| II, V | *regA* × *megA1* | 26 | 27 | 30 | 32 | 115 | 0.90 |
| II, V | *regA125* × *u-expA2* | 182 | 88 | | 97 | 367 | 0.80† |
| II, V | *regA106* × *l$^{ts}$D5* | 67 | 40 | | 33 | 140 | 0.70† |
| II, VI | *regA* × *mulD307* | 89 | 57 | | 48 | 194 | 0.40† |
| II, VII | *regA* × *expC11* | 115 | 48 | | 58 | 221 | 0.60† |
| II, VIII | *regA* × *mulB3* | 163 | 64 | | 71 | 298 | 0.30† |
| II, IX | *nitA1* × *regA* | 17 | 19 | 19 | 18 | 73 | 0.99 |
| II, X | *regA* × *frA1* | 25 | 28 | 27 | 28 | 108 | 0.99 |
| II, X | *eyeA1* × *regA145* | 34 | 29 | 20 | 28 | 111 | 0.30 |
| II, XI | *nitC12* × *regA245* | 31 | 35 | 33 | 31 | 130 | 0.975 |
| II, XII | *regA* × *rotA1* | 117 | 103 | 104 | 115 | 439 | 0.70 |
| II, XIII | *regA102* × *l$^{ts}$C4* | 75 | 24 | | 28 | 127 | 0.20† |
| II, XIV | *sex$^{c}$A, sicA* × *regA* | 123 | 127 | 113 | 120 | 483 | 0.90 |
| III, IV | *expA4* × *mesA1* | 12 | 16 | 24 | 13 | 65 | 0.20 |
| III, IV | *relA1* × *mesA1* | 39 | 31 | 22 | 25 | 117 | 0.20 |
| III, V | *mesA1* × *megA1* | 58 | 60 | 48 | 61 | 227 | 0.60 |
| III, V | *mulB3, mesA1** × *u-expA2** | 12 | 9 | 18 | 11 | 50 | 0.40 |
| III, V | *mesA1* × *l$^{ts}$D5* | 19 | 23 | 27 | 15 | 84 | 0.30 |
| III, VI | *mulD307*, mulB3* × *mesA1** | 32 | 29 | 31 | 28 | 120 | 0.975 |
| III, VI | *doA2* × *mesA1* | 16 | 13 | 8 | 15 | 52 | 0.50 |
| III, VII | *mulB3, mesA1** × *flgC11* | 37 | 23 | 22 | 36 | 118 | 0.10 |
| III, VII | *expC14** × *expA4, mesA1** | 17 | 14 | 13 | 15 | 59 | 0.90 |
| III, VIII | *mesA1* × *mulA1* | 23 | 30 | 23 | 21 | 96 | 0.60 |

TABLE 4—Continued

| Linkage groups | Parental configuration (Female × Male) | Progeny classes A | B | C | D | Total | $P<$ |
|---|---|---|---|---|---|---|---|
| III, VIII | *mulB3*, mesA1* × flgC11* | 34 | 24 | 35 | 25 | 118 | 0.40 |
| III, IX | *nitB3 × mesA1* | 25 | 21 | 20 | 34 | 100 | 0.20 |
| III, X | *frA1 × mesA1* | 14 | 10 | 7 | 10 | 41 | 0.50 |
| III, X | *eyeA1 × mesA1* | 12 | 16 | 11 | 15 | 54 | 0.80 |
| III, XI | *nitC19* × mesA1*, megA1, expA4* | 32 | 38 | 31 | 41 | 142 | 0.60 |
| III, XII | *mesA1*, expA4 × rotA1** | 29 | 29 | 31 | 35 | 124 | 0.90 |
| III, XIII | *$l^{ts}$C4 × mesA1* | 31 | 21 | 33 | 31 | 116 | 0.40 |
| III, XIV | *$sex^c$A*, sicA × mesA1** | | 47 | | 38 | 85 | 0.95‡ |
| IV, V | *megA1 × expA4* | 43 | 50 | 38 | 40 | 171 | 0.60 |
| IV, V | *relA1 × megA1* | 39 | 31 | 22 | 25 | 117 | 0.20 |
| IV, V | *u-expA2 × expA4* | 21 | 29 | 19 | 22 | 91 | 0.50 |
| IV, V | *relA1 × u-expA2* | 6 | 16 | 12 | 11 | 45 | 0.20 |
| IV, V | *$l^{ts}$D5 × expA4* | 74 | 34 | | 40 | 148 | 0.80† |
| IV, V | *relA1 × $l^{ts}$D5* | 44 | 35 | 34 | 42 | 155 | 0.60 |
| IV, VI | *mulD307 × expA4* | 26 | 20 | 22 | 22 | 90 | 0.90 |
| IV, VI | *mulD307*, relA1* × rotA1* | 56 | 55 | 62 | 66 | 239 | 0.80 |
| IV, VI | *doA2 × expA4* | 27 | 18 | 16 | 18 | 79 | 0.30 |
| IV, VII | *$sex^c$B × expA4*, flgC11*, mesA1* | 20 | 21 | 27 | 23 | 91 | 0.80 |
| IV, VII | *relA1 × flgC11* | 17 | 22 | 28 | 28 | 95 | 0.40 |
| IV, VII | *expC14* × expA4*, mesA1* | 21 | 13 | 8 | 16 | 58 | 0.20 |
| IV, VII | *relA1 × expC14* | 19 | 19 | 23 | 24 | 85 | 0.90 |
| IV, VIII | *expA4 × mulA1* | 24 | 35 | 19 | 22 | 100 | 0.20 |
| IV, VIII | *relA1 × mulA1* | 11 | 18 | 17 | 15 | 61 | 0.60 |
| IV, VIII | *mulB3 × expA4* | 21 | 20 | 23 | 20 | 84 | 0.975 |
| IV, IX | *nitA1 × expA4* | 34 | 43 | 34 | 30 | 141 | 0.50 |
| IV, IX | *relA1 × nitA1* | 23 | 29 | 26 | 32 | 110 | 0.70 |
| IV, X | *frA1 × expA4* | 41 | 32 | 28 | 33 | 134 | 0.50 |
| IV, X | *relA1 × frA1* | 12 | 16 | 12 | 13 | 53 | 0.90 |
| IV, X | *eyeA1 × expA4* | 30 | 31 | 36 | 31 | 128 | 0.90 |
| IV, XI | *nitC19* × mesA1, expA4*, megA1* | 37 | 38 | 29 | 40 | 144 | 0.60 |
| IV, XI | *nitC12 × relA1* | 21 | 28 | 18 | 23 | 90 | 0.50 |
| IV, XII | *mesA1, expA4* × rotA1** | 33 | 36 | 27 | 28 | 124 | 0.70 |
| IV, XII | *relA1 × rotA1* | 28 | 25 | 21 | 25 | 99 | 0.90 |
| IV, XIII | *relA1 × $l^{ts}$C4* | 29 | 39 | 38 | 38 | 144 | 0.70 |
| IV, XIV | *expA4 × $sex^c$A1* | 25 | | | 27 | 52 | 0.80‡ |
| IV, XIV | *relA1 × $sex^c$A1* | 23 | | | 20 | 43 | 0.70‡ |
| V, VI | *megA1 × mulD307* | 47 | 88 | | 55 | 190 | 0.50† |
| V, VI | *mulD307*, relA1 × u-expA2** | 160 | 69 | | 70 | 299 | 0.50† |
| V, VI | *doA2 × $l^{ts}$D5* | 36 | 42 | 44 | 33 | 155 | 0.60 |
| V, VII | *megA1 × flgC11* | 28 | 29 | 28 | 26 | 111 | 0.99 |
| V, VII | *u-expA2 × flgC11* | 36 | 32 | 30 | 47 | 145 | 0.20 |
| V, VII | *$l^{ts}$D5 × flgC11* | 25 | 23 | 23 | 30 | 101 | 0.80 |
| V, VIII | *mulB3 × megA1* | 34 | 30 | 39 | 35 | 138 | 0.80 |
| V, VIII | *mulB3 × u-expA2* | 39 | 42 | 27 | 31 | 139 | 0.30 |
| V, VIII | *mulB3 × $l^{ts}$D5* | 23 | 31 | 23 | 25 | 101 | 0.60 |
| V, IX | *nitA1 × megA1* | 29 | 27 | 23 | 28 | 107 | 0.90 |
| V, IX | *u-expA2 × nitA1* | 14 | 13 | 15 | 16 | 58 | 0.975 |
| V, IX | *nitA1 × $l^{ts}$D5* | 21 | 24 | 32 | 18 | 95 | 0.30 |

TABLE 4—Continued

| Linkage groups | Parental configuration (Female × Male) | Progeny classes A | B | C | D | Total | $P<$ |
|---|---|---|---|---|---|---|---|
| V, X | *megA1* × *frA1* | 18 | 16 | 23 | 21 | 78 | 0.70 |
| V, X | *frA1**, *flgC11* × *l$^{ts}$D5** | 17 | 16 | 14 | 21 | 68 | 0.70 |
| V, X | *eyeA1* × *l$^{ts}$D5* | 11 | 13 | 14 | 11 | 49 | 0.95 |
| V, XI | *nitC19** × *mesA1, expA4, megA1** | 21 | 23 | 15 | 15 | 74 | 0.50 |
| V, XI | *u-expA2**, *megA1* × *nitC12* | 21 | 30 | 18 | 21 | 90 | 0.40 |
| V, XI | *l$^{ts}$D5* × *nitC19* | 47 | 43 | 42 | 41 | 173 | 0.95 |
| V, XII | *megA1* × *rotA1* | 47 | 42 | 37 | 47 | 173 | 0.70 |
| V, XII | *rotA1* × *u-expA2* | 44 | 42 | 36 | 31 | 153 | 0.50 |
| V, XII | *l$^{ts}$D5* × *rotA1* | 17 | 25 | 24 | 25 | 91 | 0.60 |
| V, XIII | *megA1* × *l$^{ts}$C4* | 39 | 40 | 45 | 43 | 167 | 0.95 |
| V, XIII | *l$^{ts}$C4* × *u-expA2* | 14 | 19 | 16 | 7 | 46 | 0.30 |
| V, XIII | *l$^{ts}$D5* × *l$^{ts}$C4* | 132 | | | 35 | 167 | 0.30§ |
| V, XIV | *sex$^{c}$A**, *sicA* × *megA1** | 64 | | 58 | | 122 | 0.60‡ |
| V, XIV | *sex$^{c}$A**, *sicA* × *u-expA2** | | 18 | | 19 | 37 | 0.90‡ |
| V, XIV | *sex$^{c}$A**, *sicA* × *l$^{ts}$D5** | | 51 | | 61 | 112 | 0.40‡ |
| VI, VII | *flgC11* × *mulD307* | 35 | 25 | 24 | 47 | 131 | 0.90‖ |
| VI, VII | *flgC11* × *doA2**, *l$^{ts}$C4* | 32 | 26 | 38 | 35 | 131 | 0.50 |
| VI, VII | *doA2* × *expC14* | 25 | 20 | 25 | 35 | 105 | 0.30 |
| VI, VIII | *mulD307**, *mulB3** × *frA1, mesA1* | 145 | 154 | 172 | 182 | 653 | 0.20 |
| VI, IX | *nitB3* × *mulD307* | 38 | 34 | 31 | 36 | 139 | 0.90 |
| VI, X | *mulD307**, *mulB3* × *frA1** | 64 | 48 | 46 | 46 | 204 | 0.30 |
| VI, XI | *doA2* × *nitC12* | 37 | 37 | 34 | 39 | 147 | 0.95 |
| VI, XII | *mulD307**, *relA1* × *rotA1** | 47 | 61 | 57 | 60 | 225 | 0.60 |
| VI, XIII | *mulD307**, *mulB3* × *l$^{ts}$C4** | 61 | 72 | 66 | 59 | 258 | 0.70 |
| VI, XIII | *doA2* × *l$^{ts}$C4* | 82 | 85 | 86 | 69 | 322 | 0.60 |
| VI, XIV | *sex$^{c}$A**, *sicA* × *mulD307** | 42 | 79 | | 40 | 161 | 0.95§ |
| VII, VIII | *mulB3* × *flgC11* | 96 | 75 | 73 | 86 | 330 | 0.30 |
| VII, IX | *nitA1* × *flgC11* | 33 | 20 | 21 | 23 | 97 | 0.30 |
| VII, X | *frA1* × *flgC11* | 25 | 18 | 20 | 27 | 90 | 0.50 |
| VII, X | *eyeA1* × *flgC11* | 53 | 36 | | 28 | 117 | 0.40† |
| VII, XI | *flgC11**, *frA1* × *nitC12** | 47 | 54 | 48 | 58 | 207 | 0.70 |
| VII, XII | *rotA1* × *flgC11* | 29 | 28 | 27 | 31 | 115 | 0.975 |
| VII, XIII | *flgC11* × *l$^{ts}$C4* | 35 | 27 | 39 | 40 | 141 | 0.40 |
| VII, XIV | *sex$^{c}$A**, *sicA* × *flgC11** | 5 | 13 | 4 | 7 | 29 | 0.20 |
| VIII, IX | *mulB3* × *nitA1* | 45 | 39 | 37 | 32 | 153 | 0.60 |
| VIII, X | *mulB3* × *frA1* | 39 | 41 | 39 | 39 | 158 | 0.99 |
| VIII, X | *eyeA1* × *mulB3* | 21 | 18 | 25 | 23 | 87 | 0.80 |
| VIII, XI | *nitC12* × *mulB3* | 19 | 13 | 22 | 13 | 67 | 0.40 |
| VIII, XII | *mulB3* × *rotA1* | 75 | 73 | 60 | 80 | 288 | 0.40 |
| VIII, XIII | *mulD307, mulB3** × *l$^{ts}$C4** | 68 | 71 | 67 | 52 | 258 | 0.40 |
| VIII, XIV | *sex$^{c}$A1**, *sicA* × *mulB3** | 38 | 34 | 46 | 52 | 170 | 0.30 |
| IX, X | *nitB3* × *frA1* | 19 | 24 | 22 | 19 | 84 | 0.90 |
| IX, X | *eyeA1* × *nitA1* | 16 | 8 | 9 | 8 | 41 | 0.30 |
| IX, XI | *nitC19* × *nitA1* | 47 | | | 19 | 66 | 0.50§ |
| IX, XII | *rotA1* × *nitA1* | 32 | 25 | 30 | 31 | 118 | 0.90 |

TABLE 4—Continued

| Linkage groups | Parental configuration (Female × Male) | Progeny classes A | B | C | D | Total | $P<$ |
|---|---|---|---|---|---|---|---|
| IX, XII | *nitA1* × *l$^{ts}$C4* | 29 | 34 | 40 | 35 | 137 | 0.60 |
| IX, XIV | *sex$^{c}$A**, *sicA* × *nitA1** | | 41 | | 46 | 87 | 0.60‡ |
| X, XI | *flgC11*, *frA1** × *nitC19** | 45 | 34 | 47 | 41 | 177 | 0.50 |
| X, XI | *eyeA1* × *nitC12* | 44 | 43 | 30 | 35 | 152 | 0.40 |
| X, XII | *rotA1* × *frA1* | 47 | 41 | 40 | 46 | 174 | 0.90 |
| X, XII | *eyeA1* × *rotA1* | 43 | 33 | | 18 | 94 | 0.10† |
| X, XIII | *frA1**, *flgC11* × *l$^{ts}$C4** | 37 | 57 | 43 | 63 | 200 | 0.40‖ |
| X, XIII | *eyeA1* × *l$^{ts}$C4* | 31 | 22 | 29 | 24 | 106 | 0.60 |
| X, XIV | *sex$^{c}$A1**, *sicA* × *frA1** | | 21 | | 23 | 44 | 0.80‡ |
| XI, XII | *rotA1* × *nitC12* | 29 | 20 | 24 | 30 | 103 | 0.50 |
| XI, XIII | *l$^{ts}$C4* × *nitC12* | 65 | 58 | 52 | 61 | 236 | 0.70 |
| XI, XIV | *sex$^{c}$A1**, *sicA* × *nitC12** | | 52 | | 50 | 102 | 0.99‡ |
| XII, XIII | *l$^{ts}$C4* × *rotA1* | 28 | 43 | 47 | 29 | 147 | 0.70‖ |
| XII, XIV | *sex$^{c}$A1**, *sicA* × *rotA1** | 29 | 34 | 29 | 19 | 111 | 0.30 |
| XIII, XIV | *sex$^{c}$A1**, *sicA* × *l$^{ts}$C4** | 85 | 40 | | 43 | 168 | 0.95† |

The progeny classes are as follows: A, progeny of the female parental phenotype; B, progeny of the male parental phenotype; C, progeny of the double-mutant phenotype or, in Group I crosses, recombinant female progeny; D, progeny of the wild-type phenotype or, in Group I crosses, recombinant male progeny. Unless otherwise indicated, a $\chi^2$ value was calculated based on equal size of all four classes. This value was used to determine the level of significance, $P$.

* Indicates the mutant type scored when a multiple mutant strain was used in the cross.

† Due to epistatic interactions, two of the progeny classes are indistinguishable. The expected values were calculated using a 2:1:1 ratio with two degrees of freedom.

‡ Two of the progeny classes, one parental and one recombinant, either could not be scored or could not be recovered. The expected values were calculated using a 1:1 ratio with one degree of freedom.

§ The two parental classes and the double recombinant classes are indistinguishable. The expected values are calculated using a 3:1 ratio with one degree of freedom.

‖ Due to unequal recovery of one marker, the two nonrecombinant progeny classes were summed as were the two recombinant progeny classes. The expected values were calculated using a 1:1 ratio with one degree of freedom.

TABLE 5

*Crosses demonstrating allelism*

| Locus | Number of alleles | Number of crosses | Total progeny | Frequency of wild-type recombinants (%) |
|---|---|---|---|---|
| *regA* | 40 | 37 | 9476 | 0.01 |
| *megA* | 5 | 4 | 709 | <0.10 |
| *nitA* | 15 | 15 | 1399 | 0.07 |
| *nitC* | 5 | 5 | 324 | <0.30 |
| *expD* | 3 | 2 | 270 | <0.40 |
| *mulC* | 3 | 2 | 207 | <0.50 |
| *mulD* | 3 | 2 | 224 | <0.40 |

## DISCUSSION

The identification of 14 linkage groups is consistent with the observation of a haploid number of 14 chromosomes in *V. carteri* f. *nagariensis* (STARR, personal communication). This does not necessarily mean that each of the linkage groups is on a separate chromosome. As is evident from the crosses involving linkage group V, it is quite possible for two mutant loci to show apparent independent assortment and still occur on the same chromosome (see Table 2 and 3). Without resorting to very large numbers of progeny, the establishment of linkage by two-factors crosses becomes problematical when linked markers show 35% or more recombination. For this reason, the indicated linkage assignments must remain provisional until a complete set of three-factor crosses among all groups can be completed. And these definitive mapping crosses must await the isolation of new mutant loci that will allow the recovery of all recombinant classes.

The recombination data do show that Volvox is similar to many other organisms in terms of basic genetic processes. The recovery of equal numbers of mutant and wild-type alleles indicates that there is no apparent meiotic drive or selection. Unlinked genes assort independently in two-, three- and four-factor crosses. The frequency of recombination between linked genes is unaffected by marker coupling, and positive interference occurs between recombination events in neighboring regions. This means that a genetic approach to the solution of developmental problems using Volvox as a model system is quite reasonable. The only difficulty at present is the absence of a prolonged diploid stage necessary for dominance and complementation studies.

The use of a recombinational definition for allelism is less than perfect, but represents the only presently existing mechanism for allele designation in Volvox. This type of test could give false results for several reasons. For example, there is no way as yet to determine the relationship between recombination frequency and physical distance in Volvox, and it would not be wise to use such relationships as have been computed for other organisms. Thus, the use of a fixed recombination frequency, such as 1% or less, to determine allelism could be either too large or too small although the maximum intragenic recombination frequency seen in Chlamydomonas is 1.6% (MATAGNE 1978). Furthermore, if any of the mutants used as tester alleles were to contain multi-site mutations within the gene, intragenic recombination could occur without being detected, although judicious selection of mutants could overcome this difficulty. Finally, this method will not work for certain Volvox mutants that can not be crossed when carried in the male strain. This problem is easiest to visualize with the *flg* mutants, which produce nonmotile sperm, and the *rel* mutant, which does not release the sperm packets.

If genes of similar function are clustered in Volvox, false allelism by a recombination test would be a distinct possibility. The mutants isolated suggest that gene clustering is not readily apparent and that Volvox is similar to Chlamydomonas is having genes of similar function spread throughout the genome (LEVINE and GOODENOUGH 1970). In Volvox, the three *nit* genes are found on two linkage groups with about 10% recombination between the two linked

genes. The four mapped *exp* genes are not clustered, and two additional, partially mapped *exp* mutants (data not presented) are not linked to the *expA-D* loci. Furthermore, the three *sex*$^{c}$ mutants are not clustered even though they affect a specific developmental pathway and could be of a regulatory nature.

None of the mutants isolated exhibited strict non-Mendelian transmission patterns. Since the sperm is very small compared to the egg, it might be expected that if nonchromosomal markers had been isolated, they would show transmission to progency when carried in a female strain and no transmission to progeny when carried in the male strain. Several nonchromosomal drug-resistant mutants exist in Chlamydomonas (SAGER 1972) and Eudorina (MISHRA and THRELKELD 1968), which prompted a search for streptomycin-resistant mutants in Volvox; however, no such mutants were recovered despite numerous attempts. This failure could be due to the fact that effective NG mutagenesis requires DNA synthesis, and the bulk of Volvox chloroplast DNA is not synthesized during early cleavage but during the pre-release expansion phase (MARGOLIS-KAZAN and BLAMIRE 1976). Since the mutageneses reported here were performed during cleavage, it is possible that chloroplast DNA was not affected.

Although the mutagenized cultures contained gonidia in the early cleavage stages, it was not possible to guarantee that all gonidia were cleaving or that all embryos were in the same cleavage stage. This difficulty makes it virtually impossible to determine the rate of killing or the rate of mutagenesis. In addition, it was observed that the mutagenized cultures exhibited large variations in the numbers of gonidia per spheroid, which would introduce further errors into rate calculations. Finally, the occurrence of partially mutant or lethal colonies reinforced the problem of quantitating survival. For these reasons, no data are presented concerning the rate of killing or of mutagenesis.

One of the primary goals of measuring the rate of mutagenesis and killing is to determine the dose necessary to maximize the recovery of mutant types without generating multiple mutations. The NG mutagenesis procedure of SESSOMS and HUSKEY (1973) used a much higher dose than is reported here and a lower pH for the culture medium. Both of these factors should result in a higher mutagenic rate. In fact, several of the mutant strains reported earlier were found to be multiple mutants involving several separate genetic loci. It could be argued that a rate of mutagenesis that produces simultaneous mutations in separate genes would have a high probability of producing multiple mutations in the same gene or in neighboring genes. This would suggest that some of the mutants isolated in this study may not be due to single base changes. However, the fact that 11 of the mutants isolated are temperature conditional argues for a single-event mutation in those cases at least. The important observation is that it is possible to isolate stable genetic variants in Volvox, despite problems in measuring killing rates or mutagenic rates. The 33 mutant loci can be grouped into 19 phenotypic categories, and using the existing mutants as models, it should be possible to design more effective screens to isolate specific mutants following mutagenesis with other agents.

The genetic analysis of the Volvox mutants has suggested a number of avenues of research concerning regulation, differentiation and morphogenesis. The application of genetic, biochemical and biophysical techniques to the study of Volvox should provide information that will be useful in the study of higher organisms.

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## LITERATURE CITED

CARLSON, P., 1973 Methionine sulfoximine resistant mutants of tobacco. Science **180:** 1366–1368.

KIRK, D. L. and M. M. KIRK, 1976 Protein synthesis in *Volvox carteri* f. *nagariensis*. Develop. Biol. **50:** 413–427.

KOCHERT, G. and L. W. OLSON, 1970 Ultrastructure of *Volvox carteri*. I. The asexual colony. Arch. Mikrobiol. **74:** 19–30.

KOCHERT, G. and N. SANSING, 1971 Isolation and characterization of nucleic acids from *Volvox carteri*. Biochim. Biophys. Acta. **238:** 397–405.

KOCHERT, G. and I. YATES, 1974 Purification and partial characterization of glycoprotein sexual inducer from *Volvox carteri*. Proc. Natl. Acad. Sci. U.S. **71:** 1211–1214.

LAWLEY, P. D., 1968 Methylation of DNA by N-methyl-N-nitrosourethan and N-methyl-N-nitroso-N′-guanidine. Nature **218:** 580–581.

LEVINE, R. P. and U. W. GOODENOUGH, 1970 The genetics of photosynthesis and of the chloroplast in *Chlamydomonas reinhardi*. Ann. Rev. Genet. **4:** 397–408.

MARGOLIS-KAZAN, H. and J. BLAMIRE, 1976 The DNA of *Volvox carteri*: a biophysical and biosynthetic characterization. Cytobios **15:** 201–216. ——, 1977 The effect of Δ-tetrahydrocannobinol on cytoplasmic DNA metabolism. Biochem. Biophys. Res. Comm. **76:** 674–681.

MATAGNE, R. F., 1978 Fine structure of the *arg-7* cistron in *Chlamydomonas reinhardi*. Molec. Gen. Genet. **160:** 95–99.

MISHRA, N. C. and S. F. H. THRELKELD, 1968 Geneitc studies in *Eudorina*. Genet. Res. **11:** 21–31.

OLSON, L. W. and G. KOCHERT, 1970 Ultrastructure of *Volvox carteri*. II. The kinetosome. Arch. Mikrobiol. **74:** 31–40.

PALL, M. L., 1975 Mutants of Volvox showing premature cessation of division: Evidence for a relationship between cell size and reproductive cell differentiation. In: *Developmental Biology: Pattern Formation, Genet Regulation*. Vol. 2, ICN-UCLA Symposia on Molecular and Cellular Biology. Edited by D. MCMAHON and C. F. Fox. W. A. Benjamin, Menlo Park, California.

SAGER, R., 1972 *Cytoplasmic Genes and Organelles*. Academic Press, New York.

SESSOMS, A. H., 1974 The isolation and characterization of morphogenetic mutants of *Volvox carteri* f. *nagariensis* Iyengar. Ph.D. dissertation, University of Virginia.

SESSOMS, A. H. and R. J. HUSKEY, 1973 Genetic control of development in *Volvox*; isolation and characterization of morphogenetic mutants. Proc. Natl. Acad. Sci. U.S. **70:** 1335–1338.

STARR, R. C., 1969 Structure, reproduction and differentiation in *Volvox carteri* f. *nagariensis* Iyengar, strains HK9 & 10. Arch Protistenk. **111**: 204–222. ——, 1970 Control of differentiation in *Volvox*. In: *Changing Synthesis in Development*. Edited by M. N. RUNNER. Academic Press, New York. ——, 1975 Meiosis in *Volvox carteri* f. *nagariensis*. Arch. Protistenk. **117**: 187–191.

STARR, R. C. and L. JAENICKE, 1974 Purification and characterization of the hormone initiating sexual morphogenesis in *Volvox carteri* f. *nagariensis* Iyengar. Proc. Natl. Acad. Sci. U.S. **71**: 1050–1054.

VIAMONTES, G. I. and D. L. KIRK, 1977 Cell shape changes and the mechanism of inversion in *Volvox*. J. Cell Biol. **75**: 719–730.

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