Competition Study on two species of Paramecia

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Abstract—For the investigation of validity of the Lotka-Volterra model for competition between species, a simple competition was setup between two species of Paramecia which are phenotypically distinguishable. It was found that both these species could co-exist and further, it was observed that their phenotypes began to 'interchange'. An evolution study was planned to test if differentially speedening the process of evolution for one species could lead to its better relative survival.

Keywords—*Paramecium*, competition study, lotka-volterra, evolution, co-evolution

1 Introduction

Evolutionary biology often explains the observed and very rarely predicts, roughly speaking. This experiment was aimed at putting evolutionary ideas to work for predicting the outcome of an experiment. We have been taught that organisms have co-evolved and it therefore makes sense to imagine that the organisms that could evolve faster, would've out-survived their competitors. To test this, we chalked out a two stage plan. The first was to test the prevailing Lotka-Volterra model for competition between two species of *Paramecium*. Second stage was to increase the rate of evolution, selectively for one species and observe the densities, as they compete.

2 Materials

For setting up one culture of a 100 mL volume, we used:

1) Beaker/Conical flask 100 mL
2) Elix Water (roughly 100 mL)
3) Yeast Powder (0.04 g per 100 mL)
4) Wheat Beads (3-4 per 100 mL)
5) *Paramecium* Sample

For finding the Paramecia from the 'wild', the following were used

1) Gloves
2) Centrifuge Tubes (essentially seal-able containers)
3) Pro-pipetter
4) Permanent Marker

3 Method

3.1 Counting *Paramecium*

Since counting is the heart of this experiment, repeatability was a very important concern. We did the following to ensure reliability of the data.

1) Volume for counting was taken to be very small ($10\mu L$ initially, and then dropped to $5\mu L$ finally) to ensure the total number of *Paramecium* are readily countable.
2) Videos were recorded using a digital camcorder, by pointing it through the lens of the eye piece of the Ziess microscope. [1]

We took a total of $100\mu L$ volume from the sample to estimate the densities. The counting was done manually.

3.2 Creating the culture

1) Took $40mL$ of Elix water in the flask.
2) Added 4 wheat seeds to the beaker and boiled it for 2 minutes, using a micro-wave oven.
3) Further, to it, $0.04g$ of finely grinded yeast powder was added immediately and stirred
4) The contents of the beaker were cooled to room temperature.
5) To this, the *Paramecium* culture was added and mixed
6) The volume was made $100mL$ by adding Elix water and mixed gently.
7) The culture created was incubated at 25^oC.

1. The process involved careful, strategic positioning of the right kind of camera, which was discovered experimentally

3.3 Finding the Paramecia

1) At first we decided a location like a small pond, puddle, etc. and then after wearing gloves, extracted some water using pipette and pro-pipetter and dumped the water in a fresh centrifuge tube and marked it using appropriate label.
2) We repeated the above procedure for different depths and also at various other locations in the water body.
3) Then we took a drop of water from each tube in a glass slide and observed it under a microscope and we reported our observations.
4) Once, a particular species of *paramecium* was found, we reported it to Dr. N.G. Prasad [2] and then we had setup the cultures.

4 Apparatus

The following were available and used from the Biology Teaching Laboratory.

1) Zeiss Stemi DV4
2) Pathological Microscope

5 Experimental Procedure

The experiment was designed to run in two stages. The first stage was intended to verify a standard result and the second to test an aspect of evolution.

For both stages, we need model organisms. We had found *Paramecium* in a total of 10 samples collected from the Nala and nearby puddles. $50mL$ cultures were made for each of the 10 samples. (refer Materials and methods) To every $40mL$ of water, $10mL$ of the sample water was added. These cultures were checked after two days of having set up.

Our requirement was to have two samples of Paramecia which were phenotypically (for instance size) distinguishable. We observed relatively large *Paramecium* in one of the samples collected from the Nala, but we did not find *Paramecium* which were much smaller in size than this. So, we used another species of *Paramecium*, available with another group in our lab, which had a fairly smaller size.

We set up $100mL$ cultures for both of these *Paramecium* species. The cultures are referred to as:

1) Flask P: small *Paramecium*
2) Flask Q: large *Paramecium*.

Both the flasks were checked on the next day.

2. He was our Biology Lab Course's Insructor

1) Flask P: Under 4X, only *Paramecium* were seen.
2) Flask Q: Under 4X, small round organisms (referred to as 'Organism X') were seen in high numbers apart from the large *Paramecium*.

Isolating *Paramecium* from Flask Q

$10uL$ of the culture was taken on a glass slide. The drop was diluted with $200uL$ of water. Under 4X magnification, organism X were seen in the bottom part of the drop, while the *Paramecium* were seen floating on the top part. We used a $20\mu L$ micro-pipette, to carefully extract the top part of the drop. This isolated drop was used to set up a $25mL$ culture. However, on the following day, both organism X and the *Paramecium* were found in significant numbers in the flask. Thus the large *Paramecium* could not be isolated.

Motivation behind next step

In competition, the presence of the organism X can be taken as a component of the environment.

5.1 Stage 1: Setting up the Cultures for *Paramecium* Competition Study

Both the Paramecia cultures were allowed to grow for about 7 days after which we safely assumed that both the populations had reached their corresponding carrying capacities. We determined the population densities of Flask P and Flask Q, which essentially gave us the values of their carrying capacities, K_1 and K_2.

Two replicates were set up; namely Flask A and Flask B, with the following parameters

1) Total volume: $50mL$
2) Volume of Flask P culture added: $2.29mL$
3) Volume of Flask Q culture added: $3.92mL$
4) Ambient temperature of the cultures: 25^oC

They were counted daily for a period of 14 days.

5.2 Stage 2: Differentially Speeding up Evolution

For the next stage, our objective was to speed up evolution for the species which was not competent [3] To achieve this, it was decided to expose a set

3. In case only one species survives, the not competent refers to the one that perished. In case both do, the not competent is the one that is not dominant in number.

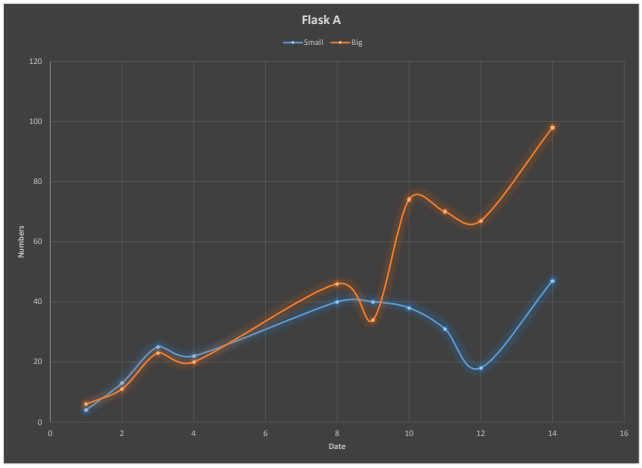

Fig. 1. Flask A

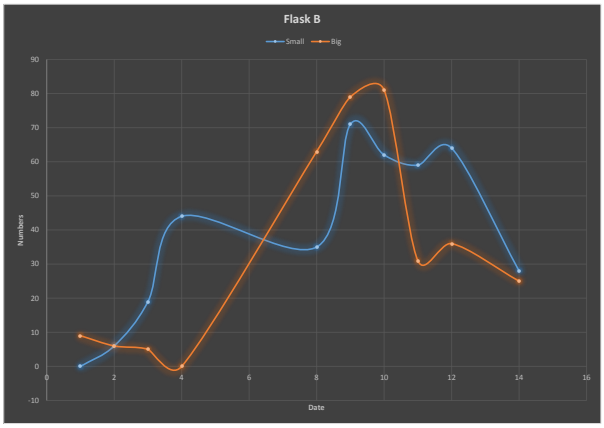

Fig. 2. Flask B

of *Paramecium* to UV, to increase mutation rate, and then immediately use this for competition. This step was expected to increase the variation, and thereby speed up evolution, of the said species. The competition study from here on was to be carried out as described in the first stage.

6 Observations

The observations were made daily for a period 14 days, however not necessarily at at the same time of the day. The time was noted and adjusted for, in the plots drawn.

6.1 Densities

Initial exponential growth was observed in both flasks. Flask A showed a distinct dominance of the larger Paramecia, while in Flask B, no such comment can be made.

Please refer to table 1 and 2 for details. Graph 1 and 2 correspond to the observations given in the table.

6.2 Qualitative Observations

There were two kinds of distinctions between the *Paramecium* species that could be observed initially.

Size: The sizes were roughly in the ratio 3:4

Phenotypic: The smaller one, had a characteristic white spot at the extreme of its body, which was opposite to it's direction of movement. The larger one had, on a large scale, uniform distribution of the white colouration.

However, afterwards, the following distinctions were observed:

Size: There were atleast three different sizes [4]

Phenotypic: The white spot could be seen at times, at the characteristic location for both large and small *Paramecium*. It is implied here, that there were both large and small Paramecia, that didn't have any clear spot.

Spore Formation

Since the water drop size was small, so after evaporation, at extremely low volumes [5], the Paramecia were observed to fuse into one another, viz. conjugate.

Realtime Size Change

Certain Paramecia, increased their size, while under observation.

7 Result and Discussion

The experiment failed in the classical sense of the word, since the observations were 'negative', yet non-trivial. Our first objective was to test the Lotka-Volterra model for two species of Paramecia, which could not be accomplished since the phenotypic distinction between the Paramecia seems to have distorted, to render the results unreliable. While this makes the experiment non-conclusive in the

4. These are most likely not 'baby' Paramecia since in the stock culture, the 'baby' Paramecia were not dominantly visible

5. when the surface area approximately equalled the volume

testing of the model, it does expose the unexpected response to adverse situations, of the Paramecia, which we have neither heard of, nor studied so far.

ACKNOWLEDGMENTS

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APPENDIX

Table 1

DATE (X-04-2013)	Time		1	2	3	4	5	6	7	8	9	10	sum (small)	sum (big)	MAG
01-04-2013	23:00	Small	1	0	0	0	0	1	0	1	1	0	4		32X
20X		big	3	0	0	0	1	0	1	0	0	1	6		
													0		
02-04-2013	17:10	Small	2	0	2	2	0	0	2	0	3	2	13		
32X		big	3	1	0	2	0	0	0	2	2	1	11		
													0		
3	7:40 PM	Small	2	6	1	3	0	2	2	1	4	4	25		
		big	0	3	2	0	3	2	3	3	4	3	23		
													0		
04-04-2013	4:08 PM	Small	3	1	3	2	1	1	3	4	3	1	22		
		Big	5	1	4	0	0	2	4	1	1	2	20		
													0		
5		Small											0		
		big											0		
													0		
6		Small											0		
		big											0		
													0		
7		Small											0		
		big											0		
													0		
08-04-2013	8:05 PM	Small	0	0	5	0	0	5	1	1	5	2	19		< Prashansa
		Big	4	1	2	3	0	3	2	4	3	2	24		
								8		5	8		21		
		Small 2	3	0	4	3	2	1	2	3	2	1	21		
		Big 2	0	0	5	4	2	2	2	3	1	3	22		
													0		
09-04-2013	11:00 PM	small	1	1	8	0	1	2	3	0	0	1	17		
		big	1	3	1	5	2	2	1	0	0	3	18		
													0		
		Small 2	3	0	2	0	3	2	1	4	3	5	23		
		Big 2	2	2	2	0	4	1	3	1	0	1	16		
													0		
10-04-2013	7:30pm	small	0	7	1	1	1	0	1	3	1	1	16		
		big	1	2	6	8	5	3	1	4	14	2	46		
													0		
		small more	2	3	5	2	4	1	1	0	2	2	22		
		big more	3	13	0	0	2	4	2	0	0	4	28		
													0		
11-04-2013		small	3	5	1	2	1	4	1	4	2	1	24		
		big	8	3	2	1	2	3	2	2	2	6	31		
			11	8				7		6	4	7	43		
		small more	1	0	1	0	1	1	1	2	0	0	7		
		big more	4	3	1	3	6	3	7	4	1	7	39		
							7	4	8	6			25		
12-04-2013		small	2	1	2	1	3	1	2	2	2	2	18		
		big	9	9	9	2	6	8	6	7	7	4	67		
			11						8		9		28		
		small more											0		
		big more											0		
													0		
13-04-2013		Small											0		
		Big											0		
													0		
													0		
													0		
													0		
													0		
14-04-2013	22:31	small	3	1	4	4	10	6	3	3	5	8	47		
		big	15	9	5	13	8	7	9	15	10	7	98		
		small more													
		big more													

Table 2

DATE (X-04-201	Time in Hrs		1	2	3	4	5	6	7	8	9	10			remarks
b	23:00	Small	0	0	0	0	0	0	0	0	0	0	0		0
20X		big	3	0	1	0	2	1	0	0	2	0	9		
													0		
															checked under compound microscope no
02-04-2013	17:10	Small	0	1	2	1	2	0	0	0	0	0	6		video taken except for drop 2
		big	0	1	0	0	0	3	0	1	1	0	6		
													0		
03-04-2013	8:15 PM	Small	2	0	0	2	3	1	1	5	4	1	19		
		big	2	0	1	1	0	0	0	0	0	1	5		
													0		
04-04-2013	16:08:00	Small	1	5	8	2	8	4	3	4	5	4	44		
		big	0	0	0	0	0	0	0	0	0	0	0		
													0		
08-04-2013	7:30 PM	Small	2	1	2	0	4	2	4	1	1	2	19		
		big	7	5	0	4	1	1	2	3	4	1	28		
															< Each drop is 5 ul, so total drops are 20, and
													0	35	total volume is still the same
		Small more	1	1	1	2	0	1	2	6	1	1	16	63	
		big more	1	1	4	1	2	6	4	7	3	6	35		
										13		7	20		
	7:55 PM	Small	3	3	0	1	2	1	1	1	1	0	13		
		Big	3	6	0	1	1	4	2	3	0	0	20		< These are totals
09-04-2013	10:30 PM	Small	4	1	2	1	6	8	3	3	3	4	35		
		Big	2	5	2	4	4	3	3	1	2	4	30		
								11					11	71	5 uL each- PRASHANSA
		small more	3	3	6	3	3	3	3	2	3	7	36	79	
		big more	9	14	8	2	5	2	3	1	5	0	49		
			12	17	14	5	8	5			8		69		PRASHANSA
													0		
10-04-2013	7:30pm	small	1	4	4	4	3	6	2	3	2	3	32		
		big	3	8	2	1	3	3	1	1	1	1	24		
				12			6	9	4	4		4	39	62	Prashansa
		small more	4	3	3	6	0	5	3	1	1	4	30	81	5 ul each
		big more	12	11	3	18	4	0	1	3	3	2	57		
			16	14		24	4						58		200ul H2O + 10ul etOH
													0		No difference based on spot.
11-04-2013	23:30	small	1	3	3	1	2	4	2	3	6	3	28	59	
		big	3	5	2	1	3	1	2	1	2	0	20	31	
													0		
		small more	3	1	3	3	3	4	4	5	3	2	31		5 ul each
		big more	3	0	0	1	1	2	2	1	0	1	11		
			6										6		
12-04-2013	22:16	small	4	8	6	1	2	0	1	2	2	5	31	64	
		big	0	2	3	1	1	1	2	1	1	4	16	36	
			4										4		
		small more	2	4	6	4	3	2	3	3	2	4	33		
		big more	0	1	1	1	0	0	3	4	8	2	20		
													0		

Table 2

															0	
															0	
															0	
															0	
															0	
															0	
															0	
14-04-2013	22:31	small	0	5	0	2	0	2	1	2	1	3	16	28
		big	1	1	2	0	4	0	3	2	1	0	14	25
													0	
		small more	0	1	1	0	0	3	2	1	2	2	12	
		big more	1	2	2	1	1	1	0	0	0	3	11	