GENETIC VARIABILITY IN POPULATIONS OF FRESHWATER SPONGES ASSESSED USING HISTOCOMPATIBILITY BIOASSAYS

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ABSTRACT

Assays based on histocompatibility were used to test whether sponges belonging to the species Ephydatia muelleri and Spongilla lacustris occupy single clonal populations. The histocompatibility assay consists of two individual sponges of the same species being bound together to form a sample pair. After seven days the sample pairs were observed as exhibiting fusion or non-fusion. Two individuals that did not fuse with each other were considered to be of different strains. The results show that neither species of sponge, E. muelleri or S. lacustris, grow as a single clonal population in Salmon Lake. Three strains of E. muelleri and two strains of S. lacustris were identified.

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INTRODUCTION AND LITERATURE REVIEW

Sponges, among the most primitive of animals, are capable of self-recognition (Hildemann et al., 1978), and self-recognition can serve as a basis for assessing genetic variation in these animals (Neigel and Avise, 1983).

Tissue coalescence (or fusion) occurs between sponges of the same species but not between sponges of different species (Wilson, 1907). Non-coalescence (or non-fusion) of tissues between individuals of the same species has also been observed (Van de Vyver and Curtis, 1971). For instance, Van de Vyver and Curtis (1971) noted that many individual masses of the freshwater sponge, Ephydatia fluviatilis, fail to fuse with other sponges of this species when placed side by side. Mukai and Shimoda (1986) showed that two types of reactions, compatible fusion and cytotoxic rejection, which causes non-fusion, could be distinguished for Ephydatia muelleri and Radiospongilla cerebellata.

As stated previously, non-fusion occurs between some sponges of the same species. This histo-incompatibility between sponges is due to genetic variability among those sponges that do not fuse. Non-fusion between sponges of the same species could be explained if sponges that did not fuse with each other had different alleles for the gene or genes responsible for histocompatibility. In my study, sponges that did not fuse with each other were considered to

be different strains. The number of different strains in a sponge population was used to describe that population's genetic variability.

Other studies have used populations of dissociated sponge cells to explain the nature of the adhesive elements at the cell surface and the mechanisms responsible. When cell suspensions from two different species are mixed, cell aggregates form. Each resulting aggregate appears to be composed of cells of one of the species alone (Hildemann et al., 1978). These populations of dissociated sponge cells consist of several different types which may have dissimilar adhesive properties. It seems that the presence of archaeocytes is necessary for aggregation to take place, but species specificity in the sorting out process is conferred by mucoid cells in their interaction with the archaeocytes (John et al., 1971).

Non-fusion between different strain types of sponges is, at least in part, the result of changes in a cell's adhesiveness (Curtis, 1979). Each strain produces a soluble factor that increases the adhesiveness of homologous cells but decreases that of cells of heterologous strains (Van de Vyver and Curtis, 1971). This soluble factor affects the specificity of adhesion among the cells so that cells from different strains can not stick to one another (and non-coalescence is observed).

Two main cell types are involved in the rejection

reaction associated with non-coalescence between different strains of sponges: collencytes and archaeocytes (Van de Vyver et al., 1990). The rejection reaction always begins with the disappearance of the external pinacoderm in the zone of contact and by a cell migration towards this zone.

Whenever two individuals belonging to two different strains come into contact while they grow, they always reject each other by building a non-merging front (Van de Vyver et al., 1990). Van de Vyver and De Vos (1979) demonstrated that a non-merging front is made of a thin layer of collagen, laid down between the two sponges. This reaction provides one explanation for non-fusion between different strains.

Sponges are capable of reproducing either by vegetative propagation or sexual reproduction. It is the latter type of reproduction that is largely responsible for genetic diversity within the same species of sponge. Additionally, sexual reproduction in sponges produces motile larvae. Thus, sponges that have been produced by sexual reproduction may have a different combination of alleles for the histocompatibility gene or genes than their parents, and the motile larva have the capability of separating themselves from their parents by a distance greater than outgrowths produced by vegetative variation can expand. Conversely, sponges produced by vegetative propagation will have the same genome as their parents, and these sponges will be

located in close proximity to their parents since they are produced by marginal outgrowths. As a result, the frequency of graft acceptance between individuals should decrease as the distance between individuals increases. For example, for Verongia longissima, 68 grafts between individuals greater than 10 m apart exhibited rejection responses (Neigel and Avise, 1983). Mukai and Shimoda (1986) found that all combinations of Ephydatia muelleri coming from the same locality, each locality varying in size and being separated by 40-80 km, were of the same strain, but those between specimens coming from different localities displayed a conspicuous cytotoxic rejection reaction.

In this study, I consider the histocompatibility responses exhibited in populations of two freshwater species of sponge, Ephydatia muelleri and Spongilla lacustris. I extend the work of Willardson (1993). Willardson performed a preliminary assessment of genetic variability of Ephydatia muelleri. In his study, only one strain of Ephydatia muelleri was found.

MATERIALS AND METHODS

Species used

The following two species of the family Spongillidae were used: Ephydatia muelleri and Spongilla lacustris. All specimens were obtained from Salmon Lake (T15N, R14W, S8), a lake in western Montana where these two species grow abundantly.

Ephydatia muelleri and Spongilla lacustris were distinguished by the appearances of spicules that were associated with the skeletons and gemmules. Skeletal spicules are classified as either microscleres (length to 130 um) or megascleres (length to 350 um). The gemmule spicules in E. muelleri are double wheel-like (birotulate) structures. Its megascleres have minute spines and no microscleres are present. S. lacustris is characterized by gemmule spicules being more or less javelin shaped and slightly curved. The megascleres of S. lacustris are smooth and its microscleres are spiny.

Field collections

Sponge samples were collected during the months of June, July, and August, 1994. The size of the sample removed from a sponge varied according to the size of the sponge. An ideal sample size was 2 cm long by 2 cm wide.

After collection, specimens were placed in small film vials containing Salmon Lake water. Vials were stored in an ice cooler for the two-hour trip back to the laboratory. At the laboratory, they were stored at 5°C in a refrigerator. All assays were begun within 24 h of collection.

Preparation of spicules

Spicules were used to identify all specimens used in this study. A piece of tissue from each specimen was placed on a glass slide and digested with 50% bleach. The residual material, containing spicules, was transferred to another glass slide, cover slipped, and examined with a light microscope.

Histocompatibility assays

Samples were cut with a razor blade to produce 1-1.5 cm square specimens. The pinacodermal surfaces of two samples were placed in contact with each other and the two were tied together with thread. Thread of different colors was used to identify specific pairings which were then placed in a tank (14.5x22.0x15.0 cm). Two bubblers were placed in opposite corners of the tank and remained on throughout the entire assay period. The tank was filled with water obtained from Salmon Lake and was replaced with fresh Salmon Lake water after 4 days. The water temperature was kept constant at 20°C by running a steady stream of tap water through a reservoir which surrounded the tank

containing the assays.

After seven days, the paired samples were observed to determine if fusion had taken place. Fusion was defined operationally as the tendency of the two sponge samples to stay attached after both threads were cut and one member of the pair was lifted by forceps. Assays with pieces of the same sponge served as positive controls.

RESULTS

From six Ephydatia muelleri specimens and nine Spongilla lacustris specimens collected, a total of 21 and 25 assays, respectively were performed.

Ephydatia muelleri

The results of the histocompatibility assays for Ephydatia muelleri are presented in Tables 1 and 2. All E. muelleri specimens were collected from the Salmon Lake outlet. All pairings involving pieces from the same sponge showed fusion. Specimens one through four were all located in the same area (Figure 1) and histocompatibility assays between various combinations of these specimens all showed fusion (Table 2). Specimens one and two of strain A did not fuse with specimen five (Table 2). The locality containing strains one through four was situated approximately 60 m upstream from specimen five (Figure 1).

Among pairs that fused exhibited coalescence by union of the contours of the two sponges at the graft interface. The original graft interface was not apparent after seven days in assays exhibiting fusion (Figure 3). Pairs that did not fuse separated at the original graft interface after seven days.

Conflicting results were observed in the assays involving specimens one and six. Of the three assays

Table 1. Results of the histocompatibility assays with Ephydatia muelleri. Fusion and non-fusion are represented by + and -, respectively. The number of symbols in the results column corresponds to the number of assays done for that particular pairing.

Pairing	Result	Pairing	Result
1-1	+,+,+	1-2	+,+,+
2-2	+,+	1-3	+
3-3	+	1-4	+
4-4	+	1-5	-
5-5	+,+	1-6	-,-,+
6-6	+	2-5	-
		5-6	-

Table 2. Summary of histocompatibility assays with Ephydatia muelleri. Fusion and non-fusion are represented by + and -, respectively.

	1	2	3	4	5	6	Strain
1	+						E.m.,A
2	+	+					"
3	+		+				"
4	+			+			"
5	-	-			+		E.m.,B
6	-*				-	+	E.m.,C

* Two of the three assays for the pairing of the specimens 1 and 6 showed non-fusion.

Table 3. Results of the histocompatibility assays with Spongilla lacustris. Fusion and non-fusion are represented by + and -, respectively. The number of symbols in the results column corresponds to the number of assays done for that particular pairing.

Pairing	Result	Pairing	Result
1-1	+	1-3	+
2-2	+	2-3	+,+
3-3	+	3-9	-,-
4-4	+	4-5	+
5-5	+	4-9	+,+
6-6	+	5-6	+
7-7	+	5-8	+
8-8	+	5-9	+,+
9-9	+,+,+	6-7	+
		6-8	+

Table 4. Summary of the histocompatibility assays with Spongilla lacustris. Fusion and non-fusion are represented by + and -, respectively.

	1	2	3	4	5	6	7	8	9	Strain
1	+									S.l.,A
2		+								"
3	+	+	+							"
4				+						S.l.,B
5				+	+					"
6					+	+				"
7						+	+			"
8					+	+		+		"
9			-	+	+				+	"

performed, two showed non-fusion and one showed fusion (Table 1). Since two of the three grafts between specimens one and six showed non-fusion, the final result of this assay was considered non-fusion.

The results of the histocompatibility assays with Ephydatia muelleri were used to estimate the degree of genetic variation in this species in the Salmon Lake outlet. The observation that not all grafts fused suggests that the population of E. muelleri in Salmon Lake is not a single clonal population. For E. muelleri non-fusion between individual sponges, which represented different strains, occurred both in the same locality and in different localities (Figure 1). Three strains, A, B, and C, were identified. Different strains of E. muelleri were found to inhabit the same rock in the field (Figure 5).

Spongilla lacustris

The results of the histocompatibility assays for Spongilla lacustris are compiled in Tables 3 and 4. All S. lacustris specimens were collected from the Salmon Lake outlet. All pairings involving pieces from the same sponge showed fusion. Specimens one through three, fused with each other (Table 4) and were located in the same area (Figure 2). Similarly, specimens four through nine fused with each other (Table 4) and were located in the same place (Figure 2). However, specimens three and nine did not fuse

with each other (Table 4). These specimens were separated by approximately 15 m (Figure 2).

Fusion responses and non-fusion responses displayed in assays with Spongilla lacustris were similar to those exhibited in assays with Ephydatia muelleri.

The results for Spongilla lacustris were used to estimate the genetic variation in this species in the Salmon Lake drainage. The observation that not all grafts fused suggests that the population of S. lacustris in Salmon Lake is not a single clonal population. Two strains, A and B, identified.

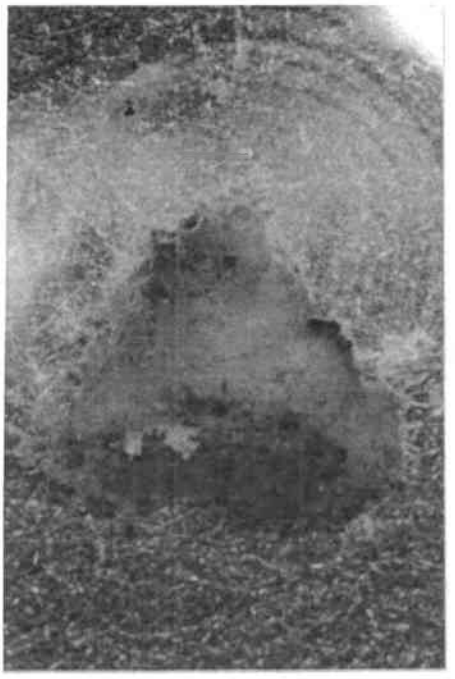

Fig. 3. Graft fusion in Ephydatia muelleri

Fig. 4. Graft fusion in Spongilla lacustris

Fig. 5. Two strains of Ephydatia muelleri occupying the same rock in Salmon Lake

Fig. 1 Strain distribution of Ephydatia muelleriat the Salmon Lake outlet.

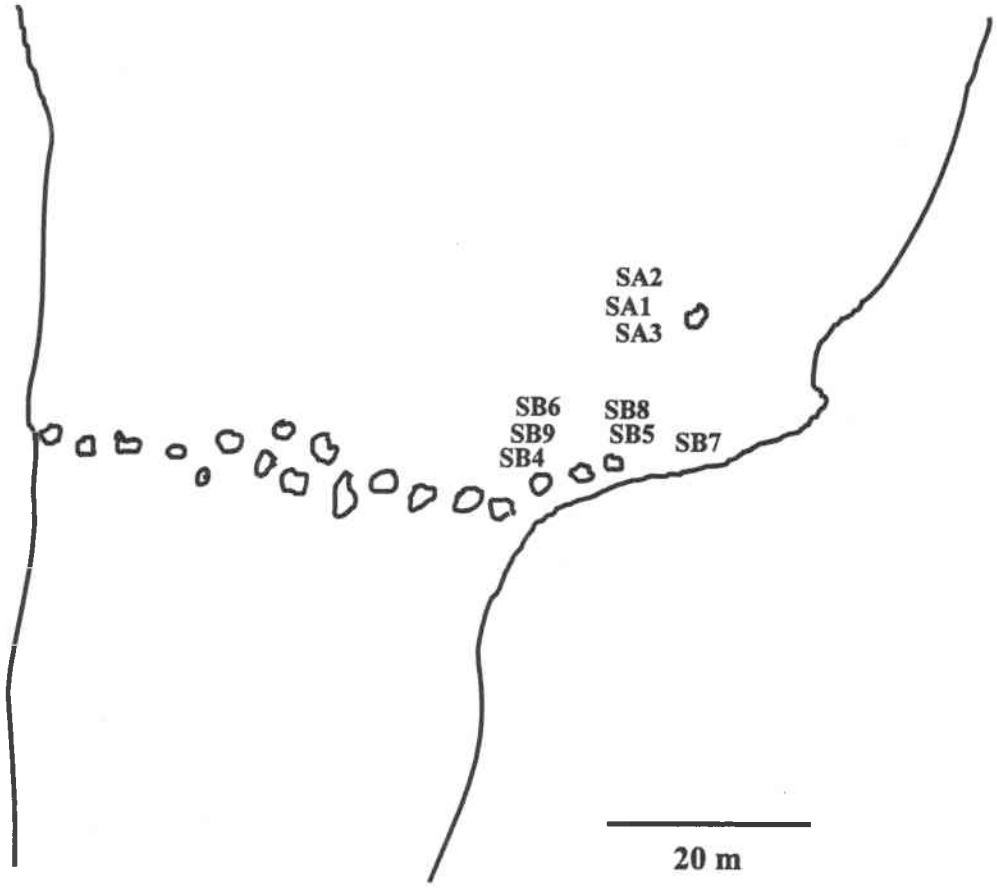

Fig. 2. Strain distribution of Spongilla lacustris at the Salmon Lake outlet.

DISCUSSION AND CONCLUSIONS

The specimens of Ephydatia muelleri used could be divided into three strains, A, B, and C, based on the results of the histocompatibility assays. Additionally, the specimens of Spongilla lacustris could be divided into two strains. Since non-fusion is evident between sponges, it seems that sponges have developed an active process that prevents allogenic overlap.

A rejection reaction occurs between individual chimeric sponges of Ephydatia muelleri growing in the same pond (Mukai, 1992). Since the rejection reaction always begins with the disappearance of the external pinacoderm in the zone of contact (Van de Vyver et al., 1990), the rejection reaction results in non-fusion in the zone of contact.

Of the three strains of E. muelleri found in this study, strain C had unusual characteristics, and was, in fact, unique in appearance. Strain C was light tan, and its body was particularly incompressible and brittle compared to strains A and B. In contrast, the latter two strains of E. muelleri were similar to each other in appearance, both being brownish green and having a softer body than strain C. The rigid body of strain C may have interfered with the histocompatibility assay. For example, one of the three pairings of strain C with strain A showed fusion.

Conceivably, intertwining of spicules accounted for one of the three pairings of strain C with strain A showing fusion. On the other hand, strain C's rigidity could have interfered with fusion. If this were the case, than A and C could actually represent different growth forms of the same strain. Providing evidence against the latter, however, was the observation that sponges showing the characteristics of strains A and C did not fuse when growing adjacent to one another on the same rock (Figure 5).

Histocompatibility assays between specimens of Spongilla lacustris that were separated by approximately 15m in Salmon Lake did not fuse while assays between specimens separated by a distance less than 15 m did fuse. A similar phenomenon occurred between the strains A and B and the strains C and B of Ephydatia muelleri which were separated by approximately 60 m in Salmon Lake. However, strain C of E. muelleri, which is composed of those sponges with the unusual characteristics discussed earlier, was found in the same location as strain A of E. muelleri. These results (with the exception of strain C) suggest that the probability of selecting two individuals of different strains increases with increasing distance between the two individuals. This spatial distribution of genetically unique populations can be explained in terms of clonal propagation (Neigel and Avise, 1983). Iotrochota

birotulata clones appeared to be restricted to single coral heads or solid patch reefs, the discrete "patches" of the habitat used by this species (Neigel and Avise, 1983). Vegetative extension of clonal colonies in Ephydatia muelleri and Spongilla lacustris may also be constrained to specific habitats as in the case of I. birotulata where clonal colonies were restricted to specific coral heads.

It is possible that certain strains are better adapted to specific habitats than other strains. For example, strain C was the only strain of Ephydatia muelleri that was found growing in shallow slow moving water. It was found growing in as little as 0.1 m of water. Since the temperature decreases as the depth of the water increases, it is possible that strain C of E. muelleri is capable of withstanding higher temperatures and greater fluctuations in temperature than other strains of E. muelleri without showing adverse effects. Additionally, if the area around a sponge colony is not conducive to growth of that specific sponge, it would act as a barrier for vegetative propagation, thus isolating that particular strain of sponge. Therefore, habitat may limit clonal distribution.

In addition, sexual reproduction potentially could have the same effect clonal propagation had on genetic population structure, if gamete and larval dispersal were so limited as to almost completely restrict gene flow within a population (Rohlf and Schnell, 1971). This event could occur in Salmon

Lake if a unique strain were located in water that was less affected by the water currents. In this case inbred patches of closely related individuals, monomorphic for the genetic determinants of histocompatibility, would result since gametes could not be displaced without a water current. These inbred individuals would appear as single clones.

Markezich and Francis (1991) proposed a model to explain the genotype of sexually reproducing sponges. This model can also illustrate how one gene for histo-compatibility could account for the existence of the three strains of Ephydatia muelleri. The model assumes that larva and adult sponges are diploid, and that sex in adult sponges is either male or female, but not both at the same time. The model is based on a single gene that is responsible for histocompatibility characteristics. This gene would have several functional alleles of two types: type I and type II. The model permits fusion as long as the strain genotypes are the same.

The Markezich and Francis model could be used to explain how one gene for histocompatibility could account for the presence of three strains in Ephydatia muelleri. For simplicity, in this presentation of the model I will use three type I alleles and one type II allele:

Type I alleles: a1, b1, c1

Type II alleles: d2

Three viable diploid strain genotypes are possible with the

four alleles:

a1d2

b1d2

c1d2

A genotype composed of two alleles of the same type, such as a1b1, would not be viable. One of each of the three genotypes could specify strain A, stain B, and stain C. In this model, strains A, B, and C could exist if there were four alleles for the gene responsible for histo-compatibility.

Another model could be proposed to explain the genotype of sexually reproducing sponges. This model would also be based on a single gene that is responsible for histo-compatibility characteristics. This gene would have several functional alleles that were either dominant or recessive.

This model could also explain how three strains could exist in Ephydatia muelleri if there were only one gene for histocompatibility. In this presentation of the model, three alleles will be used: X, Y, and Z. The X allele is dominant to both the Y and Z alleles. The Y allele is dominant to the Z allele. Six viable diploid strain genotypes are possible with these three alleles:

XX, XY, XZ

YY, YZ

ZZ

The dominant X allele could represent strain A of

Ephydatia muelleri. Therefore, three of the six genotypes would designate strain A. The Y allele could specify strain B of E. muelleri. Therefore, two of the six alleles would designate strain B. Lastly, the Z allele could specify strain C of E. muelleri. Since the Z allele is recessive to both of the other alleles, only one of the six genotypes would designate strain C. Thus, in a given population of sponges having these three alleles in equal frequencies, strain A would represent 50% of the population, strain B would represent 33% of the population, and strain C would represent 17% of the population.

Larvae from sponges of different strains fuse with each other. Markezich and Francis (1991) argued that early fusion of the larva tissue would be advantageous to the sponge because the greater number of cells should more successfully colonize the available substrate upon settlement. Ilan and Loya (1990) not only demonstrated that larva from different sponges fuse, but larvae from sponges that were thought to be genetically different fused. The phenomenon of larval fusion is completely different from the results of histocompatibility assays that used adult sponges of different strains. In assays involving adult sponges, adult sponges of different strains did not fuse. In order to explain why the sponge histocompatibility system may be turned off in larva but functional in the adult, it is necessary to look at the potential advantage the larva and

adult would gain from manipulating this system.

Sponge larva capable of fusing with other larva of different strains would have a selective advantage. When several genetically different larva fuse, they produce a chimera that is larger than any of the individuals that created it. Small body size in marine invertebrates is often accompanied by high mortality, whereas larger size results in higher survivalship (Ilan and Loya, 1990). Additionally fusion of larvae should result in reduced competition within the species because fewer cells of a single sponge would be involved in competitive interactions with adjacent sponges of the same species. Since sexual maturity is often size-related (Ilan and Loya, 1990), another possible benefit a chimera would have is early reproduction. This would reduce the generation length and produce more offspring per unit time. Lastly, a chimera would have a compound genotype. As a result, a chimera might gain more physiological resistance to different environmental conditions than any of its members separately.

The adult sponge has the ability to express its histocompatibility gene, and as a result, sponges of different strains will not fuse. This phenomenon would be a selective disadvantage to the larva, but it is selectively advantageous to the adult sponge. Adult sponges have already reached a substantial size and do not need to fuse

with others to raise their survivalship or to reproduce early. Instead, in the adult sponge, it is probable that the disadvantages of the total large size produced by fusion of unlike strains would outweigh the chimeric benefits.

By expressing the histocompatibility response, the adult sponge avoids the disadvantages of the chimeric relationship. One member of the chimera could be what Ilan and Loya (1990) referred to as "parasitic" to the other members of the chimera. This parasitic member could differentiate its germ cells to gametes, taking advantage of the other members of the chimera by using their somatic tissue for maintenance (Ilan and Loya, 1990). This arrangement would put the non-parasitic members of the chimera at a disadvantage since their ability to pass on their genotype to the next generation would be hindered.

Selection would probably act against individuals with strain alleles which determine histocompatibility that were different from strain alleles present at relatively high frequencies in the population. The sponges having the alleles present in high frequency would be able to fuse to each other. These larger sponges would be able to out-compete the smaller sponges possessing the "low frequency" strain alleles. They could not be victims of a parasitic relationship since all members of the sponge are of the same strain. Therefore, because of natural selection, sponges having the same strain allele will tend to occupy a given

locality in the absence of sponges having different strain alleles.

Histocompatibility assays between every possible specimen pairing would have strengthened the results of this work. Unfortunately two factors made specimens scarce. First, many colonies were too small to produce an adequate amount of tissue to make an assay for every possible specimen pairing. Second, the water level dropped during late June 1994, causing many sponges to die. As a result many of the colonies being used for these assays died. A future study should strive to complete specimen collection after specimens have reached their maximum size, but before water levels drop during midsummer.

The results indicate that certain members of the same species will not fuse with each other. It is concluded that there is a genetic basis for the phenomenon of non-fusion between sponges of the same species. If different strains of sponges do in fact exist, it is interesting to speculate on the origin of these different strains in the Salmon Lake outlet. I will offer two possible explanations for the occurrence of these strains which, with the exception of strain C of Ephydatia muelleri, seem to be isolated from each other. First, there could have been a mutation in one of the genes for histocompatibility to produce a new allele for that gene. Second, the different strains could have been introduced into the lake by an outside source. In

certain instances, gemmules, "seed-like" asexual reproductive structures, could be transplanted into the lake from sponge colonies in other locations by a motile animal such as a bird. Each gemmule would then grow into an adult sponge of the particular strain from which it originated. Regardless of how these strains originated in Salmon Lake, their presence suggests that populations of sponges, Ephydatia muelleri and Spongilla lacustris, in this lake are genetically diverse.

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