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Effect of ultraviolet light on reproduction in *Hydra littoralis*

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The University of Arizona, 1989

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EFFECT OF ULTRA VIOLET LIGHT ON REPRODUCTION IN *HYDRA LITTORALIS*

by

LOREN GUERRERO LADIN

A Thesis Submitted to the Faculty of the

DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY

In Partial Fulfillment of the Requirements
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1989

STATEMENT BY AUTHOR

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7/24/89
Date

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The "DNA Damage Hypothesis" pertaining to the evolution of sex was tested using Hydra littoralis. DNA damage was produced by irradiating whole live hydra with ultra violet light. A curve of uv light dosage vs. survival was constructed. Estimations of threshold fluence and LD50 were made from the survival curve. In four separate experiments, using various combinations of environmental temperatures, uv doses, and number of doses, frequencies of asexual and sexual reproduction were observed and compared. The hydra that recieved uv treatments did not show an increase in the consequent amount of sexual reproduction, and actually showed a decrease. An increase in the amount of sexual reproduction following DNA damage is predicted by the DNA damage hypothesis, therefore these results do not support this theory. The data was also used to make contradictory observations regarding the "stress hypothesis" for the occurance of sexual reproduction in hydra.

INTRODUCTION

In order to explain the maintainence of sex in a particular group of organisms, it is essential to examine what type of reproduction occurs in that organism. For example, there is a difference between competition between obligate sexual reproduction and obligate asexual reproduction (parthenogenesis or fission) and with facultative asexual reproduction (such as the case of hydra and many other cnidarians). There is also a difference between competition between outbreeding sex and obligate inbreeders and facultative inbreeders (as is probably the case with hydra). Selection for facultative asexual reproduction is an intrapopulation process requiring constantly intensive selection (Kondrashov,1985). This is in contrast to selection against obligate asexual reproduction or obligate inbreeding which may have to be explained by group selection, or what may be more appropriately called "moderate-time selection", involving more than one

generation (Kondrashov, 1985). Is it possible then to determine what is driving selection for the maintainence of sex at the intrapopulation level in the case of Hydra littoralis?

The factors that initiate sexual reproduction in freshwater hydra are not well understood. Asexual reproduction by fission (budding) is adequate for at least one of hydras cnidarian relatives, the sea anemone Haliplanella luciae (Schick, Hoffman, and Lamb, 1979), which is known to reproduce only by asexual means. In this study I have tested the "DNA damage hypothesis" (Bernstein and Michod, 1985), having initially wondered if it was not DNA damage and the need to repair this damage that was driving selection to maintain sexual reproduction in Hydra littoralis at the intrapopulation level. In the case of hydra, it did not seem necessary to invoke group selection arguments, such as those that will be reviewed in the next section of this manuscript. Sexual reproduction must confer some immediate advantage to the parental genotype since each individual has the option of reproduction by

undergoing somatic division. Asexual reproduction in hydra apparently eliminates senesence (Strehler, Crowell, 1961). Hydra is occasionally compelled for environmental reasons, and not always internal, cyclic or seasonal reasons, to produce gonads and form gametes from cells otherwise destined to be somatic cells. At an increased energy expenditure to the parent polyp, which in extreme cases may perish from sexual reproduction, "terminal sexuality" (Burnett, 1961), the production of gametes must be significant to the survival of the parent genotype.

In the following section, I will briefly describe some arguments for the maintainence of sex, and for the initiation of sex in hydra. At this point, I tend to suspect that sexual reproduction confers some evolutionary advantage to those organisms that employ it, as opposed to sexual reproduction being maintained as a by-product of meiotic DNA repair, as in accord with the DNA damage hypothesis. Although I do tend to agree with the idea that DNA mutation, and or damage, is important to the evolution of sex.

The following statement from Maynard Smith (1986) that "the main theoretical explainations have already been formulated, and our task is to decide their relative importance", may be accurate although it suggests an unnatural ability to foresee future development in this line of study. The present study is a part of this task to decide the relative importance of existing theories.

In the case of hydra, where there exist so many explanations for the persistence of sexual reproduction with very little evidence besides anecdotal evidence to support any one explanation, it is especially intriguing to sift through the peculiarities of the modes of reproduction of these animals, and thus join the ranks of people who are curious about them.

The experiment that will be described in this manuscript is primarily an inquiry of the effects of ultra violet light on the process of sexual reproduction in hydra, and what that might suggest about sexual reproduction in general.

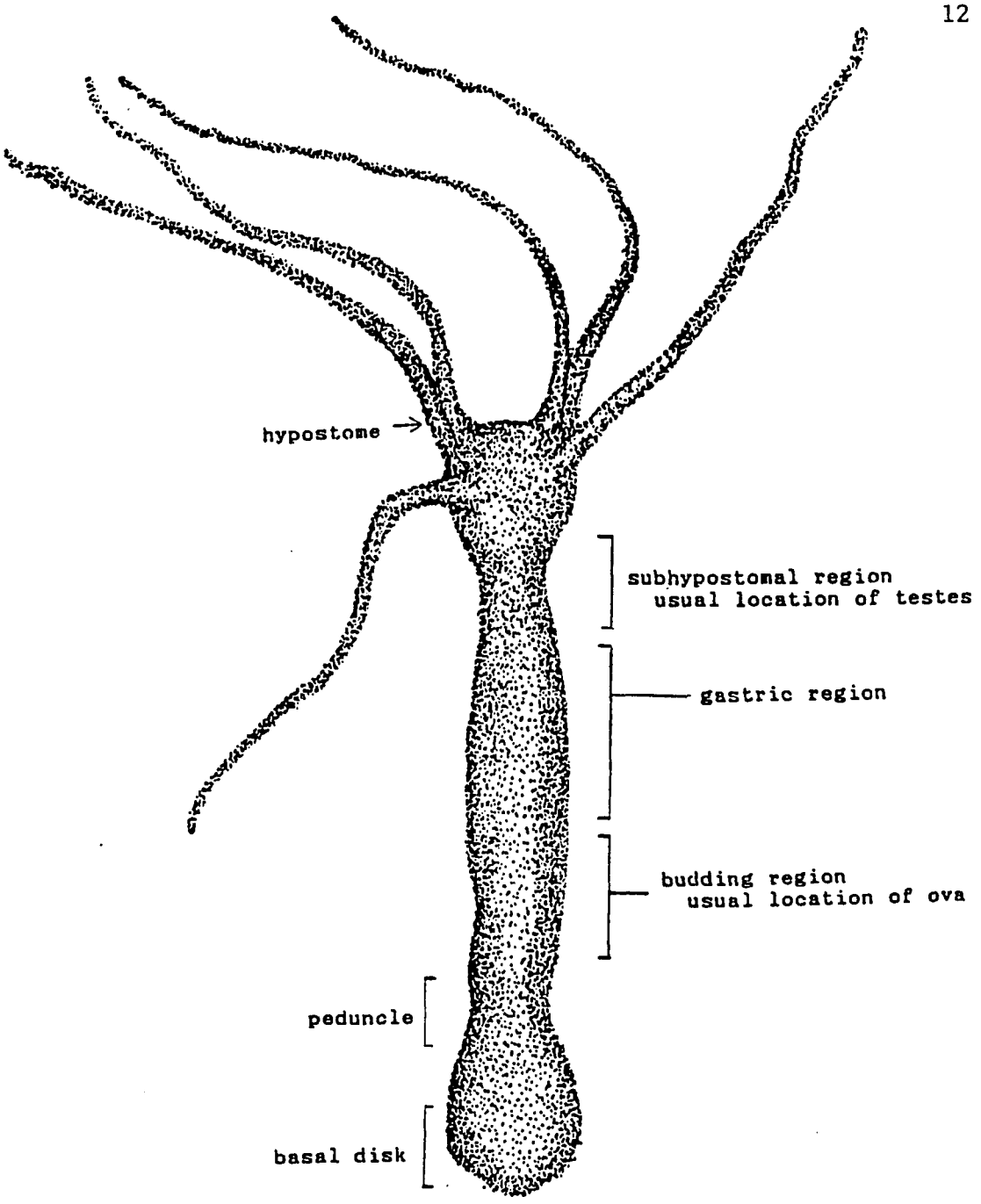

FIGURE 1. Terminology of hydra morphology

REVIEW OF LITERATURE

Literature Pertaining to Hydra

Hydra is a freshwater member of the Phylum Cnidaria (Coelenterata). These are metazoan animals with relatively simple body plans. Hydra do not have the cnidarian medusa stage. Although they are in polyp form, they are not immobile. They can move, more or less passively, vertically and horizontally through water. They can also move by inchworm and sommersaulting motions.

Hydra are composed of three tissue types, the ectoderm, the endoderm, and the acellular mesoglea. Hydra is a "naked animal" in the sense that all or most of its cells are in contact with the external environment. Hydra is constructed in the form of a tube, with a whorl of tentacles at the oral end, and a basal disk with which they can adhere to a substrate at the aboral end. They are 5 - 20 mm long and .03 - 1.0 mm wide. The body wall consists of two concentric epithelia, the ectoderm and the

endoderm, that are separated by the mesoglea (basement membrane). There are 15 - 20 cell types in hydra, which can be described as coming from three cell lineages. These cell lineages, the epitheliomuscular, interstitial, and endodermal, all have self-renewing stem cells and differentiated cells derived from stem cells. The interstitial cell lineage gives rise to nematoblasts and nematocytes, which are responsible for hydra's stinging capablities, nerve cells, and germ cells, both spermatazoa and ova. Hydra interstitial cells have been described as the most poorly differentiated cell type known in animals (Sapaev, 1977).

Regeneration in hydra is remarkable to the degree that some researchers have defined them as morphogenically "immortal" (Brien, 1953, Burnett, 1961), and as showing no signs of aging (Hyman, 1928, Stevenson and Buchsbaum, 1961). Usually hydra reproduces by asexual budding. Buds arise as evaginations of the body wall, along a certain region of the body column (figure 1, p 12). Hydra can produce more than 1000 asexual buds (hydranths) in a month

(Novak and Lenhoff,1981). Increased feeding results in increased bud formation. Hydra made up of only epithelial cells are able to live indefinitely by budding if they are hand fed, since no nerve cells or nematocytes, which are interstitial cell derivatives, are present to capture prey. It has been suggested that doubling time in hydra (budding rate) is a direct consequence of epithelial cell doubling time, both being around 3-4 days in well fed hydra (Takano, Fujisawa, Sugiyama, 1979). The interstitial cell cycle is about 1 day. In H. attenuata, 60% of interstitial stem cell daughters divide to yield more stem cells, this fraction of self renewal stem cells is designated as P(s). 30% of the interstitial stem cells differentiate into nematocytes. The remaining 10% of stem cells differentiate into nerve cells (David, 1979). In the gastric region, P(s) is regulated by negative feedback from neighboring stem cells, presumably by a diffusible factor secreted by these stem cells. Thus, when stem cell concentration is high, there is a corresponding high

concentration of "diffusible factor", and a consequent low P(s). The ratio of epithelial cells to interstitial cells in the gastric region is 1:1. Even when epithelial cell population size is increased 7 fold by increased feeding, this ratio remains 1:1 (Bode, 1979). There are relatively much fewer interstitial stem cells in the hypostome and basal disk regions. This is thought to be due to locally enhanced nerve cell and nematocyte cell differentiation effectively removing stem cells from the P(s) pathway (David, 1979). There is then competition for the same target stem cell population in the regions of the hypostome and the basal disk. Of course, the fractions of interstitial stem cell derivatives and P(s) changes if the animal is in a sexual reproductive mode. I wonder if the perturbance or imbalance of the asexual fractions, or their relationship to the epithelial cell populations might not be a trigger for sexual reproduction.

It has been suggested that sexuality in hydra is intimately linked with growth in the animal, and that

anything which inhibits cell division in the sub-hypostomal growth region initiates sexuality (Burnett, 1961). There is convincing evidence that this may be true for certain species of hydra (Burnett and Diehl, 1964). The further claim by these authors, that any other "specific evocator of sexuality need not exist", is refutable since, in their own paper, they make reference to the fact that at least two species, H. littoralis, and H. viridis, not only enter sexuality regularly under various conditions, but continue to bud, which is a sign of continued growth in the sub-hypostomal region, during the sexual process. Another argument against the above hypothesis which would need to be explained is why then do not all hydra that are not growing in the sub-hypostomal region become sexual? Temperature is important in both bud formation and gonad formation, and an uniquely different response will be obtained by varying temperature for different species of hydra (Park, Sharpless, Ortmeyer, 1965).

The occurence of sexual reproduction in hydra is not well understood. The differentiation of interstitial stem cells into either germ cells or somatic cells is determined by some environmental factors, rather than by the germinal plasm itself (Noda and Kanai, 1977). Interstitial cells with dense bodies (germ plasm) are primordial germ cells and posess the potency to differentiate into somatic cells, such as cnidoblasts. These cells probably should not be refered to as "primordial germ cells" (Weismann 1980), since they are multi-potent, if not totipotent. The development of nematocysts from interstitial stem cells is intrinsically programmed in the sense that it takes place wholly within the tissues of hydra. Only the case of sexual differentiation can be experimentally manipulated by varying external cultural conditions (Loomis, 1961).

Thus, sexual reproduction appears to be initiated by environmental indicators, and so does sex determination appear to be linked to environmental

factors. Although hydra is basically hermaphroditic, the sex displayed by any individual polyp is dependant on the environmental history of the animal (Burnett and Diehl, 1964). Spatial proximity would not be a factor influencing fertilization in hermaphroditic hydra, but hydra with gonochoristic tendencies would be likely to experience some difficulty in encountering gametes of the opposite sex since those polyps which are together in time and space in nature would be likely to experience similiar environmental influences, and therefore would be more likely to all be of the same sex. In hermaphroditic species, the first gonads formed are testes, and then ova, which may or may not be present on the polyp at the same time. Sex determination in hydra is controlled by the presence or absence of a subpopulation of interstitial cells in H. oligactis males (Littlefield, 1986). A monoclonal antibody was used to establish the relationship between this subpopulation of interstitial cells, AC2+ cells, and the sex of the individual. It was deduced by using grafting studies that the presence of these cells

programs the individual to be male, in the absence of AC2+ cells the female phenotype is expressed, and there are no AC2+ cells present in phenotypic females. AC2+ cells seem to arise from AC2- cells. It is not known what factors initiate the production of AC2+ cells in animals which lack them.

Environmental factors that have been found to influence the production of gonads in hydra include increased temperature, decreased temperature, starvation, overfeeding, and changes in photoperiod, pH, O2 tension, CO2 tension, and stagnation of culture medium, cessation of neurosecretion, cyclical phenomenon independent of immediate environment, redistribution of food resources, and the cessation of growth processes. Gonad formation is reversible, and in some cases (as in Hydra littoralis), budding continues during the sexual phase.

Sexual reproduction in hydra is thought to be a mechanism which can enable a hydrid population to persist through prolonged environmental crises that are

inescapable by plankters (Reisa, 1973). Fertilized eggs form a somewhat inpenetrable protective coating that is resistent to dessication (McConnell, 1938). In the variable conditions of limnetic habitats, temporary crises (thermal, chemical, desiccatory) occur. Sexual reproduction in such temporary crises would involve great energetic waste, and therefore be an undesirable defense against temporary crises. If hydra is induced to reproduce sexually by an environmental factor, such as a change in temperature, much time is required for the formation of gonads. Gonad formation has been reported at between a couple of weeks to a month. It has been observed that "cyclic sexuality in hydra is not phase synchronized" (Reisa, 1973). Presumably this is an "adaptive temporal variability at the population level" so that at least some animals will be capable of producting energetically expensive embryos at short notice. Furthermore, it is suggested that "if any common environmental influence were capable of entraining such cycles in nature, it would probably do so" Reisa, 1973). This argument does not seem

reasonable to me since there would be an elimination of "temporal variability at the population level" with each crisis.

The "stress hypothesis" explaining the existence of sexual reproduction in hydra is largely untested. This hypothesis, that predicts that hydra will start forming gametes when conditions worsen (when "stressed") probably seemed to make so much sense intuitively that few people were inclined to question it, let alone test it. Maybe it is not easy to define what is stress in hydra. The following description of a study (Strehler, Crowell, 1961), will clarify what is meant by saying that stress probably remains undefined in hydra, especially if longevity is to be used to indicate stressful conditions. In the late 1950's, high energy radiation was thought to be analogous to time in its effect on aging. A large radiation dose was expected to decrease the mean life span of an organism. In a study using Campanularia flexuosa, doses of 100,000 rads of x-ray irradiation did not decrease the mean life span of the Campanularia polyps (mean life span being 2.7 days without radiation) but

instead increased the mean life span to 6.3 days. Although Campanularia flexuosa is not a freshwater hydroid, but a marine colonial hydroid with a relatively short lifespan, this study does demonstrate the unusual degree of unpredictability, and therefore the great need for experimental testing of hypotheseses, in this group.

There has been at least one experiment described, again using Campanularia flexuosa, that has tested "stress" as it relates to growth rate, and has introduced various "noxious" agents. Unfortunately, I am unconvinced with the interpretation of the data in this study (Stebbing, 1979). In this experiment, it was concluded that the administration of "noxious" conditions, such as exposure to copper and reduced salinity, induced gonozooid production. Refering to the graph presenting the data in the Stebbing paper (1979), if the graph of gonozooid frequency (%) is superimposed over the graph of specific colonial growth rate (as compared to controls), it will be seen that the highest peak of gonozooid frequency in both copper and the salinity studies corresponds to treatments that result in more than 100% specific colonial

growth. The interpretation that this was evidence of gonozooid formation under stressful conditions is not warranted, since an increase in growth rate should not be interpreted as stressful. It is possible that this interpretation arose because of the fact that at higher copper concentration or at lower % salinity than that at which gonozooid production is maximal, growth rate falls off precipitously, but notice that so does gonozooid production. Because these extremes are noxious, does not mean that there is not a range at which these treatments are not stressful, and are indeed beneficial, as is implied by the data (Stebbing, 1979). This does bring to mind the fact the oxygen at high concentrations in noxious in man, but is certainly beneficial at other concentrations. The study went further to test the effects of various metals and organic pollutants, but resulting gonozooid formation was not great under these conditions and statistical tests for significance were not presented.

It is, in fact, in this next study of the scyphozoan Aurelia, a fellow Cnidarian, but of a different Class,

that there is presented data that may be contradictory to the "stress" hypothesis. Strobilation is the formation of medusae in which the sexual phase of the scyphozoan is realized, and therefore may be analogous to gonad formation in the Class Hydrozoa. Cessation of strobilation and reversal to polyp condition has been induced in Aurelia by sudden drops in temperature, sudden rises in temperature, from the transfer of organisms to laboratory medium, and finally by the administration of petroleum-related aniline and phenol. All of these conditions, if they would have had the opposite effect of switching the polyp condition to strobilation, would probably have been interpreted as "stress" and have been used to support the "stress hypothesis".

For most organisms in the wild, and I would suspect that hydra is included in this generalization, energy is at a premium. Hydra are known to go into "depression-estivation" when environmental conditions are poor. In this state, tentacles shorten, the body column recedes into a small lump with a feathery flock, the

mucous that previously covered the animal, becomes thicker, and a quick recovery to the budding condition ensues with improvement in environmental conditions. Animals which enter depression have quicker recovery times than animals which undergo sexual reproduction, and much quicker recovery times compared to embryos and hatchlings, (which generally are accompanied with high mortality).

Another possibility for the role of sexual reproduction in hydra is the reduction of competition between offspring in spatially heterogenous environments (Bell and Wolfe, 1985). In their census of a natural population of H. pseudoligactus, and using glass slides as substrate, they found that population size grew by asexual reproduction, followed by a decline in budding rate, which lagged two weeks behind the initial increase in population density. It was interpreted that high local densities on glass slides reduced rates of budding and caused dispersal of hydra. Sexual individuals appear in the middle of the growing season, near the time of maximal population density on the slides. 66% of the variance of sexual

individuals was "explained" by variance in the number of buds borne by asexual individuals. This is not a conclusive argument. Firstly, the postulation that sexual reproduction is a mechanism to reduce sibling competition with this example overlooks the importance of the mechanism of passive or non passive dispersal of the adult polyps, which would be accompanied by a much lower energy expenditure and mortality than that of forming sexual offspring. Secondly, should there have been some undescribed impetus for the formation of sexual individuals due to some environmental effect(s), the resulting production of gonads could have produced the effect of reduced rates of budding, provided that food consumption remained the same or decreased. Finally, the reduction in sibling competition may be a result of sexual reproduction, but this hypothesis would not explain why sexual reproduction occurs in solitary polyps or polyps that are not surrounded by kin, and furthermore, leaves to the imagination why crowded conditions do not always

result in sexual individuals. Of course, this argument is one of group selection, which may well be important to hydrid survival in the long run, but, as was previously explained, in the case of facultative sexuality, intrapopulational selection must be acting continuously to maintain sexual reproduction.

Literature Addressing the Question of the Evolution and the Maintainence of Sexual Reproduction

It is a well accepted notion that sex is advantageous because it is responsible for the source of individual variability which may be acted on by natural selection (Weismann, 1887). More recently, this idea has been in question. At least three disadvantages to sexual reproduction must be outweighed by the benefits it confers to the parental genotype, or possibly to the group of organisms that reproduce sexually. These disadvantages are described below, followed by a definition of them by theoreticians that have worked on the problem of the evolution of sex.

The Cost of Meiosis. Outcrossing sexual hermaphrodites would be selected to devote as much reproductive effort to male as to female gametes, even though this would amount to a great overproduction of sperm (if the organism is anisogamous). If the same individuals were to self-fertilize, they could produce just a few sperm and about twice as many eggs. Competition between outcrossers and selfers would greatly favor selfers (Maynard Smith, 1971).

The Cost of Males. In a sexual population (anisogamous), a female produces offspring at an average of one female to one male, whereas if a mutation appears causing females to produce two asexual females, its frequency will double in each generation (Maynard Smith, 1978).

Recombinational Load. Extremely fit genotypes will have no permanent significance as long as fitness depends at all on epistasis and complex gene interactions among gene loci, since these will be broken up by recombination during sexual reproduction (Williams, 1975).

Additional inherent disadvantages would be the energy put into morphological structures whose role is solely for sexual reproduction, display appartati for attracting the opposite sex both in terms of morphology and predation risks, and acquisition of territory and rivalry among sexes for mates. Sexual reproduction is not only disadvantageous in terms of the amount of energy involved in the process, but also in the amount of time consumed by the process, when compared with the higher intrinsic rates of asexual reproduction (Daly, 1978). At least one group

of people disagree with the idea that sexual reproduction involves greater amounts of energy than asexual reproduction. It was argued that "under benign conditions with plentiful food, sexual reproduction might outstrip fission because more offspring are formed per unit of assimilated energy, since assimilated energy can be converted more efficiently and more quickly into undifferentiated gametes than into differentiated somatic tissue (Calow, Beveridge, and Sibly, (1979). In response to the above argument, and aside from the fact that mortality is greatest in hatchlings compared to buds in hydra, the process of sexual reproduction in hydra requires the formation of gonads, and in the case of females, one gonad per ova per time period until maturation. This process is not likely to involve less energy than that of bud formation.

Some other availiable theories regarding the maintenence of sexual reproduction can be broken up into two categories for simplicity (Kondrashov, 1988). First will presented some "evolutionary explanations". If there

is continuous selection for an optimum genotype and the optimum keeps changing, as in the case of temporal and spatial variation in the environment, recombination can be advantageous (Maynard Smith, 1978). Sexual reproduction facilitates the combining of beneficial mutations between different genotypes, but it also breaks up favorable combinations and thus favorable combinations are not heritable, as they would be in the case of asexual reproduction (Williams, 1975). Sexual reproduction facilitates the elimination of deleterious mutations, since several mutant genes can be eliminated by a single genetic death, and by recombination, a non-mutant genotype can be preserved (Muller, 1950). The former argument can only explain the disadvantage of obligate asexual reproduction, but an extension of this idea, known as the deterministic mutation hypothesis (Kondrashov, 1982,1985,1988), states that if the deleterious mutation rate per genome per generation is great enough (>1), then the greater efficiency of selection against the accumulation of mutations, by recombination and truncation

selection, in sexually reproducing populations may be responsible for its advantage over asexual reproduction. The model goes on to predict that a deletereous mutation rate of about one mutation per genome per generation allows for maximal diversity of reproductive mode and that one would expect to see mutation rates of about one in plants, fungi and hydra. Survival data on H. magnipapillata suggest that an average of 3.5 - 4.0 lethal equivalent units of recessive deleterious genes are present per gamete, which corresponds to 7.0 - 8.0 per diploid animal (Sugiyama and Fujisawa, 1977). Data for mammals indicate mutation rates at about 100 per diploid genome per generation. Invertebrate mutation rates are around 10 per diploid genome per generation.

The second category of theories explaining the significance of sexual reproduction is that of "non-evolutionary theories". This would include ideas such as that the role of meiotic recombination, and thus sexual reproduction, is in the reprogramming and maintainence of the germ line (Holliday, 1986). This hypothesis

stresses the differences between the mortality of somatic cells and the immortality of germ cells. It suggests that recognition and repair of defects in epigenetic controls that are responsible for normal program development at the level of the DNA occurs during meiosis and recombination. Another similiar, although more simplistic argument is that sex is maintained not because of "mixis" during recombination but because of the connection of meiosis with morphogenesis (Margulis, Sagan, Olendzenski, 1885). According to this idea, sexual reproduction is the only known pathway to extensive tissue differentiation in most animals and plants. Finally, there is the DNA damage hypothesis (Bernstein, Hopf, Michod, 1988), that suggests that the primary reason for recombination is to repair double strand DNA damage, and that outcrossing in diploid organisms serves the purpose of restoring heterozygosity that is destroyed during repair, and thus masking deleterious recessive alleles. In support of this theory is the fact that most physical recombination (66%) does not result in allelic recombination. It is suggested that

meiotic recombination is designed to promote repair of DNA while keeping only a low level of allelic exchange.

MATERIALS AND METHODS

My experiment was aimed at testing the DNA damage hypothesis by inducing DNA damage in Hydra littoralis with ultra violet light exposure (240 nm). Since H. littoralis is facultatively sexual, and the production of gonads, which is the forerunner to sexual reproduction, is determined environmentally, I set out to measure this phenomenon as a response to uv light exposure.

Hydra littoralis were obtained from Carolina Biological Supply Company, and then set up in a ten gallon aquarium to increase population size. Four groups of hydra were used to evaluate the effects of raising them in four different culture solutions. Of these four solutions, "M solution" (Loomis, 1961), KNC solution (Lenhoff, 1985), Tucson city well water, and well water from the University of Arizona campus of known composition (appendix p 67), both solutions of well water did equally well and were apparently superior to either artificial solution. Therefore, all following experiments were done using the

University of Arizona well water, which will henceforth be refered to as culture solution or medium.

Constructing the Ultra-Violet Light Exposure vs. Survival Curve.

In order to have a range of uv light doses, and to know what effects this has on hydroid survival, which should correspond to DNA damage, it was necessary to construct a dosage response curve. At intervals of 50J/m2, two groups of hydroids with ten individual polyps each, were irradiated with a one hit dose and then left in the dark for 24 hours after the treatment to preclude light activated repair of DNA damage. These animals were then fed with Artemia salina nauplii three times per week and the medium was changed three times per week for two weeks, at which time survival was quantified. If hydra reverted back to normal morphology by two weeks they were counted as "survivors". Two weeks appears to be an adequate amount of time for regeneration of damaged tissues according to a study on Hydra attenuata treated with 5-azacytidine (Maharajan, Petrocellis, Garguilo,

Marino, 1988). According to my own observations, if hydra had not regenerated into normal polyps by two weeks post uv treatment, they eventually "disintergrated", and thus, did not survive. From the survival vs uv dose curve (figure 2), threshold dosage and LD50 dosage was estimated (Harm, 1980).

Effects of a One Hit Dose on Sexual Reproduction

The next step I took was to set up four groups of hydra with uv treatments corresponding to the threshold dose of 200J/m2, the LD50 dose of 700J/m2, and a dose of 80% mortality (high dose) at 900J/m2, and a control group that recieved no uv treatment. These groups were kept 24 hours in the dark post uv treatment and then set up in one gallon glass jars with continuous airation via air stone at room temperature and ten hours of white light per 24 hour period. They were fed three times a week with either Artemia nauplii, Daphnia pulex, or Tubefex sp. At the end of four weeks, which is an adequate amount of time for hydra to form gonads after they have been induced to do so

by some environmental stimulus (1-4 weeks), there was no development of gonads detected in any of the experimental groups or in the control group.

The Effect of Continuous UV Treatments on Sexual Reproduction in *Hydra Littoralis*

The next step that was taken was to treat hydra with daily doses of uv light. For six days, hydra were set up in groups with two replicates and sample sizes of 50 hydra per replicate. One group was given 50 J/m2 each day, and another group was given 100J/m2 each day and a third group was the control with no uv treatments. Upon the observation of the detrimental effects of these daily doses on the hydra morphology (at around five days), the treatments were switched to every other day at the same doses for the remainder of one month. After each dose, all groups were kept in the dark for 24 hours. They were fed three times per week with *Artemia*, and the medium was changed three times per week. Unfortunately, the control group was diminished for unknown reasons, (perhaps from the lack of light due to long periods in the dark with the

treatment groups). During the first three weeks post uv treatment, when data collection is most important, the control group was doing very poorly with a sample size of 38, whereas the pooled sample sizes for the 50J/m2 group was 96, and for the 100J/m2 was 104.

The second experiment of this nature was the irradiation of hydra with 50J/m2, three times per week for one month and a subsequent observation time of four weeks. There were control and treatment groups originating with two replicates of 60 hydra each. It is interesting that, although the occurence of sexual reproduction in the control group was almost twice as great as in the uv treatment group, the uv treatment group produced only females and the control group only males (table 3, p 49).

In the final experiment hydra were divided into two groups, the difference between the groups being the temperature regime used. One group was stationary at 21 C, like all previous experiments. The other group underwent a temperature change to increase the amount of sexual reproduction by up to 50%. This is a standard

proceedure to obtain sexual forms (Park, Sharpless, Ortmeyer, 1965). During the four weeks of uv treatment, this group was kept at 16 C. At the end of uv treatments, the group was brought up to 21 C and remained at 21 C throughout the one month period of observations. This group will be henceforth refered to as the 16 C group. Four treatment groups were set up with two replicates each of 50 individuals, corresponding to 21 C control, 21 C treatment with 100J, 16 C control, 16 C with 100J. These groups were fed two times per week with Artemia, and the medium was changed three times per week. After one month of treatments of 100J/m2 three times per week and 24 hour dark following treatments for both 21 C and 16 C groups, observations were made for four weeks. Upon the detection of a polyp with gonads, the polyp was placed in a separate container to avoid the possibility of it effecting the surrounding polyps. The incidence of both asexual and sexual reproduction was recorded and compared for these four groups.

RESULTS AND DISCUSSION

I Constructing the UV Light Exposure vs Survival Curve (Figure 2, p 43)

The range of uv doses used was between 0 and 1200 J/m2. The threshold dose appears to be in the vicinity of 200 J/m2. A reasonable estimate of LD 50 is around 700 J/m2. Bivariate statistics of linear regression of the uv survival curve are:

y intercept (a) = 1.15
slope (b) = -8.85
r2 = 0.72
standard error of regression = 0.17

Any uv dose greater than 150 J/m2 produces morphological changes in the animal. Lower doses result in smaller hydra with short tentacles. In the middle range of uv doses (approximately 600 - 800 J/m2) unusual morphologies were present, which then reverted back to "normal" morphology in many cases. At the high end of the uv doses (more than 800 J/m2) hydra tissue appeared to "dissociate" to some degree, and then reaggregate. Counting "survivors" from the high uv dose treatments may have been confounded by the possibility that more than one survivor

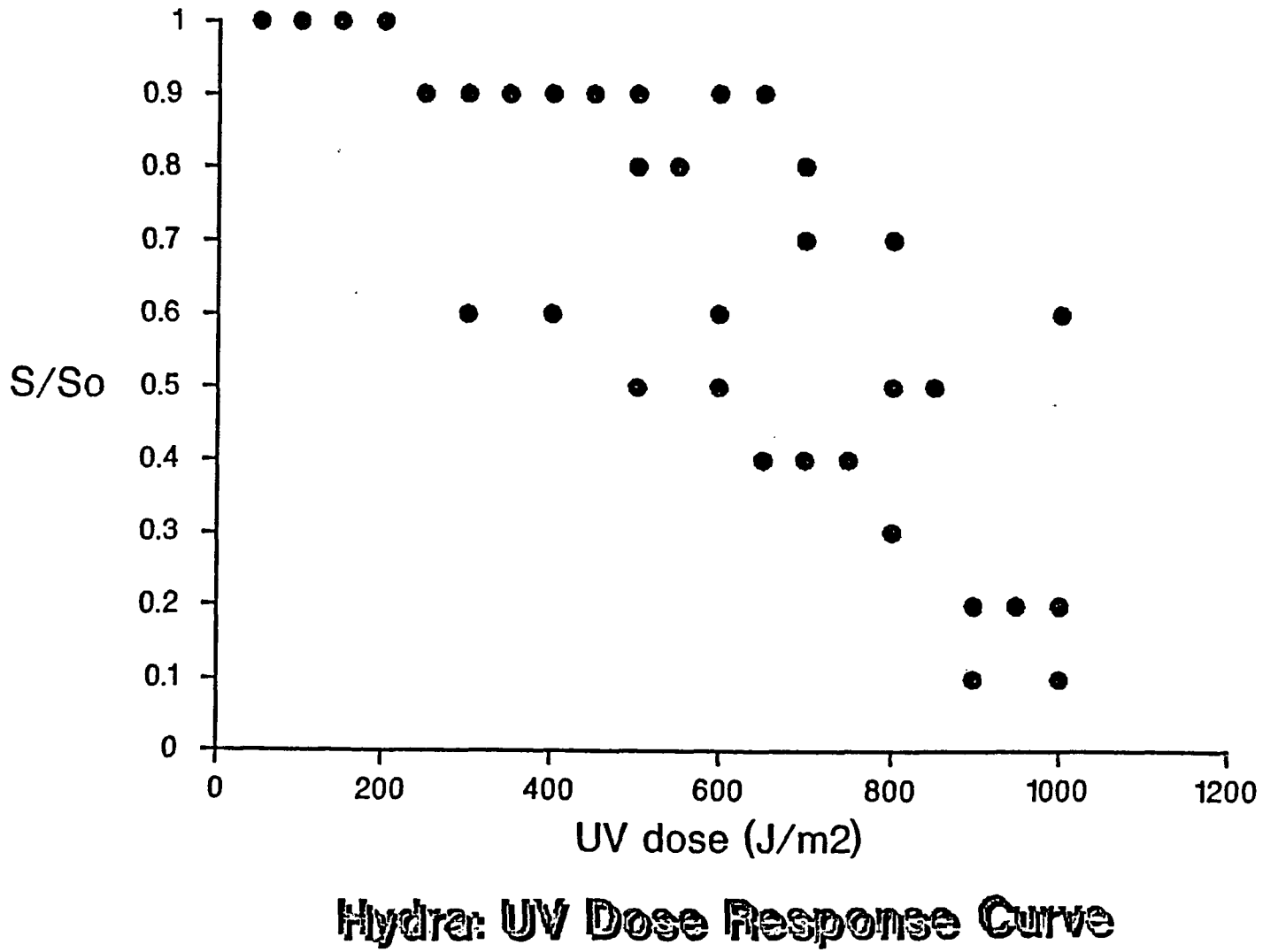
S/So
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
UV dose (J/m2)
Hydra: UV Dose Response Curve

Figure 2

could have resulted from what was originally only one individual. This explaination, if it proved to be true experimentally, may acount for some of the "variance" of the regression curve. For the interest of those who are familiar with standard survival curves, additional terms were estimanted. These are known as certain "characteristic terms" to discribe a "shouldered curve" in a "multitarget single hit case" (Harm, 1980). The resulting characteristic terms were derived from the equation: $S/So = e^{-(F-Ft)/F0.37}$

a. Threshold fluence (Ft) is about 200 J/m2

b. Mean lethal fluence (F0.37) is about 600 J/m2

c. Extrapolation number (n) is about 1.39

II Effect of One Hit Dose on Sexual Reproduction

The first experiment (experiment 1) was to give uv treatments to hydra corresponding to low dose (threshold) at 200 J/m2, LD 50 dose at 700 J/m2, and high dose (about 80% mortality), at 900 J/m2, and 0 dose for control. The

pre-treatment sample size varied between the groups due to differences in the expected mortality of the different groups. The initial sample sizes, mid-experiment sample sizes and final sample sizes are given in table 1 (p 46). During this first experiment, sexual reproduction was 0 in all groups.

III The Effect of Continuous UV Treatments on Sexual Reproduction

The first experiment of this nature, using three groups at 50 J/m2, 100 J/m2, and control, was done with variable time sequences of treatments because the original daily dose proved to be too much. At the time that it was noticed that hydra populations were diminishing, treatments were changed to every other day for the remaining time period. The results of this experiment are presented in table 2 (p 47). If the population number increased more than 10% between groups (except when the group was diminishing for unknown reasons), hydra buds were removed from the experiment. This resulted in about a maximum of 10% difference in population size between groups.

Table 1

One hit dose folled by 24° dark feeding and cleaning 3 times per week

day 1

low dose (threshold)
100 hydra at 200 J/m

medium dose (LD 50)
200 hydra at 700 J/m

high dose (80% mortality)
500 hydra at 900 J/m

control
100 hydra at 0 J/m

day 14

low dose	N = 88 ± 8.8	
medium dose	N = 95 ± 9.5	
high dose	N = 300 ± 30	
control	N = 92 ± 9.2	

day 30

low dose	N = 80 ± 8	0 sexual reproduction
medium dose	N = 90 ± 9	0 gonads
high dose	N = 200 ± 20	0 gonads
control	N = 90 ± 9	0 gonads

Experiment 1

Table 2

Continuous dose, UV doses every day for 5 days then every other day for 1 month.

day 1

2 groups of 50 individuals

50 J group
100 J group
control

day 30

50 J group	N = 96 ± 9.6
100 J group	N = 104 ± 10
control	N = 34 ± 3.4

day 50

50 J group	N = 110 ± 1.1	0 gonads
100 J group	N = 120 ± 1.2	0 gonads
control	N = 38 ± 3.8,	one male (2.6%)

Experiment 2

The result in sexual reproduction was 0 in both treatment groups and 1 male in the control, which due to small sample size (N=38) represents 2.6%. The control group may have done poorly because of a lack of ambient light. The 24 hour dark period post uv treatments used for all groups left the control with very little light, especially during the first week of the experiment when treatments were daily. Treatment groups recieved the uv doses, and intense as they may be, it may have to provided sufficient light energy necessary for biological function.

The second experiment of continuous uv dose (experiment 3) was to use 50 J/m2, three times per week for one month with one month post treatments for observations. Again, hydra buds were removed when between group sample sizes deviated by more than 10%. Table 3 (p 49) illustrates the results of this experiment. The total number of hydra with gonads (the measure of sexual reproduction) in the control group was 16 (13.9%) and in the experimental group was 9 (7.8%) at the end of 24 days. Although the total number of hydra between control and

Table 3

Irradiate hydra 22 groups of 50 individuals 3 times per week for 1 month

Post irradiation treatments

Days →	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Control																									
total	120		120		130		120		110			110			110					110		110			100
males	2		0		1		6		2			3			1					0		1			0
females	0		0		0		0		0			0			0					0		0			0
(% sex)	1.7		0		.8		5		1.8			2.7			.9					0		1.0			0
50 J/m																									
total	130		130		140		130		120			100			100					100		100			100
males	0		0		0		0		0			0			0					0		0			0
females	0		0		0		0		7			2			0					0		0			0
(% sex)	0		0		0		0		5.8			2			0					0		0			0

	total male	female	(%)	
Control	16	0	13.9	
50 J/m	0	9	7.8%	(all 13-16 days after treatment)

Experiment 3 (room temperature 21° ± 1° C)

experimental groups is not significantly different, the amount of sexual reproduction in the experimental group is almost half that of the control group. Curiously, all sexual forms in the control group were male, and all sexual forms in the experimental group were female. My initial interpretation of this result was that the uv treatments may have somehow altered the AC2+ cells described by Littlefield 1987, so that only phenotypic females were present. Since the following experiment does not have this explicit difference of sexes between groups, although only the uv treatment group had females but not only females (tables 4 and 5), I was unable to provide more evidence that the AC2+ cells were being effected by the uv dose.

The final continuous uv dose experiment (experiment 4) represents the bulk of the data obtained from the experiment, and was used to make most of my conclusions (tables 4 and 5, pp 52 and 53). Groups were divided into room temperature (21 C), control and experimental groups, and change in temperature (16 -21 C)

control and experimental groups. By keeping the hydra at 16 C for one month during uv treatments and then placing them at room temperature for one month, sexual reproduction was induced, and would therefore be expected to be greater than in the room temperature groups. The experimental groups recieved 100 J/m2, three times per week. Fluctuations in group sample sizes were greater than in previous experiments, therefore removal of hydra was done when deviation of total sample size was greater than 20% between groups.

The result of this experiment is that the 16 C control group had by far the greatest total number of hydra with gonads, this total being 38 (34.9%), compared to 4 (4%) in the 21 C control, and 3 (2.5%) in the 16 C experimental groups.

Table 4

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
Control																																			
total (Nt)	100		110	110	100		100		100		180		100		100		100		120		120	120		150			110	100		100			100		
buds (Na)	40		40	40	40		40		50		70		40		40		40		40		40	40		40			40	20		10			20		
gonads (Ns)	0		3	2	1		0		21		2		1		0		0		0		0	0		4			4	0		0			0		
% asexual	40		36	37	40		40		50		39		40		40		40		33		33	33		27			36	20		10			20		
% sexual	0		2.7	1.8	1		0		21		1.1		1		0		0		0		0	0		2.7			3.6	0		0			0	total	34.9%
100 J/m²																																			
total (Nt)	100		100	100	100		100		110		110		110		130		130		130		130	130		130			120	120		180			200		
buds (Na)	20		30	30	30		50		70		70		70		60		60		40		40	40		30			30	30		40			30		
gonads (Ns)	0		0	0	0		0		0		0		0		0		0		0		0	0		0			3	0		0			0		
% asexual	20		30	30	30		50		64		64		64		46		46		31		31	31		23			25	25		22			15		
% sexual	0		0	0	0		0		0		0		0		0		0		0		0	0		0			2.5	0		0			0	total	2.5%

Note that all sexual forms for both groups were male except for the 16°C control group which had 2 females, 1 on day 2 and 1 on day 3

Group 16° C Treatment (± 1°)

Table 5

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
Control																																			
total (Nt)	100		100	100	100		100		100		100		120		120		120		120		150	140		140			100	70		70			70		
buds (Na)	30		30	30	30				30		10		20		20		20		40		30	30		30			10	10		10			10		
gonads (Ns)	1		0	0	2		0		0		0		0		0		0		0		0	0		0			1	0		0			0		
% asexual	30		30	30	30		30		30		8		18		18		18		28		21	21		21			10	14		14			14		
% sexual	1		0	0	2		0		0		0		0		0		0		0		0	0		0			1	0		0			0	total	4%
100 J/m²																																			
total (Nt)	100		100	100	100		100		100		100		160		160		160		160		100	100		100			90	100		100			100		
buds (Na)	20		20	20	20		25		25		20		30		30		35		335		30	30		30			10	20		20			20		
gonads (Ns)	0		0	0	0		0		0		0		0		0		0		0		0	0		0			0	0		0			0		
% asexual	20		20	20	20		25		25		20		19		19		22		22		30	30		30			11	20		20			20		
% sexual	0		0	0	0		0		0		0		0		0		0		0		0	0		0			0	0		0			0	total	0%

Note that all sexual forms in 21°C control were male
No sexual forms found in 21°C experimental (100 J/m²) group

Group 21° C Treatment (± 1°)

ANALYSIS OF VARIANCE TABLE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio
Total Sample Size				
Among groups (21 con, 21 exp)	1	2.94	2.94	0.00
Within groups	32	19294.12	602.94	
Among groups (16 con, 16 exp)	1	105.88	105.88	0.19
Within groups	32	17647.06	551.47	
Asexual Reproduction				
Among groups (21 con, 21 exp)	1	6.62	6.62	0.10
Within groups	32	2179.41	68.11	
Among groups (16 con, 16 exp)	1	188.24	188.24	0.84
Within groups	32	7200	225	
Sexual Reproduction				
Among groups (21 con, 21 exp)	1	0.12	0.12	2.13
Within groups	32	1.76	5.51	
Among groups (16 con, 16 exp)	1	36.03	36.03	2.77
Within groups	32	415.53	12.99	

Statistical analysis of both sexual reproduction and asexual reproduction were done using ANOVA on the NCSS computer program (table 6, p 54). Sexual reproduction in the experimental groups is significantly lower than in the corresponding control groups. Asexual reproduction is not greatly different between experimental and control groups. Asexual reproduction is significantly different however when comparing between the 21 C and 16 C control groups, and between the 21 C and 16 C experimental groups. Since the amount of sexual reproduction in the 21 C control is similiar to that of the 16 C experimental group, it is possible that any increase in sexual reproduction that may have been induced by going from 16 C to 21 C could have been partially negated by the decrease in sexual reproduction associated with the uv dose treatments.

The results of this experimentation does not support the DNA damage hypothesis, since in no case was there an increase in the subsequent gonad formation following uv light treatment, which is used in this study as a DNA

damaging agent. There was in fact significant decrease in the subsequent gonad formation in the uv treatment groups.

The observable deterioration of hydra tissue is probably mostly a function of the state of epithelial cells, since "epithelial hydra" continue to grow, regenerate and bud, but interstitial stem cells, those responsible for gonad formation among other important cell types, have cell cycles that are typically a third of the time duration of the epithelial cell cycle. Interstitial stem cells may be more susceptable to damage from uv light than epithelial cells as a result of their quicker cell cycle. The depletion of interstitial stem cells may be responsible for the decrease in gonad formation compared to controls but the total elimination of these cells is not probable since the hydra continued to feed on live Artemia nauplii without assistance, which would indicate that such interstitial stem cell derivatives as nematacytes and nerve cells were present and continually renewed for the duration of the experiment.

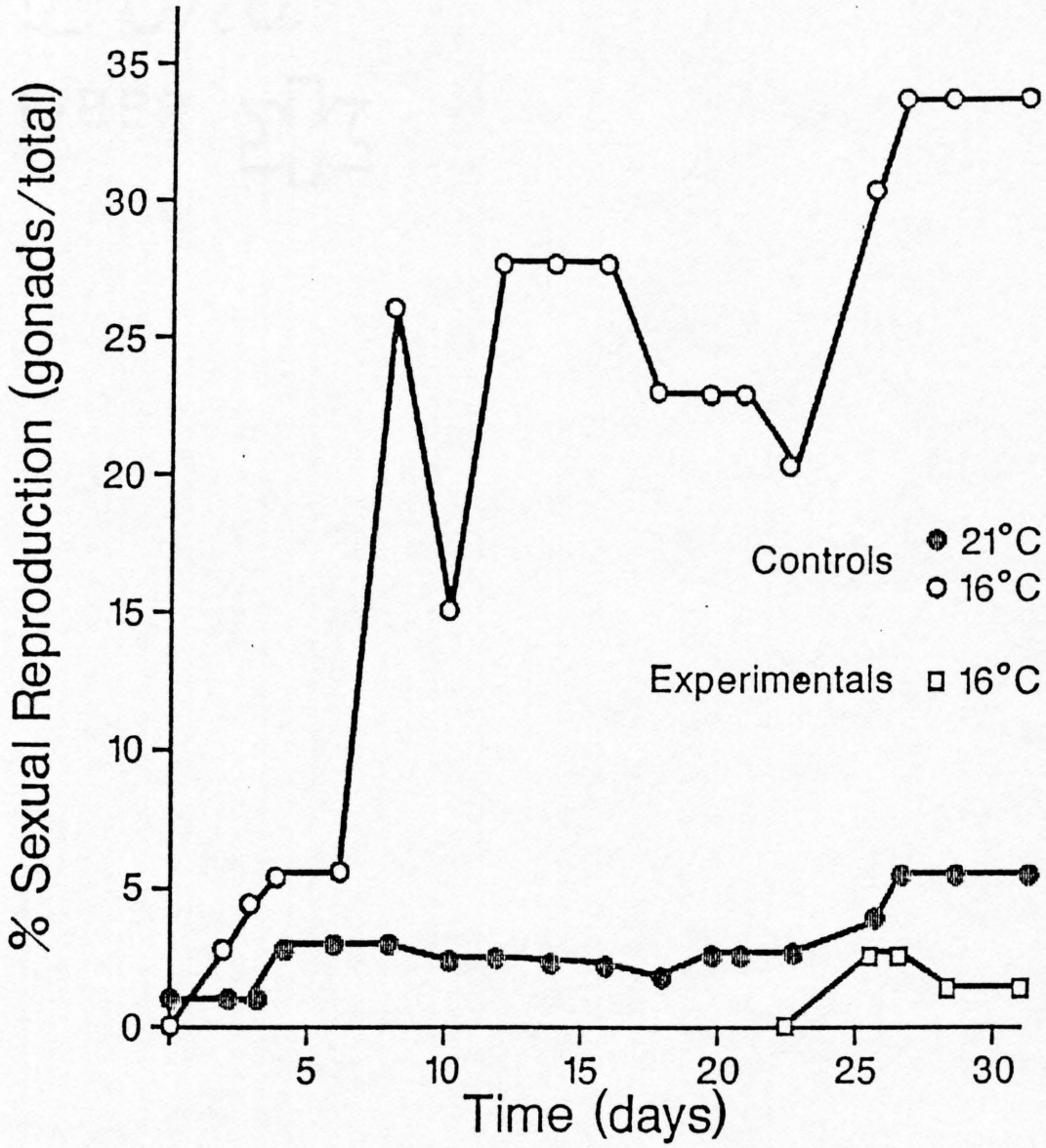
% Sexual Reproduction (gonads/total)
35
30
25
20
15
10
5
0
5
10
15
20
25
30
Time (days)
Controls
21°C
16°C
Experimentals
16°C
Sexual Reproduction

Figure 3

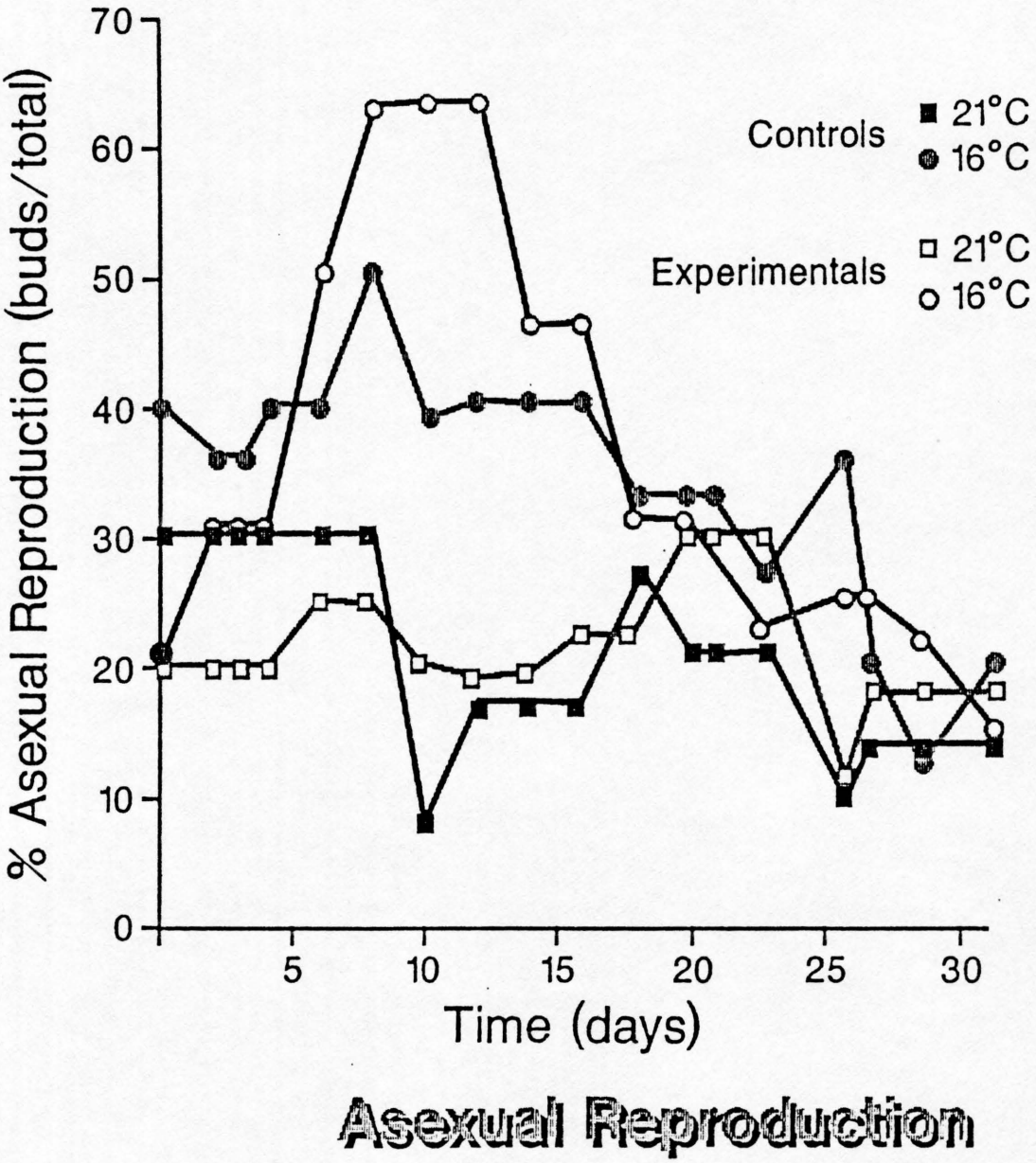
% Asexual Reproduction (buds/total)
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Time (days)
Controls
21°C
16°C
Experimentals
21°C
16°C
Asexual Reproduction

Figure 4

Although this was not a direct test of the "stress hypothesis", observations from this experimentation do not support this theory either. The hydra that showed the greatest production of gonads, concurrently and previously showed no sign of decreasing growth rates, and in fact showed signs of increasing growth rates when compared to those hydra that showed no or low gonad development (figures 3 and 4, pp 57 and 58).

CONCLUSIONS AND DISCUSSION

My data suggest that the induction of sexual reproduction is not a direct function of DNA damage by uv irradiation in hydra. There is evidence that the opposite result of an inhibition of sexual reproduction is the result of DNA damage by uv irradiation in Hydra littoralis. Perhaps the reason that DNA damage is not foremost in importance for sexual reproduction in an organism such as hydra is due to their capacity for regeneration from undifferentiated stem cells which are present throughout the individual. Cellular selection against mutation and DNA damage may be adequate in assuring that individual hydra do not accumulate lethal mutations. "Cellular selection requires that there be undamaged cells that can replicate" (Bernstein, Hopf, Michod, 1988). Both epithelial cells and interstitial cells can develop into new hydra by means of fission. Those undifferentiated cells necessary to form gametes, the interstial stem cells, are relatively abundant throughout the entire hydra polyp. Interstitial cells

represent 70% of the total hydra cells, and 4% of these are stems cells, any of which can form gonads and gametes. With such a large population of cells with this potential at any given time, cellular selection may effectively eliminate mutant and damaged cells with plenty of cells left over to serve as precursors to replication. With the rapid cell cycle of hydra cells, mutation may be increased, but so would the corresponding rate of cellular selection be increased.

So what is the purpose of sexual reproduction in hydra? If it is to escape deteriorating environments one might expect to see parthenogenesis in hydra, which is said not to have been detected to this date in any known species. We do see, however, somatic changes to cope with adverse environmental conditions in the development of morphological changes that allow the individual to persist through environmental disasters. This is the phenomenom of "depression-estivation" in hydra in both their natural habitat and in laboratory cultures.

I would like to put forth an argument that is very different from those mentioned in this manuscript by other authors, and that is the following. Reproducing by asexual means bestows the organism with functional independence among the resulting individuals. Once a single hydra polyp has cloned itself sufficiently, perhaps it is not so costly to reproduce sexually, since even if the outcome is negative in the case of any one individual, the genotype persists in the form of its clones. Hypothetically, a gene that promotes sexual reproduction when other energetic commitments have been satisfied (such as a certain degree of asexual reproduction), may persist even if any one incident of sexual reproduction resulted in no sexual progeny and the death of the parental polyp (although "terminal sexuality" does not usually happen in H. littoralis). This is possible because the parental genotype is still present in the population in many copies in the form of its asexual clones, and thus, there is persistence of the hypothetical "sex" gene. This may effectively reduce the gamble of "recombinational load"

associated with recombination and outbreeding. It would not, however, reduce the "cost of males". But hydra, not having special structures designed for the acquisition of mates, and not having territories, may have cut down on the "costs of males" significantly. It may be that in the case of hydra, the limited gamble of reproducing sexually has allowed for the persistence of sexual reproduction with its occasional production of a more fit genotype. The data presented in this study would support this theory.

At least one unexplained observation remains, and that is that I would expect to see the selection for physiological mechanisms that would prevent the occurance of inbreeding in hydra, and this has not been recorded. The precidence of asexual over sexual reproduction in hydra may correlate to the relative importance of these phenomena. If the above hyposthesis is valid, I would expect that with an abundance of resources and adequate cloning to assure the persistence of the parental genotype, sexual reproduction is not inhibited.

It is worth testing, in a more vigorous manner, the presence of a sex ratio disparity that was seen in experiment 3, and what might be the cause of it. At 50 J/m2, is there an elimination of the AC2+ cell type, and thus a stimulus for the formation of phenotypic females from those polyps that are induced to form gonads? If this inference proved to be true, it may shed some light on the mechanism for the appearance of the hydra of the opposite sex. This animal is "basically hermaphroditic", and the environmental history of the hydra determines the sex of the animal. The hydra in a subpopulation experiencing the same environment are more likely to be all of the same sex. Encountering gametes of the opposite sex may be difficult under these circumstances. If a specific amount of DNA damage (produced by a uv dose of about 50 J/m2) eliminates the AC2+ cells, a random factor would be introduced to this system, allowing for the random presence of hydra with gametes of the opposite sex. This would be testable by demonstrating a different (lower) tolerance of the AC2+ cells to uv light.

Finally, it may be worth testing the idea that the production of gonads is a response to the shifting of the 1:1 ratio of epithelial to interstitial cells within the sub-hypostomal region of the hydra. One might expect that either the reduction of epithelial cells or the increase of intersitial cells (in such a manner that the former ratio is not recoverable) may have some effect on the production of gonads. If this proved to be true, I am not sure what it would mean in terms of the ecology of hydra. It may indicate that "change" may have some bearing on the importance of sexual reproduction. It would mean that favorable changes promote sexual reproduction if the production of gonads is the result of a disruption of the 1:1 ratio due to an increase in the production of interstitial cells. It would mean that unfavorable changes promote sexual reproduction if the production of gonads is the result of a disruption of the 1:1 ratio due to a decrease in epithelial or interstitial cells. It would mean that any change, good or bad, would result in sexual reproduction if the formation of gonads was the result of

a disruption of the 1:1 ratio due to any change in the component cell types. The data in the present experiment would suggest that the possibility of a disruption in the 1:1 ratio due to a decrease in epithelial or interstitial cells would not promote sexual reproduction, since the UV treatments probably had the effect of reducing these cell populations as opposed to increasing them, especially the interstitial cell population. So if I may extrapolate from my data, I would expected that unfavorable changes would be less likely to result in sexual reproduction.

APPENDIX

WATER QUALITY ANALYSIS

"H-K Label"

State of Arizona
Department of Health Services
State Laboratory
J. M. COUNTS, Dr.P.H., Chief

SPECIMEN SUBMITTING AND REPORTING FORM 1 7

Date Rec'd.

WATER QUALITY ANALYSIS - Chemical

SPECIMEN UNSATISFACTORY 000793
Reason:

SPECIMEN NUMBER	SYSTEM NUMBER
	20-423

NAME OF CLIENT: Physical Resources
IDENTIFICATION NUMBER:
ADDRESS: Univ. Az
CITY: Tucson STATE: Az ZIP CODE: 85721 COUNTY: Pima CODE:

LAB USE ONLY — Lab Performing Test: Phoenix - 1, Flagstaff - 2, Tucson - 3; DO NOT CHARGE

MAILING ADDRESS OF REPORT: SAME
ADDRESS:
CITY: STATE: ZIP CODE:

SYSTEM OR SOURCE NAME: UA CITY: Tucson WELL NUMBER: H-K
LOCATION OF SAMPLING POINT: Pump discharge WELL NAME: Huachuca
COUNTY: Pima TOWNSHIP: RANGE: SECTION: COLLECTED BY: W.A. Witschi
LATITUDE: LONGITUDE: STATION NUMBER: DATE COLLECTED: 4 Aug 86 HOUR: 0810
STORET NUMBER: CONTROL NUMBER: PRESERVATIVE ADDED? ☐ YES ☐ NO

SOURCE TYPE (check one): [X] Well ☐ Surface ☐ Sewage ☐ Industrial ☐ Seepage

SOURCE DATA (check each applicable box): [X] Public System ☐ Individual System ☐ Treated [X] Untreated [X] Routine Sample ☐ Re-Sample ☐ Special [X] Grab Sample ☐ Composite Sample ☐ New Source [X] Existing Source

FIELD DATA -
Conductivity ____ Mhos D.O. ____ pH ____
Air Temp. ____ °C. Water Temp. ____ °C.
Well Depth ____ Static Water Level ____
Gage Height ____ Discharge ____ CFS
Depth to Surface ____ ____ GPM

SAMPLE APPEARANCE - [X] Clear ☐ Turbid ☐ Silty ☐ Floating ☐ Other

WEATHER - [X] Clear ☐ Cloudy ☐ Rain ☐ Snow ☐ Other

SAMPLER'S COMMENTS:

10 ☐ ROUTINE WATER CHEMISTRY
11 ☐ Specific Resistance (OHMS) 1620
12 ☐ pH 7.8

13 ☐ CATIONS	D	T	ME/L	Mg/L
14 ☐ Calcium				56.0
15 ☐ Magnesium				2.55
16 ☐ Sodium				63.5
17 ☐ Iron (0.3)				<0.1
18 ☐ Copper (1.0)				<0.1
19 ☐ Manganese (0.05)				<0.05
20 ☐ Zinc (5.0)				<0.05
21 ☐ ANIONS				
22 ☐ Alk P (CaCO3)				0
23 ☐ Alk MP (CaCO3)				113
24 ☐ Chloride (250)				40.4
25 ☐ Nitrates N				1.77
26 ☐ Sulfate (250)				111
27 ☐ Total PO4				
28 ☐ Fluorides				0.27
38 ☐ Nitrite				

D - Dissolved T - Total

	Mg/L
29 ☐ Solids	
30 ☐ Residue	363
31 ☐ T.D.S. Calc.	
32 ☐ Hardness (CaCO3)	149
33 ☐ Total S.S.	
34 ☐ V.S.S.	
35 ☐ Surfactants	
36 ☐ B.O.D.	
37 ☐ C.O.D.	
39 ☐ Kjeldahl Nitrogen Total	
40 ☐ Others Ba	<0.5

41 ☐ TRACE METALS (Mg/l)	D	T	Mg/L
42 ☐ Arsenic (.01)(.05)			<0.02
43 ☐ Silver (.05)			<0.005
44 ☐ Chromium (0.05)			<0.01
45 ☐ Cadmium (0.01)			<0.001
46 ☐ Lead (0.05)			<0.02
47 ☐ Selenium (0.01)			<0.005
48 ☐ Mercury (.005)			<0.0005
50 ☐ SPECIAL			
49 ☐ Boron			
51 ☐ Radiological			
52 ☐ Petroleum Res.			
53 ☐ C.C.E. (0.2)			
54 ☐ Phenols (0.001)			
55 ☐ Pesticides			
56 ☐ PHYSICAL			
☐ Color			
☐ Taste			
☐ Odor			
☐ Turbidity			

☐ Acceptable for Public Usage
☐ Provisionally Accepted for Public Usage*
☐ Rejected for Public Usage*
☐ Based on Parameters Run
*See attached for interpretation

Evaluated by ____
Bureau of Water Quality Control
Date ____

COMMENTS

Analyst [signature] Date Reported 9/23/86

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ADHS/Epidemiology and Laboratory Services-17 (5-78)

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