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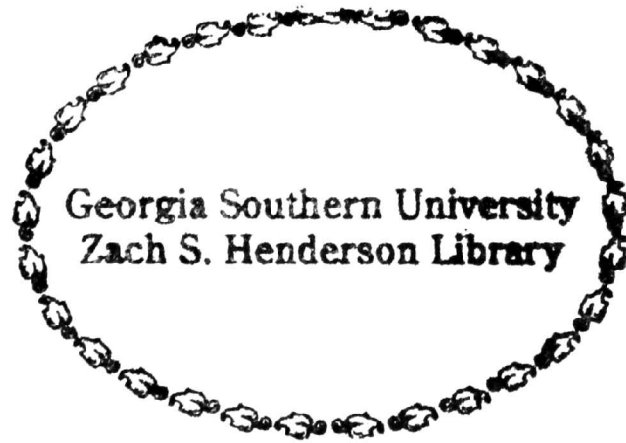
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SPONTANEOUS ALTERNATING BEHAVIOR IN
Paramecium caudatum AND
Paramecium multimicronucleatum

Nyron K. A. Bovell



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SPONTANEOUS ALTERNATING BEHAVIOR IN

Paramecium caudatum AND

Paramecium multimicronucleatum

A Thesis Presented to
the College of Graduate Studies of
Georgia Southern University

In Partial Fulfillment
of the Requirements for the Degree
Masters of Science
In the Department of
Biology

by

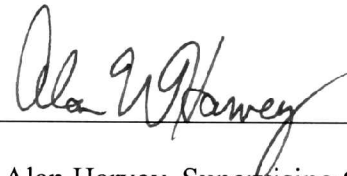
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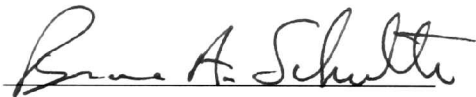
To the Graduate School:

This thesis, entitled “Spontaneous Alternating Behavior in *Paramecium caudatum* and *Paramecium multimicronucleatum*”, and written by Nyron K. A. Bovell is presented to the College of Graduate Studies of Georgia Southern University. I recommend that it be accepted in partial fulfillment of the requirements for the Master’s Degree in Biology.

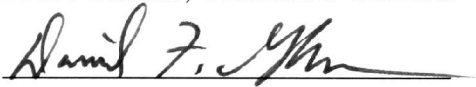


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“The race is not given to the swiftest nor to the strong but to the one that endures it all” (Ecclesiastes 9:11). First and foremost, the author would like to thank the Lord and Savior, Jesus Christ, for giving him the ability and strength to complete this work. For without Him none of this would have been possible. The author would like to give great appreciation to his Major Advisor, Alan Harvey, Ph.D., for his guidance and invaluable input that made everything seem much easier during this period. The author would also like to give thanks to his other committee members, Drs. Bruce Schulte and Daniel Gleason for the advice they provided throughout my research project and in writing my thesis. Appreciation is also given to the Deans and Associate Deans of Graduate Studies, and the College of Science of Technology for the support they provided in difficult times.

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REFERENCES

Available on request

ABSTRACT

SPONTANEOUS ALTERNATING BEHAVIOR IN

Paramecium caudatum AND

Paramecium multimicronucleatum

May 2005

NYRON K. BOVELL

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M.S. GEORGIA SOUTHERN UNIVERSITY

Directed by: Professor Alan Harvey

Spontaneous alternating behavior (SAB) describes the tendency of an organism to spontaneously select the unfamiliar direction in a two choice situation. *Paramecium* is the only microscopic genus in which SAB has been studied. The two earlier studies regarding SAB in *Paramecium* have come to conclusive, but diametrically opposed results. Designing a single new experiment that incorporates the critical differences in the designs of both studies may help to clarify the results from these earlier studies, and provide an excellent opportunity to better understand the factors that influence SAB.

The overall objective of this research project was to determine whether or not SAB exists in two previously studied species of paramecia (*Paramecium caudatum* and *Paramecium multimicronucleatum*). Specifically, the study determined whether:

- 1) maze length or species identity influence the expression of SAB in paramecia;

2) the mechanism that resulted in SAB in short mazes in the earlier experiment was intrinsic or extrinsic in nature; and 3) there were differences in swimming ability between *Paramecium caudatum* and *P. multimicronucleatum*.

SAB occurred in short mazes in both species of *Paramecium*; and maze length influenced the occurrence of SAB in both species. The number of contacts in *P. caudatum* support the use of an extrinsic mechanism to show SAB. Both species of *Paramecium* displayed similar swimming ability (speed and number of contacts on each side of the maze). This experiment further clarified the diametrically opposed findings of the two earlier studies on SAB in *Paramecium*, that is, Lepley and Rice (1952) found SAB because they used shorter mazes, and Lachman and Havelena (1962) did not find it because they apparently used mazes that were too long.

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Chapter I

Animals generally move from one place to another to minimize predation risk (the probability that the animal will be preyed upon), and maximize the quality and quantity of food ingested (Lima and Dill, 1990). Animals with minimal or no movement can suffer higher predation rates than those that are mobile (Banks *et. al.*, 2000). Animals reduce the likelihood of encountering or being detected by a predator, or improve the chances of escaping a predator, by moving and changing their microhabitats. For example, heteromyid rodents and gerbils inhabiting deserts shift their activity to safer bush microhabitats when predation risks are high (Abramsky *et. al.*, 1998).

Animals also move when food patches have abundant predators, harsh environmental conditions, and also if the quantity and quality of food within each patch are unacceptable (Marion *et. al.*, 2005). When a patch does not have enough food, the animal continues to move until it finds a favorable patch. Some studies postulated that animals search for more favorable patches by traveling in a fairly straight line (Dember, 1989; Hughes, 1989). Alternatively, it has also been proposed that some animals search for more favorable patches in a fairly random fashion (Dreisig, 1981).

Animals show two types of movement in the presence of stimuli: kinesis and taxis. Kinesis is a movement made in response to the intensity rather than the

direction of a stimulus, in which case the organism moves randomly until a better environment is found. For example, a cockroach running away from light in a random fashion until it reaches the dark displays a negative photokinetic response. A taxis is the responsive movement of a free-moving organism directly toward or away from an external stimulus, such as light. An animal that moves directly toward light shows positive phototaxis, one that moves directly away from light shows negative phototaxis. Taxes have been used to study various responses by animals to stimuli. For example, Francis and Hennessey (1995) observed negative taxes in *Paramecium tetraurellia* and *Tetrahymena thermophila* in response to an organic, chemical stimulus, guanosine 5' triphosphate (GTP).

Research regarding animal movement has either observed what makes the behavior efficient, or the purpose of the behavior in the presence of stimuli. Tolman (1925) first noted that movement in rats, in the absence of stimuli, was non-random. Later, Dennis and Henneman (1932) observed the same behavior when rats placed in t-mazes without food, alternated in turning pattern. Alternating in turning pattern describes the tendency of an organism to spontaneously select the unfamiliar direction in a two choice situation (that is, given a choice of two directions in which to turn). Dennis (1935) named the behavior Spontaneous Alternating Behavior (SAB).

The proposed advantage of SAB is that it allows movement away from hazardous environments (Taylor, 1995; Hughes, 1978; Dingle, 1965). This hypothesis was tested in woodlice (*Porcellio scaber*) by Hughes (1967) who

concluded that woodlice deprived of food or desiccated, demonstrated increased SAB compared to those that were well-fed or kept moist. Similarly, Carbines *et al.* (1992) investigated whether SAB enabled woodlice to escape efficiently from unfavorable environmental conditions. They found that woodlice displayed increased SAB in the presence of a predatory spider (*Dysdera crocata*). However, Montgomery's (1953) research reported that rats deprived of food or water for 24 hours did not show increased SAB.

Later, SAB has also been found in organisms other than laboratory rats. Most of the species have yielded positive results, but some have not (Table 1). For example, Iwahara and Fujita (1965) failed to observe SAB in oligochaets. Similarly, in a study of left-right preferences in crustaceans, MacKay (1945) reported no SAB. Species difference (some species in the same class do not show SAB) may be one reason why SAB is observed in some organisms but not in others. For example, in the class Crustacea, hermit crabs, *Calcinus herbestei* and *Clibnarius zebra* did not show SAB while terrestrial isopods, *Porcellio scaber*, did (Hughes, 1967, 1966; MacKay, 1947, 1945). Also, SAB was observed in two species of earthworm (*Lumbricus terrestris* and *Lumbricus rubellus*), but not in the species *Dugesia tigrina* (Hughes, 1987; Iwahara and Fujita, 1965; Wyner and Zellner, 1958). A second speculation is that SAB was not shown in some organisms because of behaviors unique to specific species, such as the tendency for organisms to choose the familiar rather than the unfamiliar direction. In MacKay's (1947) and (1945) experiments, hermit crabs tended to repeat the familiar turn, and did not show SAB. Similarly, Iwahara and

Fujita (1965) observed that earthworms (*D. tigrina*) tended to repeat the familiar turn after their subpharyngeal ganglion was removed, while Wyner and Zellner (1958) noticed no preference between familiar or unfamiliar direction in earthworms (*L. terrestris*) after removal of their subpharyngeal ganglia.

A third speculation for the occurrence of SAB in most species, but not all, could be because of different experimental protocols used by the researchers. For example, MacKay (1947, 1945) and Hughes (1967, 1966) used differing mazes (that is, Y- and T-mazes) with one forced turn, respectively. Rice and Lawless (1957) used T-mazes with one forced turn and did not find SAB in *Planaria dorotcephala*. When Shinkman and Hertzler (1964) used T-mazes with more than one forced turn, SAB was observed in *Dugesia tigrina*. Shinkman and Hertzler (1964) speculated that Rice and Lawless (1957) might not have found SAB in *Planaria* because mazes with only one forced turn were used. Hayes and Warren (1963) observed no SAB in two to six day old chicks placed in a Y-maze. However, Cogan *et. al.* (1979) used a t-maze with one to three day old chicks, SAB was observed. Lepley and Rice (1952) observed SAB in *P. multimicronucleatum* using T-mazes with one forced turn. However, when Lachman and Havlena (1962) used Y-mazes with one forced turn that were approximately five times as long and 20 times as wide as Lepley and Rice's (1952) mazes, SAB was not observed in *P. multimicronucleatum*.

Hypotheses regarding the mechanism in which organisms show SAB fall into two main groups, namely, intrinsic or extrinsic. Intrinsic mechanisms or intrinsic cues, such as memory, arise internally from within the organism (Hughes, 1998).

Table 1: Occurrence and Non-occurrence of Spontaneous Alternating Behavior
(SAB) in Different Species

Species	Class	Presence or Absence of SAB	Author
Human spermatozoa (male gamete)		Present	Brugger, <i>et. al.</i> (2002)
<i>Paramecium multimicronucleatum</i>	Ciliatea	Present	Lepley and Rice (1952)
<i>Paramecium caudatum</i>	Ciliatea	Absent	Lachman and Havlena (1962)
<i>Planaria dorotcephala</i>	Trematoda	Absent	Rice and Lawless (1957)
<i>Dugesia tigrina</i>	Turbellaria	Present	Shinkman and Hertzler (1964)
<i>Lumbricus rubellus</i>	Oligochaeta	Present	Hughes (1987)
<i>Lumbricus terrestris</i>	Oligochaeta	Present	Wyner and Zellner (1958)
<i>Pheretima communissima</i>	Oligochaeta	Absent	Iwahara and Fujita (1965)
<i>Armadillidium vulgare</i>	Crustacea	Present	Kupfermann (1966)
<i>Porcellio scaber</i>	Crustacea	Present	Hughes (1966, 1967)
<i>Calcinus herbsteii</i>	Crustacea	Absent	MacKay (1945)
<i>Clibanarius zebra</i>	Crustacea	Absent	MacKay (1947)
<i>Ophiulus pilosus</i>	Myriapoda	Present	Hughes (1987)
<i>Lithobius forficatus</i>	Myriapoda	Present	Schafer (1983)
<i>Trigoniulus lumbricinus</i>	Myriapoda	Present	Barnwell (1965)
<i>Dysdercus fasciatus</i>	Insecta	Present	Dingle (1965)
<i>Leptocoris trivittatus</i>	Insecta	Present	Dingle (1961, 1964b)
<i>Oncopeltus fasciatus</i>	Insecta	Present	Dingle (1965)
<i>Tenebrio molitor</i>	Insecta	Present	Dingle (1964a) ; Grosslight an Harrison (1961); Grosslight an Ticknor (1953)
<i>Forficula auricularia</i>	Insecta	Present	Hughes (1987)
<i>Blatta orientalis</i>	Insecta	Present	Wilson and Fowler (1976)
<i>Clubiona cambridgei</i>	Arachnida	Present	Taylor (1995)
<i>Helpis minitabunda</i>	Arachnida	Present	Taylor (1995)
<i>Portia fimbriata</i>	Arachnida	Present	Taylor (1995)
<i>Trite auricoma</i>	Arachnida	Absent	Taylor (1995)
<i>Trite planiceps</i>	Arachnida	Present	Taylor (1995)
<i>Carassius auratus</i>	Osteichthyes	Present	Fidura and Leberer (1974)
<i>Trichogaster trichopterus</i>	Osteichthyes	Absent	Neiger <i>et. al.</i> (1970)
<i>Gallus domesticus</i> (chicks)	Aves	Absent	Hayes and Warren (1963)
<i>Gallus domesticus</i> (chicks)	Aves	Present	Cogan <i>et. al.</i> (1979)
<i>Rattus norvegicus</i>	Mammalia	Present	Ballachey and Buel (1934)
<i>Didelphus virginiana</i>	Mammalia	Absent	Platt <i>et. al.</i> (1968)

Extrinsic mechanisms or extrinsic cues arise outside the organism, that is, the organism shows SAB due to external reasons. For example, an organism may show SAB since it is seeking food (Hughes, 1998). Intrinsic mechanisms include bilaterally asymmetrical leg movement (BALM), reactive inhibition (Hughes, 1985; Grosslight and Harrison, 1961; Grosslight and Ticknor, 1953).

The hypothesis for BALM argues that SAB might arise from relatively more stimulation of legs on the side of the body that have to travel further when negotiating a turn (Hughes, 1985). For example, if an organism made a left turn at the forced turn, legs on the right side of the body would have traveled a greater distance than legs on the left side. Therefore, legs on the right side of the body would become more tired, and as a result the organism would be temporarily biased to turn in the direction opposite to the forced turn.

Reactive inhibition, symbolized as I_R , is a theoretical construct that relates to the tendency of an animal to not make the same response twice (Hull, 1951). It is unlearned, response specific, independent of the effect of reward, and is a direct function of the time interval since the last response, and the number of preceding responses (Montgomery, 1951). Although reactive inhibition was generally considered as a mechanism for SAB in rats, some research did not support this. Montgomery (1951) designed a T-maze by blocking off one arm of a four-arm maze. A rat was placed in the starting arm that was opposite to the blocked arm, and its turning choice observed. The blocked arm and the starting arm were reversed, and the turning choice of the rat was observed a second time. If reactive inhibition was

responsible for SAB in rats, then the test rat should have ended up in the same goal arm after two consecutive trials. Instead, the test rat continued to alternate between goal arms. The test rat repeated the same response in two consecutive trials, thus ending up in different goal arms.

In a similar set-up, Glazner (1953) determined the validity of stimulus- versus response-oriented explanation of SAB (stimuli gathered by the animal while in the maze versus an innate response to the forced turn). In this study, left- and right-choice turns differed in color. Glazner (1953) found that rats alternated between brightly colored and dark alleys, even though this required repetition of turns. If reactive inhibition were responsible for SAB, then rats would have alternated in turning pattern rather than alternate based on color pattern.

Dingle (1961) also ruled out reactive inhibition in boxelder bugs (*Leptocoris trivittatus*). He found there was no relationship between the amount of time boxelder bugs took to complete mazes and whether or not SAB was observed. Dingle (1964a) failed to support reactive inhibition as a mechanism for SAB. If reactive inhibition was responsible for SAB, then the distance between the forced and choice turn should have been the only place to have an effect on the occurrence of SAB. SAB should have decreased, as the time between the forced turn and the choice turn increased, but it should not have been affected if time, and/or distance between the start of the maze to forced turn was increased. Reactive inhibition is no longer generally considered as a mechanism for SAB (Brugger *et. al.*, 2002; Hughes, 1989, 1978; Dingle, 1965).

Extrinsic mechanisms for SAB include centrifugal swing in combination with positive thigmotaxis; and intra- and extra- maze cues. Centrifugal swing describes the phenomenon in which an animal, in passing around a corner with reasonable speed, must run near the outside walls of the turns until compensatory movements restore the normal center of equilibrium (Schneirla, 1929). Thigmotaxis is the movement of an organism in response to a surface. Movement toward a surface is called positive thigmotaxis, movement away from a surface is called negative thigmotaxis. Intra- maze cues are unknown cues, within the maze, that organisms respond to in order to show SAB. Extra- maze cues are unknown cues, outside the maze, that organisms respond to in order to show SAB.

Various experiments have been designed to control for centrifugal swing. Grosslight and Harrison (1961) controlled centrifugal swing and thigmotaxis as the basic factor in SAB of the mealworm larvae (*Tenebrio molitor*). Grosslight and Harrison (1961) constructed T-mazes with v-shaped paths. These paths controlled centrifugal swing and thigmotaxis by keeping mealworm larvae in constant contact with both left and right walls of the t-mazes. Mealworm larvae could not show positive thigmotaxis to either left or right wall of the mazes and therefore should not have shown a bias at the choice turn due to positive thigmotaxis. Grosslight and Harrison (1961) concluded that centrifugal swing did not influence SAB. Dingle (1964b) eliminated centrifugal swing by placing a box over boxelder (*Leptocoris trivittatus*) bugs prior to the forced turn. The box greatly reduced the momentum of the bug, therefore reducing the probability that it would be thrown against the far

wall. When the box was removed, the bugs started from a standstill and proceeded to the forced turn, and showed SAB. If centrifugal swing was the mechanism, the bugs should not have shown SAB.

Another mechanism that has been postulated to result in SAB but not classifiable as intrinsic or extrinsic is the use of proprioceptive cues. Proprioceptive cues arise from sensory receptors that detect the motion or position of the body or a limb. Proprioceptive cues mainly involve sensory receptors whereas BALM involves motor neurons. It has been hypothesized that SAB in arthropods is a response to feedback from proprioceptive cues arising from previous responses (Hughes, 1989, 1985; Kupfermann, 1966; Dingle, 1964b).

Factors that affect SAB have been observed across species. Such factors include: (1) distance between the start and the forced turn (SF_M); (2) distance between the forced turn and the choice turn (SC_M); (3) angle of the forced turn; and (4) number of forced turns. Increased SF_M increases SAB in some species (Kupfermann, 1966; Dingle, 1965, 1964b, 1964a, 1961), but not in others (Schafer, 1982, 1975, 1972; Hughes, 1967, 1966). In most species, increased SC_M leads to decreased SAB (Schafer, 1982, 1975, 1972; Hughes, 1967, 1966; Dingle, 1964a, 1961; Grosslight and Harrison, 1961; Grosslight and Ticknor, 1953). Also in most species, increasing the angle of the forced turn increased the main angle in the alternating direction at the choice turn (Hughes, 1987, 1985, 1982; Schafer, 1983, 1982, 1975, 1972; Kupfermann, 1966). More organisms showed SAB when the number of forced turns was increased (Hughes, 1987, 1985; Grosslight & Ticknor, 1953).

Paramecium multimicronucleatum and *P. caudatum* are of the class ciliata. These species are similar in shape and size, ranging in size from 180 to 330 μm long and are quite similar in many respects. They are mainly found at the bottom of ponds where they feed on bacteria (Anderson, 1987; Kudo, 1966; Dogiel, 1965).

Only two experiments involving SAB have been conducted on microscopic species. Lepley and Rice (1952) conducted the first experiment to test the principle of reactive inhibition on maze turning behavior in *Paramecium multimicronucleatum*. They showed a significant tendency in *P. multimicronucleatum* to turn in the opposite direction of the forced turn at the choice point in a simple maze with one forced and one free turn indicating SAB. Later, Lachman and Havlena (1962) concluded that there was no evidence of reactive inhibition or SAB in *P. caudatum*. The other microscopic study of SAB involved human spermatozoa (Brugger *et. al.*, 2002). In this study, SAB was found in lower percentages, than most other SAB studies (58 versus 70%). The authors postulated that the reduced levels of SAB could be a result of the large sample size and the apparent memory difference between human spermatozoa and most other organisms studied.

Replicating Lepley and Rice's (1952) and Lachman and Havlena (1962) studies, using the same species of *Paramecium*, similar maze dimensions, and experimental conditions will allow for more meaningful comparisons, and may help resolve the conflicting results presented by these authors. Most SAB research has either focused on identifying the behavior in additional species, or determining the

mechanism involved (Dingle, 1961; Dember & Roberts, 1958; Glazner, 1953).

However, scant research has been done for microscopic organisms, and, as discussed above, results have been contradictory. Understanding the behavior in a single-celled organism may lead to a better understanding of SAB mechanisms in other, more complex organisms. Furthermore, this will provide an excellent opportunity to better understand whether maze width and length influence SAB in *Paramecium*.

The overall objective of this research project was to determine whether or not SAB exists in two species of paramecia (*P. caudatum* and *P. multimicronucleatum*) previously studied. The specific objectives were to: (1) determine whether maze length or species identity influence the expression of SAB in paramecia; (2) investigate selected factors (length of SC_M and width of maze) that may affect paramecia's ability to complete mazes; and (3) assess whether an extrinsic or intrinsic mechanism is involved in SAB for paramecia.

Chapter II
SPONTANEOUS ALTERNATING BEHAVIOR IN TWO SPECIES OF
PARAMECIA

Introduction

Spontaneous alternating behavior (SAB) has been observed in a wide variety of organisms (Dember, 1989; Table 1). SAB is thought to have evolved at least in part because it allows for efficient movement to a more favorable environment in the presence of obstacles (Taylor, 1995; Carbines *et. al.*, 1992; Hughes, 1978, 1967). It is well established that maze length affect SAB in some species. In most cases, increasing the distance between the forced and choice turn on the mazes decreases SAB (Schafer, 1982, 1975, 1972; Hughes, 1967, 1966; Dingle, 1964a). Additionally, increasing the distance between the start of the maze and the forced turn increases the occurrence of SAB in mealworms, boxelder bugs and milkweed beetles, but not in woodlice (Schafer, 1982, 1975, 1972; Hughes, 1967, 1966; Dingle, 1965, 1964a, 1964b).

Wider mazes could possibly decrease the frequency of SAB by reducing tactile cues, such that an organism may not ascertain its surroundings. Tactile cues may be partially responsible for SAB in boxelder bugs (Dingle, 1965), woodlice, and millipedes (Hughes, 1987). On the other hand, wider mazes may increase at least the appearance of SAB since an animal will have to move further to hit the far wall after the forced turn. If an animal moving along the near wall continues forward with a

change in direction less than 90° after the forced turn, it may be possible for an animal to appear to alternate when in fact a choice was never made at the forced turn intersection (Figure 1).

Lepley and Rice (1952) and Lachman and Havlena (1962) used paramecia to test the model of reactive inhibition. Although neither study found support for reactive inhibition, Lepley and Rice (1952) reported SAB in *Paramecium multimicronucleatum* while Lachman and Havlena (1962) did not find SAB in *Paramecium caudatum*. The different findings for the two experiments may have resulted from behavioral differences between the two species of *Paramecium* and/or from differing experimental procedures. For example, the mazes in Lepley and Rice's (1952) study were: 3, 1 and 0.08 mm for SF_M, SC_M and width, respectively, whereas, Lachman and Havlena (1962) mazes were 1.59 mm (1/16 inch) wide.

Extrapolating from Lachman and Havlena's (1962) diagrams, SF_M, SC_M, and the maze width were 2.1, 6.4, and 19.9 larger than Lepley and Rice's (1952) maze respectively. Lepley and Rice (1952) etched mazes into glass slides using hydrochloric acid, while Lachman and Havlena (1962) etched mazes into glass-mounted paraffin. Lepley and Rice (1952) used an average of 43 paramecia per trial while Lachman and Havlena (1962) used one paramecium per trial. No literature was found regarding the effect of the number of organisms on SAB, however most SAB studies used one organism per trial (Hughes, 1987, 1978, 1967; Dingle, 1965, 1964a, 1964b, 1961; Dember and Roberts, 1958; Glazner, 1953), except Brugger *et. al.* (2002). The discrepancy between Lepley and Rice's (1952) and Lachman and

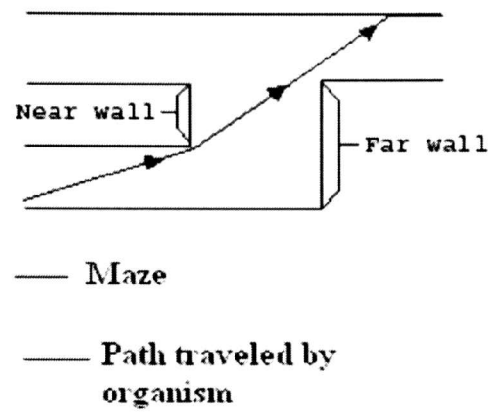


Figure 1: Potential Path of Maze Width Increases and Distance Between the Forced Turn and Choice Turn Decreases

Havlena's (1962) studies remains unresolved. It is against this backdrop that the current study was designed to determine whether maze length or species identity influence the expression of SAB in paramecia.

Materials and Methods

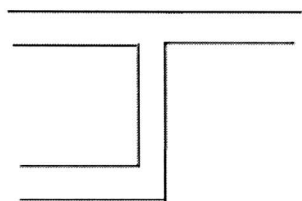
Maintenance of Paramecia

Paramecium caudatum and *P. multimicronucleatum* were obtained from Berkshire Biological Laboratories (Westhampton, MA). The paramecia were housed in their original polystyrene containers (59.15 ml). New paramecia were obtained every two weeks to prevent debris build-up in containers and to ensure that paramecia were available at all times during experimentation. No food was added to the cultures. The containers were left loosely covered at 21°C.

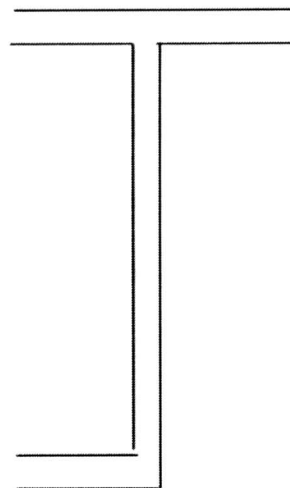
Mazes

Mazes were constructed from paraffin wax in a similar fashion to Lachman and Havlena (1962). A microscope slide was dipped into a container of paraffin wax, maintained at 85°C. The dipped slide was left to cool, and then dipped a second time in the paraffin. Paraffin on one side of the slide was then scraped off, leaving a solid layer of paraffin (0.5 mm deep) on one side of the slide into which the maze was etched. Wide mazes were etched with the tine of a metal fork (1.5 mm wide), while thin mazes were etched with the metal, sliding-cover of a 0.5 mm (3.5") floppy computer disk.

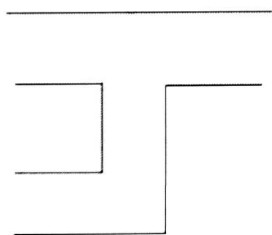
Four types of mazes were used (Figure 2): 1) Short, thin maze (STM); 2) Long, thin, maze (LTM); 3) Short, wide maze (SWM); and 4) Long, wide maze



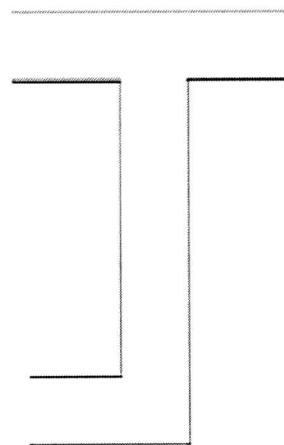
a) Short Thin Maze (STM)



b) Long Thin Maze (LTM)



c) Short Wide Maze (SWM)



d) Long Wide Maze (LWM)

12 mm: 3 mm

Figure 2: Experimental Maze Treatments

(LWM). In all treatment mazes, the length between the start of the maze and the forced turn was 3 mm. In long mazes, the length between the forced turn and the choice turn was 6 mm. Short mazes had a length of 3 mm between the forced turn and the choice turn. All forced turns were to the left. Wide and narrow mazes were 1.5 and 0.5 mm in width, respectively. The control mazes had the same widths as the experimental mazes but lacked a forced turn. Track lengths of the control mazes were 3 mm and 6 mm.

Trials

Prior to each trial, a maze was rinsed with filtered culture solution taken from cultures that were two or more weeks old. The maze was then dried with clean tissue paper. The maze was filled with filtered culture solution, using a 1.5 ml pipette. Care was taken to remove any debris, such as bits of paraffin, from the maze. Paramecia were extracted from their original containers using a 1.5 ml pipette and added to the start of the maze. If more than one paramecium was transferred, the maze was cleaned out, prepared for a second trial, and the process of adding paramecium was repeated. When a single paramecium was observed at the start of the maze, a stopwatch was started and the paramecium was observed until it entered either arm of the choice turn or for 15 minutes, whichever came first. The number of paramecia that completed each treatment within 15 minutes was taken as the measure for finishing ability. At the end of each trial, the maze was rinsed twice with tap water. The trials were observed using a dissecting microscope (Swift Instruments International, Tokyo, Japan) at 10X magnification. Mazes were bottom lit and placed

in an open Petri dish on top of a closed Petri dish filled with tap water. The closed Petri dish served to minimize heating.

Fifty paramecia from each species, *P. caudatum* and *P. multimicronucleatum* were observed in each group (treatment and control mazes), resulting in a total of 800 paramecia observed in this experiment. Mazes were run over a period of several nights. On each night, experimental mazes within each group (STM, SWM, LTM, and LWM) were run. In addition to this, control mazes within each group (3 mm long/0.5 mm wide, 3mm long/1.5 mm wide, 6 mm long/ 0.5 mm wide, 6 mm long/1.5 mm wide) were run. The number of mazes run in each group varied depending on how many successful trials were run. On no night did the total number of successful trials in each maze type exceed 10. The number of paramecia used per batch varied depending on the number of successful trials completed during experimentation.

Statistical Analysis

A chi-square test was used to compare the proportion of paramecia turning left *versus* right in each control group to the expected 50:50 ratio for SAB. If there were no significant differences in the control mazes after 50 replicates per maze type, the treatment mazes were then evaluated against the expected 50:50 ratio. If there was a significant difference in control mazes between the observed turning pattern of paramecia, and the expected 50:50 ratio for turning pattern at the choice turn intersection, then this suggested that there was a bias in turning preference at the choice turn intersection. Therefore, experimental mazes could not be compared with the expected 50:50 ratio since paramecia were showing a bias in turning preference during experimentation. Three-way contingency tables were constructed to determine

if there were any interactions among maze length, maze width, and number of paramecia showing SAB within each species.

Results

For both species there was no significant difference in the proportion of paramecium turning left versus right (left-right bias) in each control group in the narrow and wide t-mazes (Table 2). There was no bias in paramecia turning preference when the forced turn was excluded. The results indicated that SAB occurred in *Paramecium*. SAB was influenced by maze length but not maze width, while significantly more paramecia showed SAB in the short but not long treatment mazes (Table 3a and b). For the paramecia that completed STM, 71 and 70% *P. caudatum* and *P. multimicronucleatum*, respectively, showed SAB ($X^2 = 6.7$, $p < 0.05$; 8.00 , $p < 0.01$, $df = 1$). Similarly, for the SWM treatments, both species showed SAB (68% of *P. caudatum*: $X^2 = 6.48$, $p < 0.05$; 74% of *P. multimicronucleatum*: 11.5 , $p < 0.01$, $df = 1$). SAB was not observed for LTM and LWM treatments in both species.

Paramecium caudatum and *P. multimicronucleatum* showed no significant overall interaction for maze length, width and SAB. When width was held constant, significantly more paramecia showed SAB in short than in long mazes [*P. caudatum* ($G_{AC(B)} = 7.98$, $p < 0.05$, $df = 2$, and *P. multimicronucleatum* ($G_{AC(B)} = 12.26$, $p < 0.01$, $df = 2$)] Maze width did not influence the number of paramecia that showed SAB for all four mazes (Table 4). Another 3-way contingency table was done to test whether species identity in conjunction with maze length affected the number of paramecia showing SAB. SAB was the same for both species in all four maze

Table 2: Paramecia that Finished and did not Finish Control Maze and their Turning Preference (left right bias)

Species	Length between Forced and Choice Turn (mm)	Finished Maze		Did Not Finish Maze		Left Right Bias	
		Maze Width (mm) 0.5 1.5	Maze Width (mm) 0.5 1.5	Maze Width (mm) 0.5 1.5	Maze Width (mm) 0.5 1.5	L R	L R
<i>Paramecium caudatum</i>	3	36	50	14	0	18	18
<i>Paramecium multinucleatum</i>		48	50	2	0	26	22
<i>Paramecium caudatum</i>	6	28	46	22	4	14	14
<i>Paramecium multinucleatum</i>		40	50	10	0	21	19

Table 3a: Three Way Contingency Table Comparing Finishing Ability and SAB in *P. caudatum*

Maze Length (A)	Maze Width (B)	Behavior Observed (C)			Total
		Turned in Same Direction as Forced Turn	Showed SAB	Failed to Finish Maze Treatment	
Long	Thin	14	11	25	50
	Wide	25	25	0	50
Totals	-	39	36	25	100
Short	Thin	27	11	12	50
	Wide	35	15	0	50
Totals	-	62	26	12	100

Interaction	G-statistic	df	p-value
ABC	0.32	1	0.57
AB	8.01	3	0.05
AC	2.00	3	0.57
BC	9.64	3	0.02
AB(C)	1.97	2	0.37
AC(B)	7.98	2	0.02
BC(A)	0.34	2	0.65

Table 3b: Three Way Contingency Table Comparing Finishing Ability and SAB in *P. multimicronucleatum*

Maze Length (A)	Maze Width (B)	Behavior Observed (C)			Total
		Turned in Same Direction as Forced Turn	Showed SAB	Failed to Finish Maze Treatment	
Long	Thin	24	26	0	50
	Wide	24	26	0	50
Totals	-	48	52	0	100
Short	Thin	35	15	0	50
	Wide	37	13	0	50
Totals	-	72	28	0	100

Interaction	G-statistic	df	p-value
ABC	0.11	1	0.74
AB	12.34	3	0.01
AC	0.20	3	0.98
BC	12.26	3	0.01
AB(C)	0.12	2	0.94
AC(B)	12.26	2	0.0002
BC(A)	0.20	2	0.91

Table 4: Three Way Contingency Table Comparing SAB for both Species of Paramecium

Maze Length (A)	Species (B)	Behavior Observed (C)			Total
		Turned in Same Direction as Forced Turn	Showed SAB	Failed to Finish Maze Treatment	
Long	<i>P. caudatum</i>	39	36	25	100
	<i>P. multimicronucleatum</i>	52	48	0	100
Totals	-	91	84	25	200
Short	<i>P. caudatum</i>	27	61	12	100
	<i>P. multimicronucleatum</i>	28	72	0	
Totals	-	55	133	12	200

Interaction	G-statistic	df	p-value
ABC	0.09	1	0.77
AB	19.83	3	1.84×10^{-4}
AC	0.73	3	0.86
BC	20.39	3	1.41×10^{-4}
AB(C)	0.73	2	0.70
AC(B)	19.82	2	4.97×10^{-5}
BC(A)	0.16	2	0.92

treatments (Table 4). Maze length was the only factor that affected the number of alternating paramecia ($G_{AC(B)} = 19.82$, $p < 0.001$, $df = 2$).

Significantly more *P. multimicronucleatum* than *P. caudatum* completed 0.5 mm wide / 3mm long control mazes ($X^2 = 10.7$, $p < 0.01$, $df = 1$) and 0.5 mm wide / 6 mm long control mazes ($X^2 = 8.1$, $p < 0.01$, $df = 1$). Also, for the wide control mazes, significantly more *P. caudatum* completed the 1.5 mm mazes than the 0.5 mm mazes ($X^2 = 12.20$, $p < 0.01$, $df = 2$).

Significantly more *P. multimicronucleatum* completed LTM than *P. caudatum* ($X^2 = 12.5$, $p < 0.01$, $df = 1$). For *P. caudatum*, more paramecia completed STM treatments than LTM treatments (Table 3a). In the experimental maze treatments, there were no overall significant differences between the number of paramecia that completed LTM, STM, SWM and STM treatments ($X^2 = 1.66$, $p > 0.05$, $df = 1$).

Discussion

The present study revealed that short mazes increased SAB in *Paramecium caudatum* and *P. multimicronucleatum*. This finding was similar to that reported by other studies for other species, such as mealworms, boxelder bugs, milkweed beetles, and pill bugs (Hughes, 1967, 1966; Dingle, 1964a, 1964b, 1961). The fact that SAB occurred only in short mazes suggest that one of the differences between Lepley and Rice's (1952) and Lachman and Havlena's (1962) results may have been due to maze length. Lepley and Rice (1952) used short mazes, and their results were consistent with the finding of this study. Similarly, Lachman and Havlena (1962) used long mazes, and their results were also consistent with the findings of this study. The short, thin mazes used in this study had the same distance as Lepley and Rice's

(1952) for SF_M , three times the distance for SC_M , and were approximately six times as wide. The long wide mazes had half the distance used by Lachman and Halena (1962) for SC_M , and approximately the same distance for SC_M , and width.

Maze width did not influence SAB, which was an unexpected result; however, it is possible that the wider mazes reduced tactile cues, such that the paramecia could not ascertain their surroundings. It is also possible that the widths used in this experiment were not appropriate to detect an effect on SAB. Both species of *Paramecium* responded similarly to all experimental variables. Previous studies across species reported the effect of length on SAB, however, none of those studies used *Paramecium* (Carbines *et al.*, 1992). As recommended by Hughes (1989), the present study allowed for cross comparison between two species in the same study. The study also added one more microorganism to the list of species that show SAB (Table I). Also, this study reduces errors that could occur from comparisons made between different studies, such as difference in time experiments was carried out, and experimental procedures used.

SAB was studied in both species of paramecia to determine whether maze dimension (length and width) or species identity influence the expression of SAB in paramecia. The major findings are: (a) SAB occurred in short mazes in both species of *Paramecium*; and (b) there was a difference in finishing ability between the two species of *Paramecium* in some mazes. This study is the first to report the influence of maze length on SAB in *Paramecium*. It is one of the few studies in which two species have been compared within a single investigation. In conclusion, SAB in *Paramecium* is not species specific. Finally, similar factors affect SAB at the

microscopic level as observed at the macroscopic level (the effect of SC_M on whether or not SAB is observed).

Chapter III

BEHAVIOR OF PARAMECIA IN RELATION TO SPONTANEOUS ALTERNATING BEHAVIOR

Two studies have investigated SAB in paramecia using t-mazes (Lachman and Havlena, 1962; Lepley and Rice, 1952). Of these studies, Lepley and Rice (1952) reported SAB in *Paramecium multimicronucleatum*, while Lachman and Havlena (1962) did not find evidence of SAB in *Paramecium caudatum*. Since then, there have been no other published reports of SAB in any other species of *Paramecium*. My first experiment clearly demonstrated that SAB occurs in both *P. caudatum* and *P. multimicronucleatum*, depending primarily on the distance between turns. However, the mechanism for SAB in these species is still unknown.

Researchers have proposed intrinsic and extrinsic mechanisms, and proprioceptive cues for SAB (Hughes, 1998). Intrinsic hypotheses state that SAB results from cues that arise within the organism, such as memory (Hughes, 1998). Extrinsic hypotheses state SAB results from cues that arise from outside the organism. Intrinsic mechanisms for SAB that have been demonstrated in species other than *Paramecium* include: bilaterally asymmetrical leg movement (BALM) and memory (Hughes, 1985; Kupfermann, 1966).

BALM is not applicable to *Paramecium*. Because cilia on *Paramecium* are spirally arranged along the surface of the cell, and paramecia swim in a spiral motion,

no single row of cilia remains constantly exposed to one side of the maze (Dogiel, 1965). Therefore, cilia closest to inside of the corner at the forced turn intersection would not be more tired than cilia closest to the outside of the forced turn intersection, thus ruling out the hypothesis for BALM.

Reactive inhibition was negated as a possible mechanism for SAB in *Paramecium* by previous researchers (Lachman and Havlena, 1962; Lepley and Rice, 1952). In their experiments, *Paramecium* did not show increased alternation as the number of forced turns were increased. *Paramecium* would have shown increased alternation as the number of forced turns were increased, if reactive inhibition was the mechanism.

It is hypothesized, that if *Paramecia* use an intrinsic mechanism to show SAB, it is most likely to be memory. Memory identifies the ability of a system to store and recall information on previously encountered characteristics (Kurtz, 2005; Hampton and Schwartz, 2004). If paramecia show SAB due to memory, it is because they recalled the direction they turned at the forced turn, and turned in the opposite direction at the choice turn intersection (Kupfermann 1966). Researchers have shown that Protozoa possess some ability to learn (Hinkle and Wood, 1994; Berger, 1983). When Protozoa were placed in the narrow end of capillary tubes, and allowed to swim until escaping into larger volumes of medium, the time the Protozoa required to escape the tube decreased, as the number of trials increased (Hinkle and Wood, 1994; Berger, 1983). The researchers concluded that, the decrease in time taken to escape the tube was evidence of Protozoa's ability to learn. *Stentor* was reported to begin

contracting when presented with a light stimulus if the light had previously been paired with shock (Soest, 1937). However, Applewhite and Gargner (1973) reported that Soest (1937) did not use controls in their study, therefore their results might not have been valid. Paramecia were found to be more likely to attach to bare wire that had previously been baited with bacteria than to a wire that had not been previously baited (Gelber, 1952). However, Gelber's (1952) study has been criticized for poor experimental design, and the results might have been due to chemosensory responses by *Paramecium* (Hinkle and Wood, 1994).

Extrinsic mechanisms for SAB in *Paramecium* include intra- and extra-maze stimuli, and centrifugal swing. Centrifugal swing was refuted as a possible mechanism for SAB in some organisms by previous research (Grosslight and Harrison, 1961). Intra-maze stimuli (the use of sensory cues within the maze) were proposed as a mechanism for SAB in rats (Dennis and Henneman, 1935). In their experiment, Dennis and Henneman (1935) found that rats alternated between brightly colored and dark alleys indicating the use of intra-maze stimuli. Kivy *et. al.* (1961) exposed rats to color alternatives outside the maze for varying time periods. The rats did not make their choices based on the extra-maze stimuli.

It is against the foregoing backdrop that the present study was designed. The findings from my pre-experimental trials (results not shown), and Experiment 1 showed that *Paramecium caudatum* completed LTM treatments less often than *P. multimicronucleatum*. This raised the question as to whether the two species differed in swimming ability. No previous study was found which compared the swimming

ability of the two species of *Paramecium*. Therefore, it was hypothesized that: 1) there would be a significant difference between the number of contacts that paramecia made in short and long mazes, if SAB was the result of an extrinsic mechanism; and 2) there will be no significant differences in the swimming ability of the two species of *Paramecium*.

The objectives of this experiment were: 1) to determine whether the mechanism that resulted in SAB in short mazes in the earlier experiment was intrinsic or extrinsic in nature; and 2) to assess whether there were differences in swimming ability (speed and number of contacts on each side of the maze) between *Paramecium caudatum* and *P. multimicronucleatum*.

Materials and Methods

Objective 1 – Mechanisms for SAB

To investigate whether the mechanism for SAB is intrinsic or extrinsic, four maze treatments were used: LTM, LWM, STM, SWM (Figure 2). Mazes were divided into three equal sections (Figure 3). The experimental variables altered were maze length and maze width. Maintenance of the paramecia, and addition of the paramecia to the maze were the same as discussed in Experiment I. In this study, intrinsic mechanism refers to memory; BALM and reactive inhibition were not measured. The length of time paramecia took to complete each maze was taken as the measure for intrinsic mechanism (memory). Extrinsic mechanism was measured by the number of contacts paramecia made with the sides of each maze. The duration of each trial was either the amount of time it took each paramecium to enter either

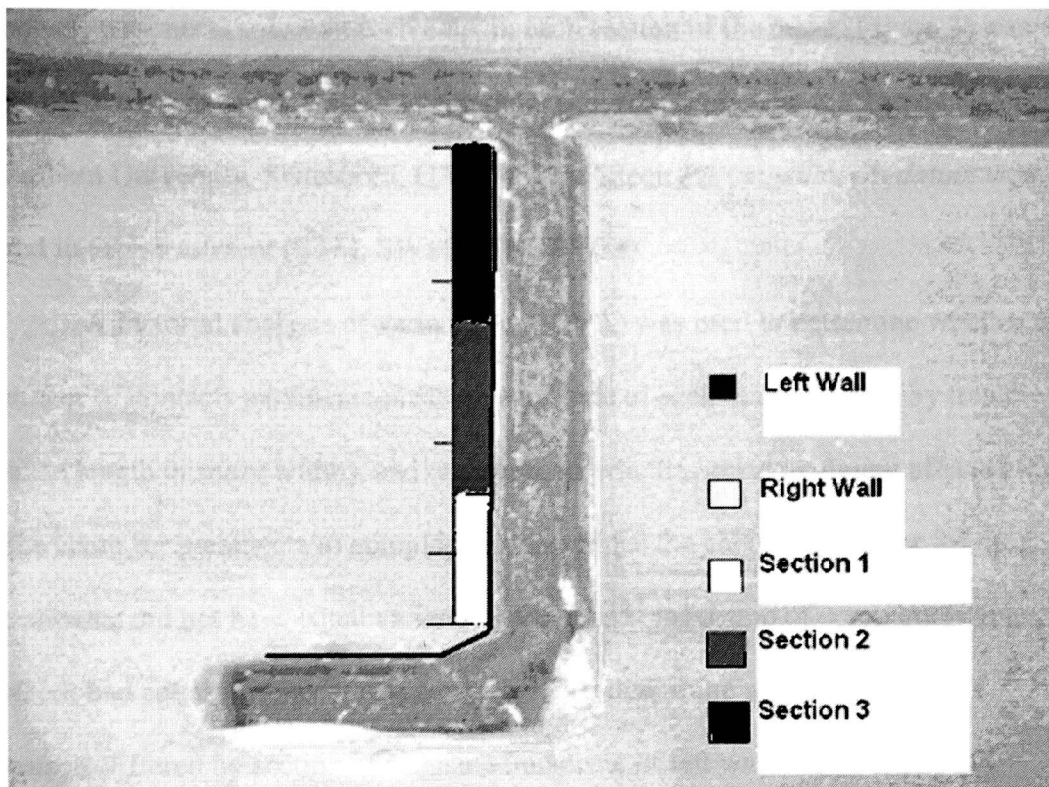


Figure 3: Long Wide Maze Displaying Section and Contacts Recorded

arm of the choice turn or 10 minutes, whichever came first. The length of time taken for each paramecium to reach the choice turn was also recorded. The number of contacts paramecia made on each side, in each section of the maze (Figure 3) was recorded using an event recording computer program (Paravent, A. Harvey, Georgia Southern University, Statesboro, GA 30460). Fifteen *Paramecium caudatum* were used in each treatment (STM, SWM, LTM, LWM).

A factorial analysis of variance (ANOVA) was used to determine whether the number of contacts paramecia made on each side of each maze differed by treatment (maze length or maze width), and to determine whether maze treatment affected the time taken for paramecia to complete mazes. Since the completion times for the four treatments did not have equal variances, the natural logarithm of completion times (which had equal variances) was compared. To determine whether number of contacts differed by section, the arcsine transform of left wall versus right wall contacts, by each paramecium, in each section of the maze was taken, and a factorial analysis of variance was used on the transformed data to compare the number of contacts in each section of each maze treatment.

Objective 2. Paramecia's Swimming Ability

In experiment I, the only experimental treatment in which significantly less *P. caudatum* than *P. multimicronucleatum* completed mazes was the LTM. Therefore, in this experiment, only LTM treatments were used to investigate paramecia's swimming ability. In this objective, swimming ability refers to swimming speed and number of contacts on each side of the maze. The time paramecia took to complete a

run, and number of contacts with the sides of the maze were taken as measures of swimming speed and swimming pattern, respectively. The number of contacts on each side of each maze, amount of time each paramecium took to complete each maze, and direction turned at the choice turn, were recorded. In each trial, a maximum of 10 minutes was allocated for each paramecium to complete each maze. Thirty *Paramecium multimicronucleatum* and 30 *P. caudatum* were used in LTM treatments.

Pearson's correlation was used to assess the nature of the relationship between number of contacts and length of time taken to complete LTM treatments for the two species of *Paramecium*. ANCOVA (with number of contacts as the covariate) was used to determine whether there were differences in amount of time taken to complete LTM treatments for both species.

Results

Objective 1 – Mechanisms for SAB

For the short mazes, paramecia made more contacts with the right wall than the left wall (Table 5). Short mazes had a significantly higher proportion of right wall contacts than long mazes (73.8% right wall contacts for short mazes while 52.6% right wall contacts for long mazes, Table 6). Paramecia made significantly more contact with the right wall, in the first section of short mazes, than in any other section ($F_{2,29} 14.22$, $P < 0.0001$, Table 7a). The mean right wall contacts for the STM and SWM were 2.07 ± 0.70 and 1.8 ± 0.77 , respectively (Table 5). In long mazes, there was no significant difference between right and left wall contacts in any section of the

Table 5: Mean Number of Contacts for All Four Maze Treatments for *P. caudatum*

	Short Mazes				Long Mazes			
	Thin		Wide		Thin		Wide	
	Mean right wall contacts	Mean left wall contacts	Mean right wall contacts	Mean left wall contacts	Mean right wall contacts	Mean left wall contacts	Mean right wall contacts	Mean left wall contacts
<i>Paramecium caudatum</i>	2.07+0.70	0.87+0.99	1.8+0.77	0.53+0.74	8.07+4.29	7.13+4.30	10.07+7.30	9.27+6.43

Table 6: ANOVA Table for Comparison of Proportion of Right Wall Contacts in All Four Maze Treatments for *P. caudatum*

SOURCE	df	SS	MS	F
Maze length	1, 29	4.53	4.53	31.56*
Maze width	1, 29	0.14	0.14	0.99
Interaction	1, 29	0.18	0.18	1.27

*p < 0.001

df = degrees of freedom, SS = sum of squares, MS = mean square

Table 7a: ANOVA Table for Comparison of Proportion of Right Wall Contacts in Each Section of Four Maze Treatments for *P.**Caudatum (Comparison of Proportion of Right Wall Contacts in Each Section of Short Mazes)*

SOURCE	df	SS	MS	F
Maze section	2, 29	13.29	6.64	14.22*
Maze width	1, 29	0.17	0.17	0.36
Interaction	2, 29	0.60	0.30	0.64

* $p < 0.0001$

df = degrees of freedom, SS = sum of squares, MS = mean square

maze (Table 7b). Thin mazes and wide mazes had similar wall contacts (Table 6). There was no significant difference in the time taken for paramecia to complete the 4 maze treatments ($F_{1,29} = 3.14$, $p > 0.05$, Table 8).

Objective 2: Paramecia's Swimming Ability

There was no significant difference in the number of contacts for *P. caudatum* and *P. multimicronucleatum* ($t = 0.08$, $p > 0.05$, $df = 29$; mean contacts SD *P. caudatum* = 12 ± 17.06 ; *P. multimicronucleatum* = 11 ± 19.97). Similarly, there were no significant differences in swimming speed ($t = 0.20$, $p > 0.05$, $df = 29$). There was a weak, statistically significant positive correlation between time taken to complete mazes and number of contacts for *P. caudatum* and *P. multimicronucleatum* ($r = 0.38$, $p < 0.05$ and $r = 0.36$, $p < 0.05$ respectively). ANCOVA revealed that the two species of paramecium did not differ significantly in swimming speed or LTM treatments ($F = 0.0$, $p > 0.05$, $df = 1$)

Discussion

There were significantly higher numbers of contacts on the right, rather than the left wall of the short mazes. As stated earlier, extrinsic mechanism for SAB in *P. caudatum* was measured by the number of contacts paramecia made with the sides of each maze. Also, paramecia were swimming more frequently in the direction of the right arm of the choice turn, and were more likely to show SAB in short mazes. Therefore, maze treatment affected whether or not paramecium showed SAB, which further substantiates that, in this study, SAB was due to some unknown interaction

Table 7b: ANOVA Table for Comparison of Proportion of Right Wall Contacts in Each Section of Four Maze Treatments for *P.**Caudatum (Comparison of Proportion of Right Wall Contacts in Each Section of Long Mazes)*

SOURCE	df	SS	MS	F
Maze section	2, 29	0.35	0.18	0.77
Maze width	1, 29	0.01	0.01	0.02
Interaction	2, 29	0.15	0.08	0.32

df = degrees of freedom, SS = sum of squares, MS = mean square

Table 8: ANOVA Table for Comparison of Time Taken for *P. caudatum* to Complete in All Four Maze Treatments

SOURCE	df	SS	MS	F
Maze length	1, 29	4.60	4.60	3.14
Maze width	1, 29	2.27	2.27	1.55
Interaction	1, 29	0.05	0.05	0.86

df = degrees of freedom, SS = sum of squares, MS = mean square

between paramecia and maze treatment, thus giving support for an extrinsic mechanism.

There were no significant differences in the amount of time the two species of *Paramecium* took to complete short and long mazes. This finding is quite counterintuitive, as one would reasonably have expected short distances to take less time to run than long distances. However, a possible explanation may be found in a quirk of the experimental design. Each of the four treatments was tested late at night on a different day, and the outdoor temperatures were coldest at those times when the two short maze treatments were run. Although the tests were run indoors, it is quite possible that fluctuations in indoor temperatures tracked those of outdoor temperatures.

Paramecium is known to be very sensitive to temperature changes, its optimum temperature being 25° to 28°C (Szeto and Nyberg, 1979; Tawada and Miyamoto, 1973). Tawada and Miyamoto (1973) provided strong evidence that *P. caudatum* detects minute changes in temperature, as little as $>0.055^{\circ}\text{C}/\text{second}$. It has been reported that for *P. caudatum*, swimming rate increased with small temperature increases ($>0.1^{\circ}\text{C}/\text{second}$) (Tawada and Miyamoto, 1973). Thus, small changes in indoor temperature, corresponding with larger changes in outdoor temperatures, might well have caused paramecia to swim at a lower rate on colder nights, which were coincidentally the nights when the short mazes were run.

The results can be summarized briefly: (a) the number and pattern of contacts in *P. caudatum* supports the use of an extrinsic mechanism to show SAB; (b) similar

completion time in the four maze treatments suggest against an intrinsic mechanism for SAB in *Paramecium*. However, daily temperature fluctuations could have influenced completion times and the possibility of intrinsic mechanism for SAB in the paramecia studied cannot be completely eliminated; and (c) both species of *Paramecium* displayed similar swimming ability (speed and pattern). Collectively, the results of the two experiments are important because further evidence of SAB at the microscopic level was shown. Earlier, Lepley and Rice (1952) and Brugger *et al.* (2002) proposed an intrinsic mechanism for SAB at the microscopic level. However, this is the first study to demonstrate that extrinsic mechanisms are responsible for SAB in two species of *Paramecium*.

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