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Effects of Continued Selfing on
Hymenolepis nana (Cestoda)[1]

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Abstract. The effects of selfing on *Hymenolepis nana* were investigated by establishing single infections in mice, thus ensuring self-fertilization. Selfing appears to increase the frequency of cysticercoid abnormalities. The proportion of eggs developing into cysticercoids in *Tribolium confusum* appears to decrease with continued selfing. Similarly, the proportion of cysticercoids developing into adults in mice decreases with continued selfing.

Selfing causes no observable variation in the size of either adults or cysticercoids, nor does it cause changes in the frequency of appearance of certain morphological variants of the adult worm. The variant of reversal of genital pore appears to cause sterility of those proglottids demonstrating this modification.

No selfed strain could be maintained beyond the fifth selfed generation. A population of twenty adult worms, the number employed in control mice, does not appear sufficient to prevent the decrease in viability caused by selfing. Cercomer damage is of little importance in the infectivity of the cysticercoid.

INTRODUCTION

This study was undertaken to gain a partial understanding of the effects of selfing on the genetic mechanisms of the cestode, *Hymenolepis nana* (von Siebold, 1852) Blanchard, 1892, and to determine the action of such mechanisms on its infectivity and morphology. The work has consisted of the continued maintenance of a strain of *H. nana* with a single worm established in each definitive host. The presence of only one worm necessitates each proglottid either to fertilize itself or to be fertilized by another proglottid of the same individual. Thus, selfing may be enforced in each generation.

The effect of this selfing on the viability of the cysticercoids is reported herein. A morphological study of cysticercoids and adults of the selfed generations was undertaken in order to investigate the possible changes induced by selfing. Cysticercoids were also studied to determine if there was a visible difference between viable and non-viable specimens.

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A colony of mice harboring multiple infections of *H. nana* was also maintained as a control.

HISTORICAL REVIEW

The effects of selfing and other types of close inbreeding have been extensively investigated since the time of Darwin. Many such investigations are cited or discussed by Gowen (1952). Thus, inbreeding in swine has been shown to have a deleterious effect on general growth rates and survival ability, although not accompanied by significant changes in body conformation. Similar results have been recorded from studies on the inbreeding of guinea pigs.

The most complete study on the selfing of a normally cross-fertilized organism, maize, was that reported by Jones (1939). He reported a decrease to 40% of the normal yield after five selfed generations, a further drop to 20% by the tenth, and a slower decline until the twentieth generation. The size of the mature plant dropped drastically during the first five generations, and thereafter remained somewhat constant. Apparently, there are fewer genes controlling size than controlling a complex factor such as yield. Because of this, the genes for size become homogeneous before those determining yield, and the deleterious effect of this homogeneity is noted earlier. No variants favorable to survival were noted in this study.

Studies on the effect of temperature on viability and development of *H. nana* cysticercoids have been reported by Heyneman (1958) and by Voge and Heyneman (1958). Morphology of the normal cysticercoid and the common variations due to temperature stress are given in these papers. Additional work on the normal development of cysticercoids of this species in *Tribolium confusum* (Duval, 1868), was presented by Voge and Heyneman (1957) and by Schiller (1959a).

In reporting the results of x-irradiation of *H. nana*, Schiller (1959c) analyzed normal and irradiated cestodes with respect to the occurance of five variants. These variants were: (1) fusion of cirri, (2) reversal of genital pore, (3) position variant of testes (two poral, one antiporal), (4) reduction of the number of testes to two, and (5) sterility. Schiller found that abnormal proglottids were present in his irradiated cestodes in direct proportion to the amount of radiation to which the worms had been exposed. This indicated that such variants were analogous to gene and chromosome mutations, and that the effect of the radiation was to increase the frequency of these genotypic changes.

MATERIALS AND METHODS

Intermediate Hosts. Cultures of laboratory-reared *Tribolium*

confusum were kept at room temperature in large petri dishes containing a mixture of wheat bran and rolled oats. Pieces of apple were added periodically to provide moisture.

Definitive Hosts. Albino mice of the Swiss-Webster strain were obtained from two commercial sources: Gauthier Animal Colony, 603 Loomis Avenue, Corning, Iowa, and Arthur Sutter, 1813 W. Phelps, Springfield, Missouri. Animals not infected with *H. nana* or other tapeworms were selected from among the purchased mice and these served as parental stock for all experimental work. Fecal pellets of each mouse were examined periodically and any individuals harboring *H. nana* were destroyed or isolated in a separate room so as to avoid possible contamination of experimental animals. Commercial feed and water were freely available to the mice.

Exposure of the Intermediate Host. The strain of *H. nana* used throughout these experiments was present in mice from the Gauthier Animal Colony and was maintained through ingestion by the mice of contaminated fecal pellets. These infected mice were kept in a separate room.

Adult H. nana were procured either from mice harboring natural infections or from experimental mice 11 days after exposure to cysticercoids. The mice were sacrificed by a blow on the head and a cervical fracture. The small intestine was resected, adult cestodes removed from the ileum, and then placed in normal saline. The posterior 8-10 proglottids were removed, placed on a small piece of moist filter paper, and teased apart. A small sample of the eggs released was examined under a compound microscope to determine if well-formed oncospheres were present.

Prior to exposure, adult beetles were isolated from food and water and kept approximately 48 hours in an incubator at 30-32° C. Five beetles were then removed from the incubator and placed in a one-inch stender dish. Moistened filter paper containing *H. nana* eggs was placed in the dish and allowed to remain for 6-8 hours at room temperature. During this period, the beetles fed randomly upon the eggs. The paper was then removed, a small amount of bran added to the container, and the cultures returned to the incubator.

Exposure of the Definitive Host. Six days after exposure of *T. confusum* to eggs, the beetles were dissected in insect Ringer's and the cysticercoids removed from the haemocoel. While cysticercoids of an experimental nature were kept separate, the control cysticercoids were pooled before infection of the definitive host. In the experimental groups, each mouse received a single cysticercoid. Simple free-hand drawings were made of such

cysticercoids. In control groups, multiple infections were established. All cysticercoids were force-fed to mice within 45 minutes after their removal from the intermediate host.

Mice of either sex, two to four months of age, were kept from water for one-day pre-exposure. Following light ether anesthesia, the mice were exposed by pipetting cysticercoids directly into the stomach. One experimental group was exposed without the use of ether. Exposed mice were kept in a room reserved for infected individuals.

Microtechnique. All cysticercoids not used in feeding experiments were fixed in AFA (alcohol-formalin-acetic acid) and stored in 70% ethyl alcohol. Some specimens were prepared as whole mounts using Mayer's paracarmine with fast green counterstain.

After removal of the posterior proglottids for the exposure of the intermediate host, adult *H. nana* were relaxed for one hour in tap water at room temperature. Unflattened worms were then fixed in AFA and later transferred to 70% alcohol. Cestodes used for whole mounts were stained with Mayer's paracarmine.

All line drawings were made from whole mounts with the aid of a micro-projector.

Feeding Experiments. In the following sections, symbols are used in designating generations of cestodes. P_1 indicates the adult worms recovered from naturally-infected mice, the P referring to cestodes serving as parental stock for the selfed generations. P_2 indicates the progeny of the P_1 generation, adult cestodes similar in heredity to P_1 for they too presumably developed from eggs derived from cross-fertilization in naturally infected hosts. S_1 refers to the first selfed generation. S_2, S_3, etc., refer to further selfed generations. Cysticercoids are designated by symbols indicating the adult generation produced by them. Thus, CS_1 refers to the cysticercoids destined to produce the S_1 adults.

Several experiments were completed during the course of this investigation. Two controls were also maintained: Control A in conjunction with Experiments 1, 2, and 3, and Control B in conjunction with Experiment 4. Control A was maintained by exposing each mouse in the control to six cysticercoids. Control B consisted of mice exposed to 25 to 50 cysticercoids. All techniques of exposure and care were similar to those employed in experimental infections, except in the number of cysticercoids used.

In Experiment 1, involving ether anesthesia, cestodes were reared through the fifth selfed generation. *H. nana* from a

naturally infected mouse with a burden of approximately 30 worms provided the P_1 generation.

In Experiments 2 and 3, cysticercoids from the fourth generation of Control A were used as the CP_2 generation. In these two experiments, cestodes were reared through the S_2 generation. Experiment 2 was handled as above with the use of ether. Exposure in Experiment 3 was made without the use of ether. All data from Experiments 2 and 3 were parallel, indicating that the use of ether as an anesthetic had little effect on the cestodes.

In Experiment 4, the strain was raised through the S_2 generation. The results of only Experiment 1 are discussed below, since this experiment provided the most extensive data on selfing.

RESULTS

Variations of Cysticercoids. In the study of cysticercoids developing from selfed generations, measurements were taken from simple line drawings made at the time of feeding. There appears to be no significant trend in the size of cysticercoids with regard to either selfing or viability. The mean size of the cysticercoids apparently does not change with continued selfing. Neither is there any significant difference in the size of viable cysticercoids and those not producing adult cestodes.

The number of cysticercoids developing in experimental beetles is dependent primarily on the random feeding of the beetle. As many as 154 cysticercoids were recovered from one *T. confusum*. When large numbers of cysticercoids were found in a single intermediate host, the cysticercoids were smaller due to the crowding effect. Since the time allowed for the development of the cysticercoids was greater than the minimum as determined by Heyneman (1958), delayed development due to the crowding effect was avoided.

Abnormalities and cercomer damage were noted at the time that the line drawings were prepared. Of a total of 14 cysticercoids with torn cercomers at the time of feeding, four or 29% were viable. Of the total cysticercoids fed singly, 35% were viable. This substantiates the conclusion of Schiller (1959b), who found that cercomer damage was of little importance in the infectivity of the cysticercoid.

Cysticercoids noted as abnormal were found in the following generations: three in CS_2, two in CS_3, one in CS_4, and two in CS_6. These abnormalities may represent delayed normal stages of development, but they appear to occur more frequently after several selfed generations. The abnormalities noted were lack of development or poor development of rostellum, suckers, or internal membrane (Figs. 1, 2). As none of these cysticercoids was

viable, any abnormality noted at the time of feeding was apparently sufficient to have inhibited development completely. Similar abnormalities have been shown by Voge and Heyneman (1958) to result from high temperature stress.

Variations of Adults. The frequency of variant characters was analyzed by studying the proglottids of each adult worm in the sample and calculating the percentage of those showing the condition under study. In addition to the five previously noted characters analyzed by Schiller (1959c), the frequency of abnormal segmentation was analyzed. Thus, six characters, described below, were chosen because of their ease of recognition in stained whole mounts. The frequency of their occurrence, as well as the average length of the cestodes is given in Table 1. These data are from Experiment 1.

Although sterility may result from a number of disturbances of the reproductive system, it was analyzed in this study by examination of that portion of the strobila normally consisting of gravid proglottids. A segment was considered sterile if it lacked eggs or if it did not contain normal eggs. Proglottids of normal shape and those showing abnormal segmentation were included as showing this condition if they met the above requirements.

The failure of one testis to develop most often results in a proglottid with the two remaining testes in a one-poral, one-antiporal configuration (Fig. 4). The two-antiporal formation has also been observed.

Infrequent variants of the normal testes arrangement (one-poral, two-antiporal) included two-poral with two-antiporal (Fig. 3), two-poral with three antiporal (Fig. 5A), one-poral with three-antiporal (Fig. 5B), and three-antiporal (Fig. 6A). The most common variant of testes arrangement was two-poral, one-antiporal (Fig. 6B). Testes variations seem to have little effect on sterility.

Abnormal segmentation is a general term, referring to several types of variations. Two segments may fail to separate completely and, in such instances, a single U-shaped uterus may be associated with the two incomplete segments (Fig. 7), or separate uteri may appear (Fig. 8) as in normal proglottids. Another type of abnormality involves the appearance of a triangular segment between two normal segments (Fig. 9). More rarely, a double set of genitalia is found in one segment. Most abnormal segments contain a normal complement of eggs.

Reversal of genital atrium refers to the abnormal location of the atrium on the left margin of the adult strobila rather than on the right. Most commonly, the other reproductive structures are
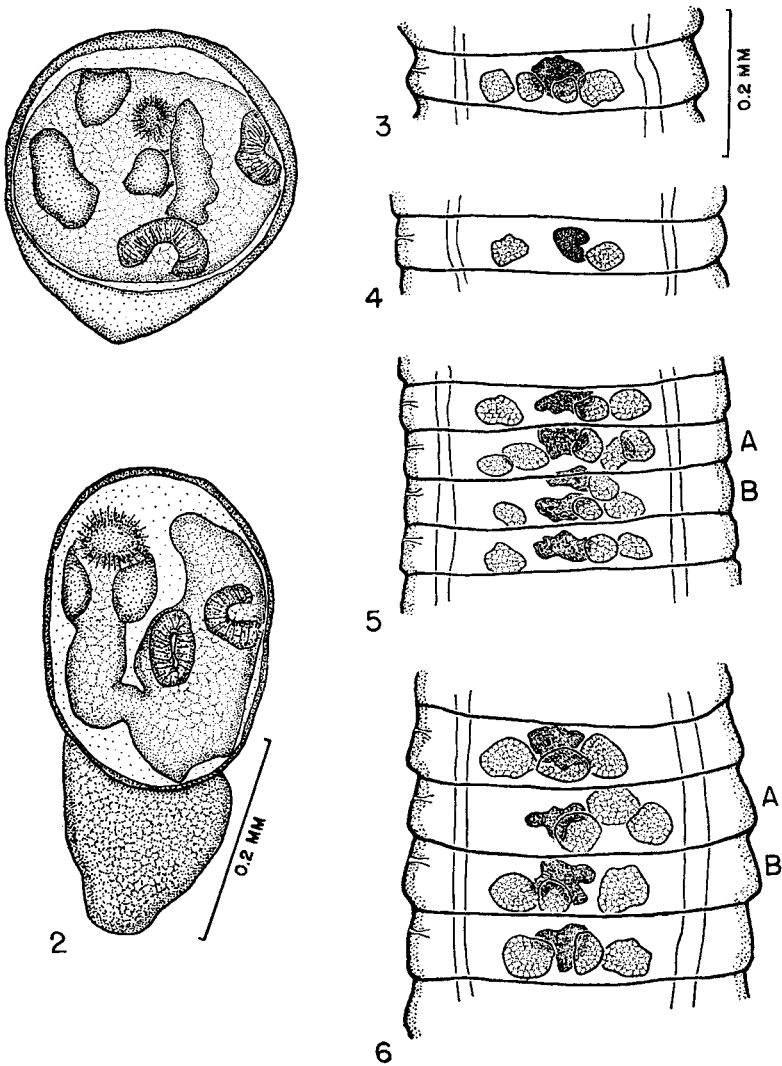
Figure 1. Abnormal cysticercoid; note lack of cercomer, failure of capsule to develop normally, and imperfect development of suckers and rostellum.

Figure 2. Abnormal cysticercoid; note evaginated scolex and abnormal sucker formation.

Figure 3. Testes variant of 2 poral, 2 antiporal.

Figure 4. Testes variant of 1 poral, 1 antiporal.

Figure 5. A. Tests variant of 2 poral, 3 antiporal.
           B. Testes variant of 1 poral, 3 antiporal.

Figure 6. A. Testes variant of 3 antiporal.
           B. Testes variant of 2 poral, 1 antiporal.

Figs. 1 and 2 are drawn to the scale shown in Fig. 2.
Figs. 3 through 6 are drawn to the scale shown in Fig. 3.

Table 1. Frequency of variants and average length of selfed cestodes

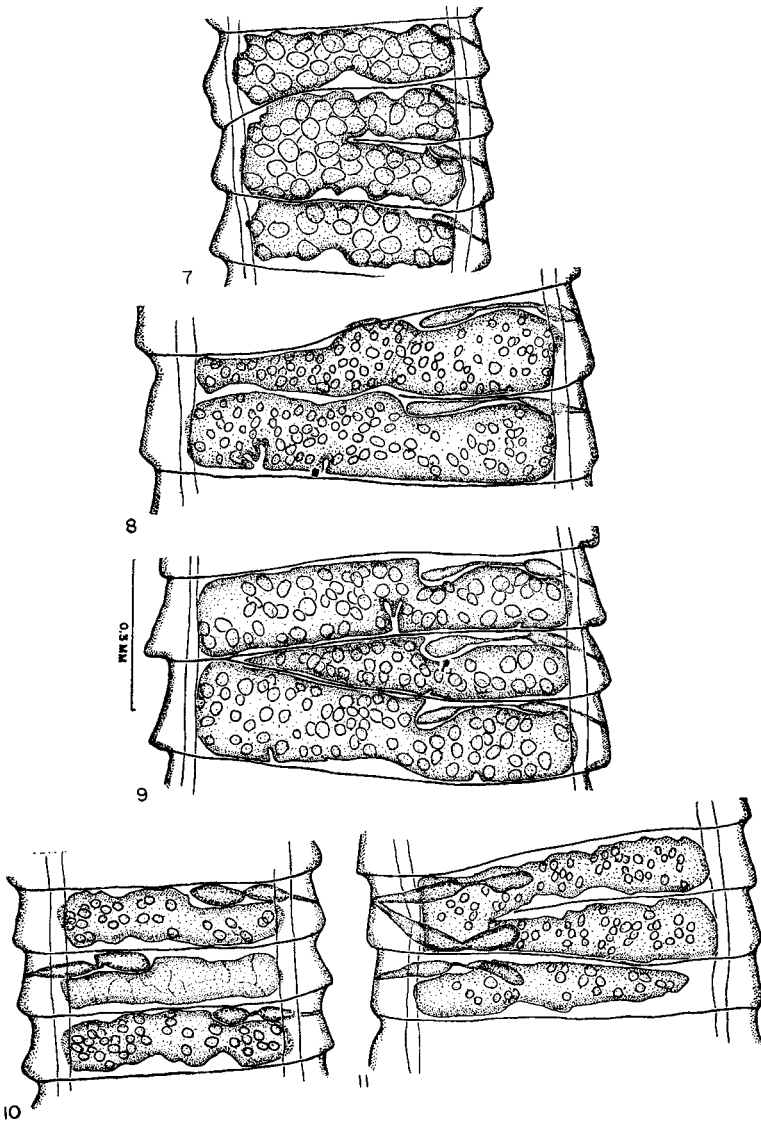| Generation	Sterility Per cent	2 testes Per cent	Testes variant 2 p, 1 ap Per cent	Abnormal segmen- tation Per cent	Reversed genital atrium Per cent	Common genital atrium Per cent	length cm	Sample size
P_1	8.60	1.94	11.1	2.34	3.32	1.35	9.0	4
P_2	0.00	0.456	5.82	1.17	0.042	0.625	13.9	3
S_1	0.131	0.727	8.62	2.05	0.127	0.802	16.3	3
S_2	0.288	0.789	4.03	1.82	0.117	0.935	16.9	3
S_3	0.140	0.704	8.55	2.14	0.104	1.21	10.4	2
S_4	0.00	0.708	5.48	1.72	0.00	1.32	14.4	1
S_5	0.457	1.32	5.00	2.20	0.00	0.884	18.9	1

Figure 7. Abnormal segmentation, U-shaped uterus.
Figure 8. Abnormal segmentation, double uterus.
Figure 9. Abnormal segmentation, triangular segment.
Figure 10. Reversal of genital atrium with accompanying sterility.
Figure 11. Common genital atrium and abnormal segmentation, U-shaped uterus.
    Figures 7 through 11 are drawn to the scale shown in Fig. 9.

also reversed so that they retain their normal orientation with re-
spect to the atrium. This abnormality appeared in 39 gravid
proglottids and was also seen in immature and mature proglot-
tids. Normal eggs were found in only three of the gravid prog-

lottids exhibiting this abnormality. In these cases, the number of eggs in the uterus ranged from one to seven. Apparently, the changes associated with the reversal of the proglottid are suffici- ent in most cases to render it sterile (Fig. 10).

The condition referred to as common genital atrium is char- acterized by a single genital pore serving the genital ducts of two or more proglottids (Fig. 11). This variant has also been termed cirrus fusion. It appears to have little effect on the steril- ity of the proglottid.

There appears to be no general pattern in the frequency of variations as studied in different selfed generations. The wide variation among samples, for instance, is shown in generations P_1 and P_2 (Table 1). These two generations should have similar values as they both are presumably the result of a normal infec- tion. The values for P_1 are not entirely comparable to those for the other generations, as P_1 designates worms taken from a multiple infection; and therefore more variations in size and oth- er characters are expected.

Changes in Viability of Eggs and Cysticercoids. Loss of vi- ability of *H. nana* due to selfing should be demonstrable at two points when the indirect path of development is used. If this loss of viability is a true effect of selfing, one would expect few- er eggs to develop into cysticercoids and fewer cysticercoids to develop into adults as selfing continues.

Although the quantity of cestode eggs ingested by *T. confusum*
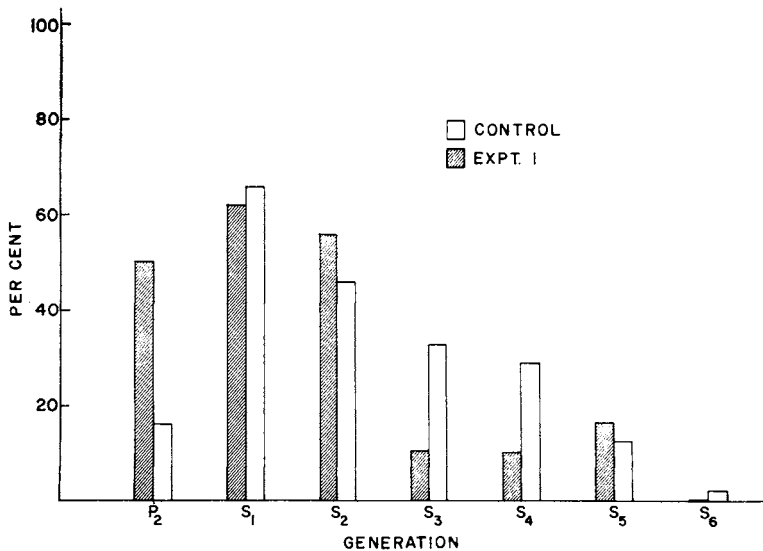
GRAPH I. VIABILITY OF CYSTICERCOIDS

is unpredictable, the number of cysticercoids developing per beetle has a fairly consistent relationship with the generation of the selfed worm. Thus, the average number of cysticercoids developing per beetle in the selfed generations varied as follows: 8.4 in CP_2, 17 in CS_1, 7.4 in CS_2, 6.8 in CS_3, 4.0 in CS_4 6.2 in CS_5, and 6.0 in CS_6. A decrease in the viability of the eggs as selfing continues is suggested by this data.

By plotting the per cent of cysticercoids developing into adults versus the generation to which they belong, a definite decrease in viability with selfing can be demonstrated. Graph 1 shows this relationship as demonstrated by Experiment 1 and Control A. A possible explanation for this loss of viability among control animals is discussed below.

DISCUSSION

With the exception of the increased frequency of abnormalities, the size and morphology of cysticercoids does not seem to be affected by selfing. There is no visible difference between cysticercoids producing adults and those not developing to maturity. Neither does there seem to be a change in the morphology of the adults as selfing continues. With the exclusion of the aberrant P_1 generation, the six abnormalities studied appear at approximately the same frequency throughout the selfed generations.

Both eggs and cysticercoids appear to suffer a loss of viability due to selfing. The loss demonstrated by the lowered production of cysticercoids from eggs ingested by *T. confusum* is difficult to present clearly. The loss of viability in cysticercoids shown by the decline in the development of adult *H. nana* can be more clearly shown because of the greater amount of control which can be exerted over the ingestion of cysticercoids.

In considering the results described above, the pronounced decrease in viability associated with selfing appears to be the most significant. The opposite of this deleterious effect is termed hybrid vigor. As mentioned before, work in this area has been summarized by Gowen (1952). Hybrid vigor is evidenced by the increased vigor and size of a hybrid oganism. Heterosis is the concept which attempts to account for this phenomenon.

Two explanations of heterosis are commonly advanced.. One of these asserts that the heterozygote is superior to either the dominant or recessive homozygote. Therefore, cross-breeding, with its accompanying increase in heterozygosity, would produce an increase in vigor. Many instances have been reported where the heterozygous condition is superior to the homozygous condition. The superiority of this heterozygous condition is from

interactions between the unlike alleles, not from the heterozygosity *per se*.

The second explanation involves the masking of deleterious homozygous recessives. The dominant alleles, which may be introduced by cross-breeding, eliminate or modify the deleterious effect of the recessive alleles.

According to each of the above explanations, an increase in heterozygosity produces an increase in vigor and, conversely, a decrease in heterozygosity would bring about a decrease in vigor.

Organisms which normally self, such as the tomato, are "self-cleaning," with selection tending to eliminate those individuals with deleterious characters. The tomato shows little hybrid vigor when two separate strains are crossed.

Cross-fertilized organisms tend to accumulate large numbers of deleterious recessives, usually in a heterozygous condition.
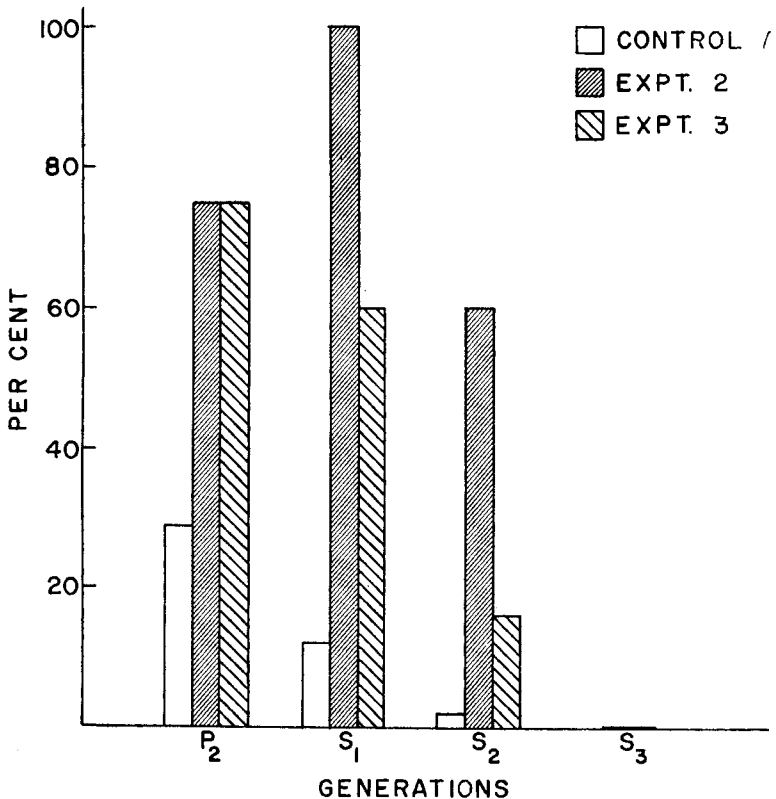
GRAPH 2. VIABILITY OF CYSTICERCOIDS
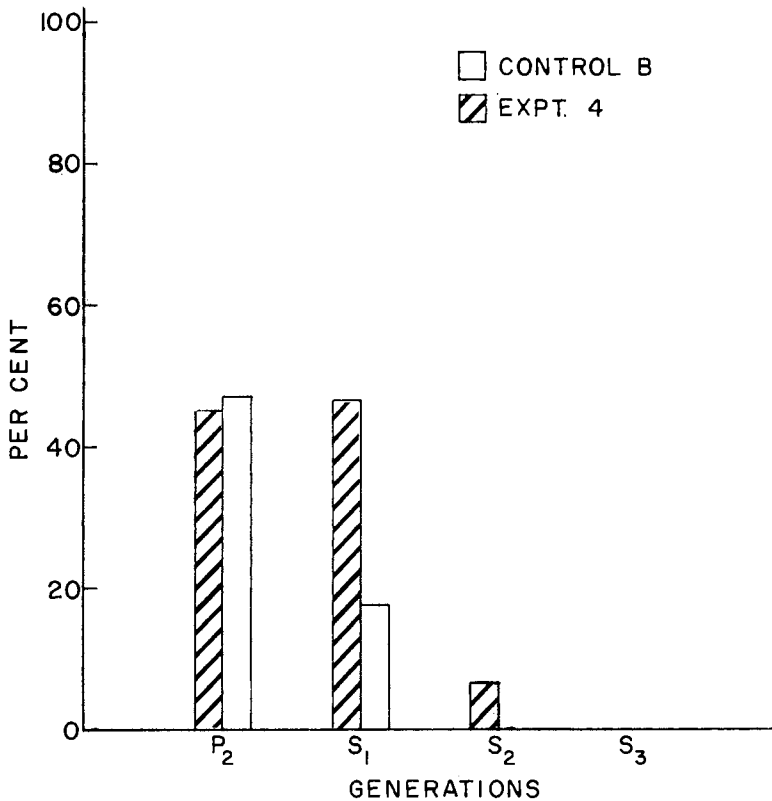
GRAPH 3. VIABILITY OF CYSTICERCOIDS

An experiment tending to produce a homozygous state would make it possible for these recessives to become manifest. Since selfing will theoretically produce a homozygous state sooner than any other reproductive method, it is possible to demonstrate the effect of this increased homozygosity within relatively few generations.

The decrease in viability associated with selfing as shown by these experiments indicates that cross-fertilization is the normal method of reproduction for *H. nana*. The high tolerance of the intermediate host enables large numbers of cysticercoids to be produced, making a multiple infection more likely in the definitive host.

Besides this indirect path of development, *H. nana* is unique in being able to infect its host directly. Viable eggs ingested by the definitive host will produce cysticercoids in the intestinal villi and these larval stages will mature subsequently in the gut lumen. This method of development also makes more probable

the presence of a multiple infection with its accompanying opportunity for cross-fertilization. Similar experiments on species such as *Dibothriocephalus latus,* commonly found in single infections, should provide additional interesting data on the effect of selfing.

The loss of viability shown in Graph 1 indicates that the control underwent considerable selfing despite the multiple infections present. In this control, the infection ranged from but two to four adult cestodes. A similar decrease in viability was noted in Control B, where a maximum of 21 adult *H. nana* was present. Although this number was believed at first to be sufficiently great to provide opportunity for cross-fertilization, experimental results indicated that selfing appears to occur commonly even when 20 worms (or less) are present in the gut.

An explanation other than the effects of selfing might be advanced for the decrease in viability of control and experimental infections shown in this study. It is suggested that *H. nana,* the only cestode capable of direct development, has developed a certain physiological dependence upon the direct cycle. Any individual whose progeny are more capable of following the direct cycle successfully would have an evolutionary advantage over other cestodes. Thus, through natural selection, the direct cycle might become the dominant mode of development. The experimental substitution of the indirect cycle might be deleterious when the experimental subject is primarily adjusted to the direct mode of development. This deleterious effect might be manifested by a decrease in viability and this decrease would become more severe with the continued use of the indirect cycle.

Further experimental work is needed to determine the number of adult cestodes necessary in a multiple infection to eliminate the effects of selfing. The possible physiological significance of the direct cycle might well be tested by studies on *Hymenolepis diminuta,* a related cestode whose development is dependent upon an intermediate host.

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Development and Comparative Morphology of the Gonopodium of Goodeid Fishes[1]

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REBECCA REITER[4]

Abstract. The Goodeidae, one of four families of the cyprinodont fishes and all of whose species are viviparous, have a modified anal fin in males for intromission of sperm. This gonopodium, with structures specific for each species, develop under the dual controls of genetically determined patterns and endogenous androgenic hormones. In males of all species of the family, rays 2 to 7 of the anal fin are shorter than the other rays and in some species they are single instead of bifurcated. The rays of this complex are strongly recurved and slender in some species. A comparative study of rays 2 to 7 in adult males of different species reveals that the primitive unspecialized structure of the juvenile fin is basic and that, in the modifications in the male fin, evolution has proceeded independently and in varying degrees in the several subfamilies.

INTRODUCTION

Four of the families of the cyprinodont fishes have developed independently ovoviviparity or viviparity. Internal fertilization, essential to these types of reproduction, occurs in each family and in each, parts of the anal fin of the male have become modified for the purpose of intromission of sperm. These specialized structures, known as gonopodia, are radically different in structure and in use in the four families. In the family Poeciliidae rays 3, 4, and 5 of the anal fin are elongated and in addition there are ray enlargements, fusions of rays and additions in specific positions of hooks, spines, serrations and other structures which are species specific. Taxonomists have taken advantage of this specificity to use the characters for the separation of genera and