Exocytosis in *Paramecium tetraurelia*: Trichocyst Function in Prey-Predator Interactions

Ana M. Eduardo
University Undergraduate Fellow, 1986-1987
Texas A&M University
Department of Biology

APPROVED

Fellows Advisor: _____

Honors Director: _____

Acknowledgments

The research in this work has been extensive and involved the following people whose contributions have been instrumental in making this research successful: Dr. Karl Aufderheide, associate professor in the Department of Biology, for the use of his laboratory and equipment and for his invaluable guidance throughout this project which were critical in carrying out the experiments; Susan Sharp, Ph.D. candidate, for her help in experimental setups, keeping stocks of specimens alive, analyzing data, and her never-ending wit; Dr. Thomas Sharp, Department of Chemistry, for the use of his computer in data analyses; Myrtle Schneller, for invaluable advice about the techniques in the handling of Paramecium; Deborah Gamble, Ph.D. candidate, who taught me how to use OBS Wylbur and the different statistical methods; Dr. Omer Jenkins, Department of Statistics, for taking the time to analyze the results of my experiments and suggesting appropriate statistical tests; and finally, my parents; my sister, Amy; and my roommate, Patricia Payne; for their encouragement and support throughout this project.

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Abstract

The ciliophoran Paramecium tetraurelia, has large exocytotic (secretory) organelles called trichocysts. Although considerable work on the development, genetics and physiology of the trichocyst as an exocytotic organelle has been done, the function of the organelle is still unknown. One venerable hypothesis of trichocyst function is that its violent exocytotic release serves as a defense against predator attack. This hypothesis was tested in the laboratory through two types of experiments. The predator used in the research was Didinium nasutum, another ciliophoran. Wild-type P. tetraurelia, \$51s, and a mutant conferring loss of trichocyst function, nd7, were used as prey. The first approach (competition survival) involved exposing mixtures of precise numbers of wild-type cells and mutant cells (unable to release trichocysts) to the predator. A fixed time for interaction between the two species was allowed and the surviving paramecia were isolated and tested for trichocyst function. Variations of this involved increasing the intensity of the predation while maintaining a constant prey population. The second set of experiments consisted of measuring the times required for successful predation. Search times were defined as the time from predator introduction to the point of successful attack (i.e. capture of prey). Handling time was the interval from the

successful attack until total engulfment of the prey. If trichocysts serve to protect cells from predation, then normal cells should have a better chance for survival than cells unable to release trichocysts. Comparison of the results suggested that the competition survival experiments were the most appropriate for testing this hypothesis. Analysis of the data obtained through t-tests indicates that wild-type cells have a slight but significantly better chance of escaping predation than trichocyst-defective mutants. The results suggest that trichocysts may have a subtle role in defense, at least from certain kinds of predators.

INTRODUCTION

The ciliophoran Paramecium tetraurelia contains exocytotic organelles known as trichocysts. Trichocysts are membrane-bound and consist of an ovate body and a pointed tip. They have been aptly compared in shape to "a carrot with a golf tee attached". Mature trichocysts are 5 micrometers long, have a diameter of about 2 micrometers, and are located perpendicular to the cell surface in the cross-ridges between cortical units (Bannister, 1972; Ehret and McArdle, 1974). Trichocyst are composed mainly of a 36,000 molecular weight protein dimer consisting of two 17,000 molecular weight monomers (Adoutte, et al., 1984). Bannister (1972) documented the ultrastructural details of the components of trichocysts: The body consists of a crystalline matrix with a periodic spacing of lattices, and the tip can be resolved into an inner sheath, an outer sheath, an outer microtubular ring. The outer sheath of the tip is composed of scattered filaments and a cylindrical array of microtubules embedded in an irregular granular matrix. This outer sheath completely encloses the tip of the trichocyst, except the base. Once the trichocyst is discharged, the outer sheath disintegrates. The inner sheath is separated from the outer sheath by a narrow space. The inner sheath consists of two concentric layers and ranges from the apex to

the base of the structure. This sheath has a loose network arrangement of fine fibrils which enlarges into areas of dense material at intersection points of overlapping layers. Upon discharge of the trichocyst (exocytosis), the inner sheath remains intact, and the outer microtubular ring and the membranous sac are left intact within the cell (Bannister, 1972). Trichocyst discharge is associated with a five fold increase in the length of the organelle, and can be accounted for by an increase in the periodic distances of the components of the paracrystalline lattice in the trichocyst body.

Trichocyst formation and maturation follows a developmental sequence which begins with the synthesis of component proteins in the endoplasmic reticulum followed by their processing through the Golgi apparatus (Beisson, et al., 1980; Lefort-Tran, et al., 1981). The membrane bound vesicle emerging from the Golgi apparatus will subsequently develop an internal paracrystalline array of the major constituent proteins. After morphogenesis is completed, the trichocyst then migrates to the cell surface (Pollack, 1974; Aufderheide, 1978). Trichocyst attachment to the cell surface is associated with the rearrangement of intramembranous particle arrays in the plasma membrane at the attachment site (Beisson, et al., 1976, 1980). One array, the "rosette", is an intramembranous set of protein particles in the plasma membrane which has calcium-activated ATPase activity (Plattner, et al., 1973; 1977). The developmental

stages of trichocyst morphogenesis have been reconstructed by detailed phenotypic analysis of various mutant cells unable to produce functional trichocysts (Lefort-Tran, et al., 1981). Trichocysts are discharged, or exocytotically released, following various physical or chemical stimuli (Wessenberg and Antipa, 1970; Pollack, 1974). The mutant strain of Paramecium tetraurelia used in this study (nd7) lacks a complete rosette of proteins in the trichocyst insertion site at the cell surface (Lefort-Tran, et al., 1981). This defect produces inserted trichocysts that lack the ability to be discharged when cells are exposed to standard exocytotic stimuli.

Despite the knowledge concerning the development and the discharge of trichocysts, there is still little agreement as to their function. A number of hypotheses for the function of trichocysts have been suggested. One theory states that trichocyst discharge occurs through abnormal stresses and serves as a way for the cells to attach to the various environmental surfaces (Saunders, 1925; Hyman, 1940; Jones and Cloyd, 1950). Another theory suggests an osmoregulatory role for the trichocysts to remove salts from the cell interior (Wohlfarth-Botterman, 1950; 1953). Still other theories of trichocyst function include injury expression (Jennings, 1906), as a structural component of the cell cortex (Ehret and McArdle, 1974), or as a response to excitation (Vivier, 1974). The most commonly expressed theory of trichocyst function is that they serve as defensive

organelles (Pollack, 1974). This theory was based upon observation of trichocyst discharge by Paramecium when attacked by a predator Didinium nasutum (Mast, 1909). It was reported that the predator became entangled in a mat of trichocysts which aided the prey to escape (Mast, 1909; Wessenberg and Antipa, 1970). It has also been reported that Paramecium capable of expelling trichocysts are less likely to be caught by a predator (Root, 1914).

The ciliophoran Didinium nasutum was the predator of choice in this experiment due to earlier studies of predator-prey selection using Didinium and Paramecium. When Didinium is fed exclusively one species of Paramecium, Berger (1979; 1981; 1982) reported that it will tend to prefer that species when presented a choice of different prey species. This phenomenon is termed dietary imprinting (Berger, 1981). D. nasutum has two rows of cilia which aid in a clockwise movement (Mast, 1909). Anteriorly, the Didinium has a proboscis which is used for attacking its prey. Upon attack, there is an exocytotic discharge of strand-like organelles known as toxicysts and pexicysts from the central core of the proboscis (Wessenberg and Antipa, 1970). Toxicysts are flexible and strongly attached to the prey. After Didinium has successfully captured Paramecium, the Didinium dialates its proboscis in order to swallow its prey. The prey usually dies within a few minutes if not swallowed (Wessenberg and Antipa, 1970). A third type of extrusive organelle found in Didinium is called a cyrtocyst. These are located randomly

throughout the surface with the exception of the proboscis (Wessenberg and Antipa, 1970). Unlike Paramecium trichocysts, physical or chemical stimuli were unable to cause discharge of the extrusive organelles in the proboscis of Didinium (Wessenberg and Antipa, 1970).

As stated previously, the popular theory to be tested concerns the defensive function of trichocysts (Mast 1909; Wessenberg and Antipa, 1970). The hypothesis of this research is that trichocysts confer a survival advantage for the Paramecium under predation from Didinium. The results expected in the experiments would then show a significantly greater chance for survival of wild-type cells (trichocyst competent) over that of the trichocyst defective mutants.

METHODS

The organisms used in this research include: wild-type Paramecium tetraurelia, stock 51s, mating type 0 (ATCC 30300); a mutant of P. tetraurelia lacking trichocyst discharge competence, nd7 (from the laboratory of J. Beisson, CNRS, Gif-sur-Yvette, France); Paramecium caudatum, syngen 3 (from the laboratory of K. Hiwatachi, Biological Inst., Tohoku University, Sendai, Japan); Paramecium multimicronucleatum, syngen 2 (from Carolina Biological Supply Co.); and Didinium nasutum (from Wards National Science Est.).

The medium used for culturing and washing the organisms is a standard 0.25% Cerophyl extract (Sonneborn, 1970), buffered with 5.25 mM sodium phosphate (pH 7.2) and augmented with 5 mg/l stigmasterol. The medium is bacterized before use with a non-pathogenic strain of Klebsiella pneumoniae (ATCC 27889) and incubated at 34 degrees Celsius overnight.

Prior to all the experiments, the predator Didinium nasutum was reared on various types of Paramecium for approximately four days to produce dietary imprinting (Berger, 1979, 1980, 1981, 1982) upon a particular prey type. Before use, the didinia were starved in bacterized media at 27 degrees Celsius for approximately twenty-four hours.

Competition survival tests were done by introduction of five didinia ("very light predation") into a small volume

(about 45 microliters) of medium containing fifteen wild-type and fifteen mutant paramecia. After approximately twenty minutes, a drop of saturated aqueous solution of picric acid was added to the drop containing the surviving prey and predators. The picric acid kills the cells and also causes the discharge of trichocysts from those paramecia capable of responding (Pollack, 1974). The numbers and phenotypes of paramecia which had escaped predation were determined using dark field microscopy (Pollack, 1974). Variations of this experimental approach involved increasing the intensity of predation. A "light predation" test consisted of 15 didinia added to the 30 paramecia, while "heavy predation" used 20 didinia. The time of interaction for these latter two versions of the competition test was two hours.

Statistical analysis consisting of arc sin transformations of the ratio of survivors/15 for each run, followed by paired t-tests comparing wild-type and mutant survival. The formula for the transformations is: arc sin (survivors/ 15) (Fisher, 1948; Sokal and Rohlf, 1969). The t-value obtained was then used to estimate significance.

Another approach to measuring differential predation susceptibility consisted of measurement of the times of attack and feeding as defined by Berger (1982). To a small volume containing thirty paramecia of one phenotype, one Didinium was introduced, and the times of attack and feeding were noted and recorded. Search time was defined as the time from introduction of the predator until a successful attack

is accomplished. The handling time was measured from the successful attack until total engulfment of the prey (Berger, 1982). The search and handling times for two hundred trials were recorded. The prey were either wild-type or mutant P. tetraurelia and the didinia were imprinted upon one of the four Paramecium types described above. Analysis of variance tests and comparisons of means and standard deviations on the means of the different prey types and different imprinting types were used to determine significance in the defensive role of trichocysts (Fisher, 1948; Sokal and Rohlf, 1969). These values were determined through use of SAS and OBS Wylbur.

RESULTS

The very light predation introduced 5 didinia into a prey population containing fifteen cells each of wild-type (\$51s) and fifteen cells of the mutant nd7. Prior to the trials, the didinia were imprinted on either \$51s or nd7. The experiment was repeated 20 times for each imprinting type.

The results (Table I) of the experiments, using didinia imprinted on \$51s, showed a significantly better survival rate for wild-type prey over that of the mutant ($t = 2.72$, $P < 0.01$). Tests using a pooled chi square test (Fisher 1948, Sokal and Rohlf, 1969) were abandoned because the direction of the didinia's preference can not be taken into account. The data indicate that there was a tendency for the \$51s to survive in slightly greater numbers in most of the trials (Table II). The raw values (Table I) were converted via arc sin transformations prior to analysis using the paired one-tailed t-test (Fisher 1948, Sokal and Rohlf, 1969). With this significance value, we cannot reject the hypothesis that trichocysts confer an advantage in survival from predation. However, in the data obtained from the didinia imprinted on nd7 (Table III), no significance was obtained ($t = 0.37$, $P > 0.25$). The data also showed \$51s survived in greater numbers in only slightly more than half of the twenty trials (Table

IV). With the lack of significance in this experiment, the strong significance seen in the previous data set may indicate a subtle advantage for the wild-type Paramecium.

Variations of the competition survival experiments involved an increase in the intensity of the predation (Table V and Table VI). The experiment consisted of twenty trials in which a fixed number of didinia were introduced into a population of 15 $51s and 15 nd7 cells. The levels of predation were somewhat arbitrarily set as: heavy predation = 20 didinia and light predation = 15 didinia. Following predator introduction, a interaction period of two hours elapsed. In the data set involving didinia imprinted on $51s (Table V), values for light predation and heavy predation were: $t = 0.42$, $P > 0.25$; $t = 0.57$, $P > 0.25$ (18 degrees of freedom), respectively. And for the didinia imprinted on nd7 (Table VI), the values obtained were $t = 0.27$, $P > 0.25$ for the light predation. The heavy predation using the didinia imprinted on nd7 yielded the following values : $t = 1.33$, $P < 0.10$. These values were obtained through arc sin transformations and one-tailed t-tests at nine degrees of freedom. The pattern differed from the very light predation studies in that the nd7 survived in greater numbers in the majority of the trials run.

Another set of experiments were performed to eliminate the possibility of influence by imprinting and starvation on the data. These experiments were similar in setup to the competition survival involving very light predation. The

didinia introduced to the prey population were previously imprinted on P. multimicronucleatum (Table VII). The data indicated a significantly increased chance of wild-type survival over that of the mutant ($t = 2.42$, $P < 0.025$). This experiment would tend to eliminate imprinting as an influence in the results because of the small number of runs performed. Also, the pattern of \$51s prey as the majority survivors in a majority of the runs was evident. To test the effect of a 24 hour starvation of the predator upon the data, didinia imprinted on P. multimicronucleatum were fed once on P. multimicronucleatum prior to the competition survival experiments (Table VIII). It was possible that extremely hungry didinia would not exercise any prey selection but would attack any potential prey. This effect could mask any subtle defensive advantage conferred by functional trichocysts. However, this experiment produced no significant trends in prey selection ($t = 0.73$, $P > 0.25$). From this experiment, the starvation duration appears to play little role in the data obtained.

The second major approach towards testing the hypothesis involved search and handling times. Search times were measured as the time from introduction of the predator until the predator achieves a successful attack. The handling times were defined as the interval from the time of successful attack until total engulfment of the prey (Berger, 1979, 1980, 1981, 1982). Prior to actual testing, the didinia were imprinted on four types of Paramecium: nd7

$51s, P. caudatum, and P. multimicronucleatum. A population of 30 cells of one phenotype (nd7 or $51s) were used in each of the 25 trials of each type of imprinted didinia. The mean search times and standard deviations are recorded on Table IX for each of the four imprinted Didinium nasutum types. A general linear model analysis performed on the data following a conversion to logarithm values, produced an F value of 1.96 with a P value of 0.06 (Fisher 1948, Sokal and Rohlf, 1969). The conversion to logarithm values allowed the distribution curve to be normalized and suppresses the long tail of the data at higher times. When analyzing the source of the slight significance, the interaction of the prey and the imprinting showed a P value of 0.02 and F= 3.44. An expected observation of search times, assuming that trichocysts have a defensive role, would show a greater search time for the prey $51s than for the mutant. However, this did not prove to be true as seen in the GLM analysis. The general linear model analysis pools all the data and calculates an F-value for the model, prey, imprinting, and prey-imprinting interaction. From the F-values, significance is determined.

The handling time data (Table X) were treated in a similar manner with the conversion to logarithm values followed by a GLM analysis. An F value of 6.83 and a P value of 0.0001 was obtained. The sources of the significance was found in the imprinting (F= 9.05, P= 0.0001) and the interaction of the prey and the imprinting (F= 6.90, P=

0.0002). However, the prey type yielded no significance (F= 0.66, P= 0.4164). The expected result for handling times, assuming that trichocysts confer a defensive advantage, would be a longer value for the wild-type than the mutant. From the data reported in Table X, one can see that the data are consistent with regard to the influence of imprinting. From dietary imprinting studies, didinia imprinted on a certain Paramecium type preferred that type when given a choice (Berger 1979, 1981, 1982). This is evident in the didinia imprinted on \$51s and nd7. As previously stated, the \$51s was expected to have greater search time and handling time mean values than the nd7 mutant, indicating a defensive role for trichocysts. Overall, the nd7 prey required a greater search time than the \$51s prey (30.04 sec compared to 23.74 sec). However, the handling times show that the \$51s prey had a smaller mean time than the nd7. The mean handling time values for nd7 and \$51s regardless of imprinting are 41.42 sec and 39.49 sec, respectively. Because of the small difference in the means of both the search times and handling times between the two prey types and the large variability in the data sets, this experimental approach provided no critical means to test the hypothesis.

DISCUSSION

From the results, the experiments done involving the very light predation provided the most useful information. The experiment allowed didinia to make a choice between the two types of prey. The statistical manipulations used (i.e. arc sin transformations of ratio of survivors) permit one to determine the direction of preference the didinia seemed to show for attack of the prey, nd7. As seen in the results, Table I and III, significance was obtained for the didinia imprinted on $51s. This may indicate that trichocysts confer a slight advantage in defense against predation by D. nasutum. Whether this defense function operates against other species of predators is unknown.

The work involving the search and handling times did not prove helpful in testing the hypothesis because the standard deviations obtained (Table IX and X), were too large to identify any possible significance in the mean values. Evidence of dietary imprinting as described by Berger (1980) was seen in the search times (Table IX).

The experiments dealing with light and heavy predation, did not provide significance (Table V and VI). It was believed that because the interaction time was increased to two hours, the didinia would have time for multiple meals and therefore might show a preference for a particular type of prey. This did not prove to be true as seen from the t-

values determined for the data (Table V and VI). Many of the values obtained for the heavy predation yielded no survivors and therefore could not be calculated accurately in the one-tailed paired t-tests.

Whether the imprinting or the starvation of the predator influenced the results were also tested. Didinia imprinted on P. multimicronucleatum were used in competition survival tests at the level of very light predation. The values (Table VII) obtained, showed a trend for better survival of wild-type cells, similar to those obtained from Table I and III. This experimental approach should be tested further to see if the survival pattern continues.

Starvation of the didinia for twenty-four hours may have influenced their behavior such that they attacked the first prey they came upon, regardless of prey defenses. A limited number of trials were run in which didinia imprinted on P. multimicronucleatum were fed once on P. multimicronucleatum and allowed to digest their prey. After 1-1/2 to 2 hours, the didinia were put into the competition survival test with very light predation. An interaction time of 20 minutes and the addition of picric acid was done. The results obtained again showed no difference in survival patterns (Table VIII). The paramecia with trichocysts survived in greater numbers in a slight majority of the trials completed. Further testing will have to be done in this area also to show no evidence of any effect of starvation upon prey choice.

Statistical analysis permits one to discern from the

very light predation competition survival tests that cells with trichocysts may have a slight defensive advantage over cells without trichocysts. Given the usually sparse, clumped distributions of paramecia and didinia, we have reason to feel that the very light predation regimen may be a useful model for the interaction of these species in the wild.

Trichocysts make up nearly ten percent of the cell's protein content (Adoutte, et al., 1984). Evolutionarily, the large amount of protein allotted for this organelle would seem to indicate an important function for that organelle. The results presented here in the very light predation seem to suggest that one aspect of trichocyst function may be the provision of a slight defensive advantage from Didinium predation. Though the evidence is slight, further testing and experiment design may prove to beneficial in assessment of the hypothesis. The repeat of these tests using other paramecial predators (e.g., suctoria, Bursaria trumcatella, and Chaos carolinense) may be of use.

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The following shows sets of data obtained from the three types of experiments performed. Abbreviations used for the different types of Paramecium: nd7 = mutant P. tetraurelia with no trichocysts; $51s = Stock 51s of P. tetraurelia (wild-type).

Table I.
COMPETITION SURVIVAL EXPERIMENTS: VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON $51s (TRICHOCYSTS)

RUN	NUMBER OF SURVIVORS	
	nd7	$51s
1	13	14
2	11	12
3	9	11
4	11	13
5	11	12
6	11	12
7	11	12
8	10	14
9	10	12
10	13	11
11	12	13
12	11	13
13	12	13
14	11	12
15	9	14
16	11	12
17	12	11
18	13	10
19	11	11
20	13	14

**t= 2.72, P < 0.01

Table II.

COMPETITION SURVIVAL EXPERIMENTS

VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON WILD-TYPE (TRICHOCYSTS)

Number of runs	Mean number of survivors	
	nd7	$51s
20	11.25 (SD= 1.21)	12.30 (SD= 1.17)

t= 2.72, P < 0.01

Runs with majority survivors:

nd7	$51s
3	16

Table III.
COMPETITION SURVIVAL EXPERIMENTS: VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON nd7 (NO TRICHOCYSTS)

RUN	NUMBER OF SURVIVORS	
	nd7	$51s
1	10	13
2	8	9
3	14	10
4	9	10
5	8	12
6	8	8
7	13	10
8	10	12
9	12	10
10	9	12
11	10	12
12	9	13
13	14	10
14	12	8
15	12	13
16	12	10
17	14	11
18	12	11
19	9	15
20	11	12

**t= 0.37, P > 0.25

Table IV.

COMPETITION SURVIVAL EXPERIMENTS

VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON nd7 (NO TRICHOCYSTS)

Number of runs	Mean number of survivors nd7	Mean number of survivors $51s
20	10.80 (SD= 2.07)	11.05 (SD= 1.79)

t= 0.37, P > 0.25

Runs with majority survivors:

nd7	$51s
8	11

Table V.
COMPETITION SURVIVAL- HEAVY AND LIGHT PREDATION

IMPRINTED ON $51s (TRICHOCYSTS)

PREDATION TYPE	RUN	NUMBER OF SURVIVORS nd7 (no trichocysts)	$51s (trichocysts)
LIGHT 15 didinia	1	6	6
	2	5	6
	3	5	6
	4	5	2
	5	11	9
	6	11	7
	7	6	6
	8	3	8
	9	7	7
	10	3	9
	**t= 0.42, P > 0.25		
HEAVY 20 didinia	1	3	2
	2	0	1
	3	0	1
	4	1	1
	5	2	1
	6	1	3
	7	0	1
	8	0	1
	9	1	1
	10	2	0
	**t= 0.57, P > 0.25		

predation type	number of runs	mean nd7	mean $51s	Runs with majority survivors nd7	Runs with majority survivors $51s
light	10	6.20 (SD= 2.82)	6.60 (SD= 2.01)	3	4
heavy	10	1.00 (SD= 1.05)	1.20 (SD= 0.79)	3	5

Table VI.
COMPETITION SURVIVAL- HEAVY AND LIGHT PREDATION

IMPRINTED ON ND7 (WITH NO TRICHOCYSTS)

PREDATION TYPE	RUN	NUMBER OF SURVIVORS nd7 (no trichocysts)	$51s (trichocysts)
LIGHT	1	2	5
15 didinia	2	4	4
	3	7	1
	4	4	2
	5	2	1
	6	2	3
	7	1	2
	8	3	4
	9	1	4
	10	1	1
	**t= 0.27, P > 0.25		
HEAVY			
20 didinia	1	5	3
	2	2	0
	3	5	2
	4	1	2
	5	7	2
	6	1	0
	7	2	3
	8	4	4
	9	2	4
	10	2	2
	**t= 1.33, P < 0.10		

predation type	number of runs	mean nd7	mean $51s	Runs with majority survivors nd7	Runs with majority survivors $51s
light	10	2.70 (SD= 1.89)	2.70 (SD= 1.49)	3	5
heavy	10	3.10 (SD= 2.02)	2.20 (SD= 1.40)	5	3

Table VII.

COMPETITION SURVIVAL EXPERIMENTS

VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON *P. multimicronucleatum*

Run	Survivors	
	nd7	$51s
1	9	11
2	10	12
3	12	11
4	10	14
5	10	11
6	10	10
7	9	11
8	11	13
9	12	11
10	9	11
Mean	10.20 (SD= 1.14)	11.50 (SD= 1.18)

$t = 2.42$, $P < 0.025$

Runs with majority survivors:

nd7	$51s
2	7

Table VIII.

COMPETITION SURVIVAL EXPERIMENTS

VERY LIGHT PREDATION (5 DIDINIA)

IMPRINTED ON *P. multimicronucleatum*, previously fed

Run	Survivors	
	nd7	$51s
1	14	12
2	13	15
3	14	15
4	15	13
5	10	15
Mean	13.20 (SD= 1.92)	14.0 (SD= 1.41)

t= 0.73, P > 0.25

Runs with majority survivors:

nd7	$51s
2	3

Table IX.

MEAN SEARCH TIMES (SECONDS)

IMPRINTED ON	PREY nd7	PREY $51s
$51s	25.40 (SD= 26.53)	23.44 (SD= 16.20)
nd7	22.36 (SD= 18.71)	24.52 (SD= 18.09)
P. multimicronucleatum	36.88 (SD= 25.13)	27.44 (SD= 22.29)
P. caudatum	35.52 (SD= 30.87)	19.56 (SD= 18.80)
All *Paramecium* types	30.04 (SD= 26.07)	23.74 (SD= 18.90)

GLM Analysis:	F-value	P-value
Model	1.96	0.06
Prey	1.95	0.16
Imprint	0.49	0.69
Prey * Imprint	3.44	0.02

(values were converted to logarithm values prior to GLM analysis)

Table X.

MEAN HANDLING TIMES (SECONDS)

IMPRINTED ON	PREY: nd7	PREY: $51s
$51s	38.32 (SD= 16.70)	46.00 (SD= 15.40)
nd7	50.92 (SD= 23.41)	41.92 (SD= 15.08)
P. multimicronucleatum	27.80 (SD= 9.53)	35.60 (SD= 11.77)
P. caudatum	48.64 (SD= 24.05)	34.44 (SD= 12.13)
All Paramecium types	41.42 (SD= 21.16)	39.49 (SD= 14.29)

GLM Analysis:	F-value	P-value
Model	6.83	0.0001
Prey	0.00	0.95
Imprint	9.05	0.0001
Prey * Imprint	6.90	0.0002

(values were converted to logarithm values prior to GLM analysis)