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EXPERIMENTAL RESULTS OF CROWDING ON THE RATE OF ASEXUAL REPRODUCTION OF THE PLANARIAN DUGESIA DOROTOCEPHALA

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Abstract

Natural selection is central to evolution and is thought to provide the mechanism for the development of new species. Pressure exerted by natural selection is thought to play a major role in the regulation of animal populations. If it could be demonstrated that natural selection is not needed for the maintenance of populations or that the harvest of prey species by predators is random, then evolution would be without a mechanism. Experimental evidence is presented indicating the freshwater planarian, Dugesia dorotocephala, *regulates its own population density at healthy levels without need for starvation, disease or predation. Data also indicate worm density is a more important determining factor of reproduction rate than food under certain conditions. Additional work is needed with other species and under natural conditions.*

Introduction

If the Creation/evolution controversy is ever to be resolved, I believe it will be done at the level of population ecology. Fossil evidence from Darwin's time to the present day overwhelmingly supports the Creation model and has little correlation with evolution, but most life scientists ignore the fossil evidence. Philosophical arguments are interesting, but offer no testable data or predictions. Few areas in science have expanded as rapidly in recent years as population ecology and evolution is central to the discipline. Indeed population ecology might be more appropriately called applied evolution. In spite of the upsurge in interest and federal support, the most basic of all population ecology questions remains unanswered: that of how the population of animals in the natural environment is regulated.

Darwin and others rightly observed that, without human intervention or environmental catastrophe, the population density of most animal species remains nearly constant from year to year. This is amazing when one considers annual differences in rainfall, growing season, temperature, wind and other environmental factors. Population stability is also striking when one considers the reproductive ability of most organisms. Darwin was impressed with the reproductive potential of animals which is seldom achieved under natural conditions; certainly not for long. It is this very point that gave Darwin the mechanism for evolution. He assumed animals reproduce at their maximum physiological potential and these excess ani-

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mals provide the raw material for species "improvement" by natural selection. Modern evolutionists go so far as to suggest that the ability to leave reproducing offspring is the only thing that natural selection ultimately favors.[1] Darwin insisted as do most modern population ecologists that population is regulated by negative outside forces such as starvation, disease, predation, and intraspecific and interspecific competition for resources. It seems unlikely that animals could maintain the highest sustainable yield from their resources without some internal limit. In order to avoid overfishing, man has established fishing limits. Increased fishing effort increases the fish caught but would soon result in over exploitation and diminished returns, Animals too regulate "harvest" of natural resources in part by parcelling out living space into territories. Unlimited reproduction by individuals as postulated by Darwin and modern population ecologists would lead to over exploitation and habitat destruction.

For evolution to be a plausible explanation for the origin and diversity of life, it must have a mechanism. Natural selection is still thought by many evolutionists to be that mechanism. If natural selection can be shown to be inoperative, unnecessary, or nonselective, then evolution would be without a rational mechanism. In contrast, the Creation model has no need for a selective mechanism for "species improvement." Invalidation of natural selection would therefore weaken the argument for evolution, while having no effect on the credibility of Creation as a rational explanation for the origin and diversity of life.

There are two straightforward ways to falsify the concept of selection. If it could be shown that natural selection lacked selectivity, i.e., it was random, then it could have no directive influence. We have long been told that predators are necessary for removal of the weak, diseased, and old prey individuals. There are few scientific data supporting such a "Wild Kingdom" or "Walt Disney" mentality. Indeed, limited observational data seem to indicate mountain lions select the strong, fast and healthy deer over the weak, sick or diseased. If that is the case, then natural selection would be mal-adaptive for the prey species by leaving the inferior as breeding stock.[2] Many fateful predator-prey encounters appear to be random, again negating the selective aspects of "natural selection."

A second way to falsify natural selection as the directive force in evolution would be to demonstrate that natural animal populations are self regulating; that is, that it is recruitment and not negative outside forces that maintain population equilibrium. Wynne-Edwards[3] and others have amassed a large data base suggesting that it is recruitment, not losses, that are regulated! Such an interpretation appears more in line with a Creationist view and has been discussed previously.[4]

The common freshwater arrow headed planarian or flatworm *Dugesia dorotocephala*, provides an excellent model for evaluating population regulation. They are nocturnal free living omnivores found in streams throughout much of North America. Reproduction is sexual and asexual. Sexual reproduction occurs in hermaphroditic adults. Fertilization is internal and stalked eggs are attached to the substrate. Asexual reproduction occurs by fissioning. The posterior end of the worm clings to the substrates while the anterior end crawls away. A tug-of-war ensues with the tail piece finally breaking off. Both pieces regenerate missing parts. No fracture plane has been described. Fissioning is thought to be under control of the brain and was found to occur in inverse proportion to population density.[6,5] Since planaria reproduce asexually, (only growth by mitosis is involved) clonal cultures can be established to reduce genetic variability.

The purpose of this study was to elucidate factors limiting asexual reproduction in planaria, *Dugesia dorotocephala.* A clonal colony was established in order to reduce variability of the data and sufficient experimental replicates were tested to obtain statistical validity. Several factors vary with population density that might alter reproduction. Such factors include worm meeting worm encounters, slime build-up on the substrate, availability of oxygen, or metabolic products such as carbon dioxide or ammonia or possibly some other population density factor. Several types of experiments were conducted in an attempt to isolate and quantify various density dependent factors.

Experimental Methods

On May 1, 1982, a single flatworm was collected from Red Rock State Park, Hinton, Oklahoma (Caddo County). This is the same population previously studied.[7] A clonal colony was established with a population of several hundred worms by October, 1982, when experiments were begun. As over 2,000 flatworms were tested, experiments were conducted throughout the remainder of 1982 and all of 1983. Stock culture and all experimental animals were maintained in non-aerated, non-chlorinated well water with a 12-hour photoperiod. Duration of each experiment was at least 60 days. The first two weeks data were discarded to allow acclimation to experimental conditions. Data are presented as days per fragment per worm. It was calculated as:

$$\text{Days/fragment/worm} = \frac{\text{Total days}}{(\text{Total fragments / number of worms})}$$

Means were compared by Student's t-test and considered different if $P < 0.01$. Semilogarithmic regression equations of the form $Y = A^{BX}$ were calculated by the method of least square. Standard errors were calculated for A and B.

Experiment 1. This experiment was designed to determine the asexual reproduction (fissioning) rate of flatworms as a function of density. Planaria were maintained in 10 ml of water in 25 ml glass vials. Worms were fed three times a week (MWF, 2.33 days) (days fed, feeding interval). Following feeding, the fragments (if present) were counted and removed to the stock culture. Water was decanted, the vial rinsed once, then refilled with 10 ml water. Worms were tested at densities of 1, 2, 4, 6, and 8 worms per 10 ml of water. Eight replicate experiments were used. Surface area for gas exchange was 4.52 cm^2. Surface area or "crawl space" was 21.1 cm^2.

Experiment 2. This experiment is similar to experiment 1 except worms were fed and fragments counted and removed twice a week (MF, 3.5 days). Worms were tested at densities of 1, 2, 4, 6, and 8. Thirty-four replicate experiments were performed.

Experiment 3. A similar experiment was done except animals were fed once a week on Monday. Feeding interval was seven days. Worms were tested at the same densities with 23 replicates.

Experiment 4. A similar experiment was done except feeding was once every other week (feeding interval 14 days). Twenty replicate experiments were conducted.

Experiment 5. This experiment is similar to the previous ones except the water volume was doubled to 20 ml. Densities tested were 1, 2, 4, and 8 worms in each vial. Worms were fed three times a week (MWF, 2.33 days). Surface area for gas exchange was still 4.52 cm^2, however surface area increased to 37.7 cm^2. Eight replicates were performed.

Experiment 6. An attempt was made to determine the relative importance of substrate or water in regulating reproduction. Worms were fed twice a week (3.5 day intervals) and maintained in glass vials as in the previous experiments. Three vials were used in each replicate as follows. Six worms were placed in a clean vial. At each feeding day one test worm was in each of two other vials. Single worms in the two vials were fed and the water discarded. Next, water from the vial with six worms was placed in a clean vial and the "water test" worm transferred into it. The six worms were then transferred into a new clean vial and fed. The "old" vial in which the six worms had been living was refilled with fresh water and the "substrate test" worm transferred into it. Fragments were counted and removed at each feeding. If something on the substrate (such as slime) inhibited reproduction one would expect the single worm moving into the vial lived in by six worms would show reduced fissioning. If, on the other hand, something in the water inhibits fissioning the worm moving into the vial with water lived in by the six worms would show reduced reproduction. Five replicates were tested.

Experiment 7. An attempt was made to determine the natural limits of the planaria under the experimental conditions of this study. Single worms were placed in 10 ml water in glass vials and fragments counted but allowed to remain in each vial. Five replicates in each group were used and four groups tested at feeding intervals of 2.33, 3.5, 7, and 14 days. Population equilibrium required approximately 90 days.

Experiment 8. An attempt was made to determine the effect of substrate or living space on reproduction. Worms were housed in small plastic boxes made for mailing microscope slides. The boxes measured 3.0 x 7.5 x 1.5 cm. Worms were placed in the boxes in 15 ml of water. Two worm densities of 2 and 8 worms per 15 ml were used. One set of slide boxes had no slides in them, the other set contained a slide. The addition of the slide increased substrate area (crawl space) from 40.8 to 61.8 cm^2 (an increase of 51 percent). Surface area in both cases was 2.4 cm^2. Five replicates of each condition were tested.

Results

Increased population density reduced the rate of reproduction in each group tested. Less frequent feeding also reduced reproduction. Results of Experiments 1-4 are summarized in Figure 1 and Table 1. Data with each experiment were highly correlated (correlation coefficient > 0.99). Table 2 contains regression equations. Intercepts (a) were significantly different, but slopes (b) were all similar. The results of Experiment 5 (see Table 2) were not significantly different from the results of Experiment 1 even though twice the water volume was used.

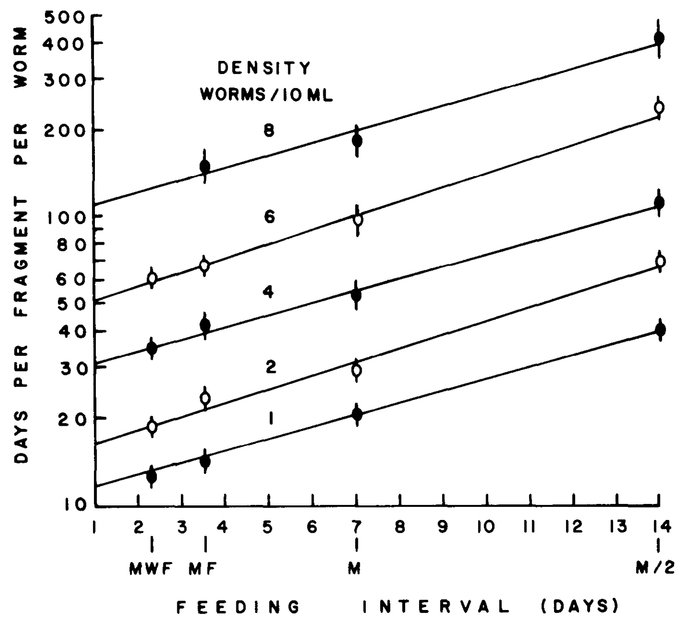

Figure 1. Semilogarithmic relation between feeding interval and rate of reproduction. Circles indicate means and vertical lines represent two standard errors of the mean. Best fit lines were calculated by the method of least squares.

The "substrate test" group of Experiment 6 produced a fragment every 23.0 ± 2.2 days while the "water test" group yielded fragments every 23.6 ± 1.7 days. Results were not significantly different.

Results of Experiment 7 after population equilibration was 7.8 ± 1.6, 11.6 ± 2.1, 7.5 ± 2.2, and 1.5 ± 3.0 respectively for animals fed MWF, MF, M and M/2.

Experiment 8 yielded the following number of days per fragment per worm.

Density/15 ml	40.8 cm^2 Substrate	61.8 cm^2 Substrate
2	40.3 ± 2.4	27.4 ± 3.0
8	90.5 ± 5.5	49.8 ± 2.0

Discussion

In each experimental group, increasing worm density reduced the rate of asexual reproduction. Experiments 1-4 (Figure 1, Table 1) clearly show the effects of density and feeding frequency. Reproduction appears to be more closely linked with density than with feeding frequency. For example, consider a density of 2 worms/10 ml fed twice weekly. If feeding is reduced to once a week, reproduction drops from one

Table I. Results of worm density and feeding frequency on asexual reproduction in flatworms, *Dugesia dorotocephala.* See text for experimental conditions. Units are day/(worm fragment/worm).

Experiment	Feeding	1	2	4	6	8
1	MWF	12.8 ± 0.7	18.8 ± 0.05	35.1 ± 2.7	61.5 ± 2.7	—
2	MF	14.5 ± 0.8	23.3 ± 1.5	42.9 ± 4.4	68.5 ± 3.8	150.2 ± 22.7
3	M	21.2 ± 1.3	29.5 ± 1.8	54.3 ± 4.2	98.6 ± 9.1	188.7 ± 19.7
4	M/2	40.7 ± 4.2	70.9 ± 8.4	115.9 ± 9.8	224.0 ± 41.4	432.0 ± 50.9
5	MWF	13.1 ± 0.6	18.8 ± 1.3	35.4 ± 0.7	93.7 ± 2.6	—

fragment every 23.3 days to one every 29.5 days — a difference of 6.2 days. If instead, one moves from 2 worms/10 ml fed twice a week to 4 worms/10 ml fed twice a week, reproduction is reduced to a fragment every 42.9 days or a change of 19.6 days. The same data (from Experiments 1-4) were replotted in Figure 2 showing the relation between feeding frequency and reproduction. Again, the effect of crowding is dramatically illustrated.

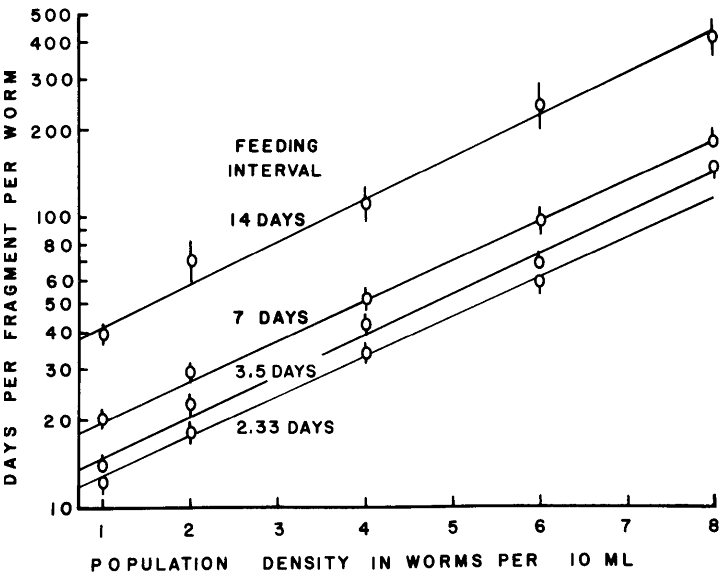

Figure 2. Semilogarithmic relation between population density and rate of reproduction. Symbols and calculations the same as in Figure 1.

The results of Experiment 5 help to isolate what factor of density is most important in reducing reproduction. In this experiment, 20 ml of water instead of 10 ml was used. Any inhibiting factor was therefore reduced by the additional water volume and the worms had more substrate (crawl space). Fissioning however was not changed significantly (Table 2). Area for gas exchange remained the same suggesting it may be some diffusible component such as O_2, CO_2, or NH_3 that limits reproduction. Following feeding, worms were often observed near the surface or actually clinging to the water surface. This was especially common in high density vials. One would expect oxygen consumption (as well as carbon dioxide production) to be highest immediately after feeding.

Experiment 6 further suggests neither substrate nor something in the water inhibit reproduction as neither "used water" nor pre-slimed substrate reduced reproduction; this was interesting as slime had been postulated as inhibiting reproduction by previous workers.[8]

Experiment 7 clearly shows that flatworms are capable of regulating their own population density without negative outside forces such as predation, starvation, disease. In three of the four feeding schedules, population densities were maintained independent of feeding frequency. Such regulation strongly suggests internal control involving feedback and some census or measure of the population as has been postulated by Wynne-Edwards.[9] It is also significant that worms maintained a healthy density with no obvious detrimental crowding effects.

Experiment 8 seems to suggest a substrate or crawl space component. Increasing crawl space and thus reducing worm/worm encounter increased the rate of reproduction.

Conclusions

Data from these experiments clearly indicate that the planarian, *Dugesia dorotocephala,* can regulate its population density apart from the so-called Darwinian checks. Negative outside forces such as starvation, predation or disease were not necessary for population homeostasis. Worm density could also be regulated independent of feeding frequency. Such data strongly suggest the role of natural selection has been over emphasized both as a factor in population control and more importantly as a mechanism for evolution. Implications of internal population regulation weaken the entire evolution scenario. Animals are not reproducing at their physiological limit, but are merely replacing losses. Natural selection, as defined by the

Table II. Experiment summary and regression equations of the form $Y = A^{BX}$ where X is the worm density and Y is the number of days per worm fragment per worm (see text).

Experiment	Feeding	Volume	A	B	Correlation
1	MWF	10	9.76 ± 0.46	0.31 ± 0.013	0.998
2	MF	10	11.36 ± 0.96	0.32 ± 0.017	0.995
3	M	10	15.67 ± 0.31	0.31 ± 0.003	0.999
4	M/2	10	32.30 ± 2.86	0.33 ± 0.02	0.995
5	MWF	20	10.59 ± 0.81	0.28 ± 0.02	0.996

evolutionist is not natural. An even grander design than evolution is evident. Animals were designed with the ability to avoid over-exploitation of their habitat without the need for disease, predation or starvation. Only recently is man learning to limit the harvest of renewable resources to assure the highest sustainable yields. Design in nature implies a Designer. "And God saw all that he had made and behold it was very good." (Genesis 1:31a).

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EDUCATIONAL COLUMN

TEACHING ABOUT ORIGIN QUESTIONS: ORIGIN OF LIFE ON EARTH

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Abstract

In the first article (CRSQ 21:115-19) in this four-part series the author stated the validity of two fundamentally contrasting viewpoints about origins; and in doing so, he provided objective, scientific data for (1) Total Creationism (based upon belief in Eternal, Personal Creator God who created all things), and (2) Total Evolutionism (based upon the belief that all things derived from some Eternal, Impersonal Matter-Energy condition,). By emphasizing limitations of proper, orderly scientific endeavor, he delineated that scientists deal with two kinds of inquiries: (a) inquiries to explain "present" natural phenomena (leading to the science of cosmology, for example), and (b) inquiries to explain unobservable origins of aspects of the "present" natural environment (leading to "Historical" Theories, such as cosmogonies). In a second article (CRSQ 21:189-94) he concentrated upon differences between the methods of cosmologists and cosmogonists, and presented itemization of circumstantial evidence for an Evolution Model and Creation Model about the origin of the universe. This article contains discussion of specific examples and illustrations of the above as applied to teaching about the origin of life on the earth.

Introduction

Science, as a proper and orderly profession, entails specifically the direct and/or indirect, *repeatable* observation(s) of *natural* objects and/or events that occur or exist in the physical environment.

Total Creationism (based upon belief in Eternal, Personal Creator God Who created all things), and Total Evolutionism (based upon the belief that all things derived from some Eternal, Impersonal Matter-Energy condition) involve unnatural objects and/or events (singularities). Thus these viewpoints cannot possibly be submitted to scientific study. Nevertheless, professionally qualified scientists of the majority do present objective, scientific facts in support of Total Evolutionism; and, also, professionally qualified scientists of the minority do present objective, scientific facts in support of Total Creationism, as listed in a previous article (CRSQ 21:115-19) in this series.

Whereas changing descriptions of the structure of the universe can be handled collectively under the term "cosmology," and ideas of scientists about the origination and generation of the universe can be subsumed under the term "cosmogony," there are at least two main ideas of scientists about the origin of life on the earth *in addition* to the majority position. Many, many modern biologists and biochemists accept the "conventional wisdom" about some sub-microscopic origin of life on the earth, but other scientists favor the idea that life came to earth from outer space; yet, a minority of scientists opt for the traditional, theistic view of origin of life on the earth (more on these latter concepts in other sections of this article).

Again, modern scientific endeavor is focused on the "present." Although developments regarding gene manipulations and synthesis and transfer of genes are "frontier" aspects of modern biology, nevertheless the ultimate origin of life on the earth is beyond application of scientific methodology. Biologists are not able to study scientifically the origin of life on the earth, as has been admitted by Bernal, Dixon, Mora, and other scientists. In short the principles of experimental science do not apply to discussions about the origin of life on the earth.

Modern Majority Position Evaluated

But what is the present position of the majority of biologists? According to their mechanistic, materialistic view of the universe, all reality came into existence through "evolution." Thus proponents of this view insist that life arose on the earth (or somewhere in the universe) from inanimate matter through chemical and physical processes still operating today. (A brief summary of this "chemical evolution"— sometimes called "molecular evolution"— is provided in Table I.) However, in order to protect the integrity of proper,

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