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AN UNUSUAL SITUATION IN THE SALAMANDER
AMBYSTOMA JEFFERSONIANUM (GREEN)¹

BY WESLEY CLANTON

IN making a study of the life history of the salamander *Ambystoma jeffersonianum* in southern Michigan during the years of 1928–1932 a situation of considerable interest was discovered.

TWO FORMS OF ADULTS.—In this region *Ambystoma jeffersonianum* is composed of two confusingly similar forms that live together with little or no differentiation in activities. For the present, one will be known as the dark form and the other as the light form.

The most striking difference in the appearance of the adults is in the pigmentation. The dark form is very black, differing particularly from the light one in being heavily pigmented on the ventral surface. The lateral and ventral spots are usually large and distinct. The light form is less heavily pigmented, and it is more gray than black on the ventral surface. The lateral silver coloration tends toward mottling rather than spotting, and the ventral spots are small or absent. The two forms are illustrated in Plate I. Coloration alone, however, is

¹ Contribution of the Department of Zoology and the Museum of Zoology, University of Michigan.

not invariably sufficient for their differentiation. Moreover, alcoholic specimens of both forms become lighter in pigmentation soon after preservation.

The most certain distinction between them is in the eggs which in the dark form are smaller in size and less heavily pigmented than in the light form. The average size of the freshly dissected, ovarian eggs of the dark form has been found to be 1.65 mm. The smallest eggs measured were 1.54 mm. in diameter, the largest were 1.68 mm. The eggs of the light form are larger, averaging 2.1 mm., with a range of 1.81 to 2.23 mm. The difference in coloration of the eggs is striking, and it has been found to be constant. The lower surface of the eggs of the dark form is unpigmented, while the lower surface of the eggs of the light form is pigmented rather heavily. The upper surface, also, is less heavily pigmented in the dark form.

There is a distinct difference between the average number of eggs produced by the two kinds of females. The dark females, although smaller, produce a greater number of eggs, from 120 to 300, with an average of 216. The dark females vary in length from 82 to 134 mm., with an average of 104 mm. By contrast the light females produce from 50 to 216 eggs, with an average of 142. The range in length of these females is 93 to 142 mm., with an average length of 120 mm. These facts are presented graphically in Figure 1. Here it may be noted that there is no overlapping in the number of eggs produced by the two forms if the number is considered in relation to the length of the female.

There are several slight external differences between the two forms. The head of the dark form is relatively shorter and narrower than that of the light form. The cloacal opening of the dark females is relatively smaller than that of the light females.

The newly hatched larvae of the dark form are about 2 mm. shorter than those of the light form. Piersol (1910) found a female which deposited small eggs. The eggs developed into

small larvae, but as they did not feed well they were considered abnormal.

SEX RATIO.—One expects to find the sexes equally numerous in the salamanders of a habitat. As collections were made in various localities, it became noticeable that the females greatly outnumbered the males; furthermore, it was found that the females in excess were of the light type. Males of the light form were collected, but only rarely, for out of about thirteen

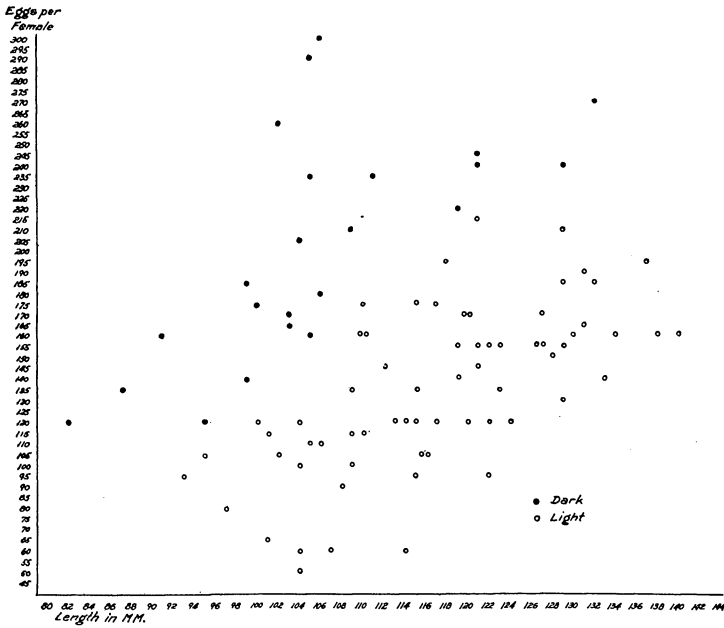


FIG. I. The relation of total length to the number of eggs produced by light and dark females.

hundred adults only three were identified as light males. In the dark form, the sexes were equally represented. There is, then, a form consisting predominantly of light females living in the same habitat with the dark form, which is composed of both sexes in equal numbers.

The sex ratio of the species as a whole varies in different habitats from an excess of 2 females to 1 male to 52 females

to 1 male. The dark individuals are most numerous in localities having a lower ratio of females. These localities are not common, for only three have been found, while many places have a large proportion of light females. The sex ratios in localities receiving study are as follows:

| Locality | Individuals examined | | Approximate ratio | |
|-------------------------------------|----------------------|-----|-------------------|---|
| | ♀ | ♂ | ♀ | ♂ |
| Scio Twp., Washtenaw Co. | 692 | 322 | 2 | 1 |
| Pittsfield Twp., Washtenaw Co. | 205 | 4 | 51 | 1 |
| Lodi Twp., Washtenaw Co. | 104 | 2 | 52 | 1 |
| White Oak Twp., Ingham Co. | 52 | 28 | 2 | 1 |
| Other places | 158 | 32 | 5 | 1 |

The abnormal sex ratio is not confined to the vicinity of Ann Arbor, Michigan, but has also been found to occur in other parts of the range. Females outnumber males 3 to 1 at Buffalo, New York, according to Humphrey (1928), who was first to report the condition. Bishop (correspondence to Blanchard) reports an abnormal ratio at Rochester, New York, his records showing 8 females to 1 male.

The number of individuals in a habitat is noticeably greater when the proportion of females is less. Logically, increase in population is handicapped by great irregularity in the sex ratio. At Pittsfield and Lodi, adults were not numerous; most of the individuals found, but not all, were newly transformed. From other localities, only adults were considered in determining the sex ratio. The fact that newly transformed individuals and adults give similar sex ratios precludes the possibility that the inequality is caused by some factor in the environment of the adult or some peculiarity in the collecting. Evidently the cause of the inequality is operative before transformation. No explanation of the unequal sex ratio was found until the two forms were mated together.

RESULTS OF CROSSING.—Since both forms migrate to the same breeding pond at the same time, and as light males are rare, cross matings must necessarily occur between dark males

and light females. In 1931, the available crosses, that is, a light female and a dark male, and a dark female and a dark male, were made in the field. Light males were not available, but since they are rare, their matings can be of little significance in the population. Environmental conditions were as nearly identical as possible; matings were made under the same conditions and at the same time. The spawn was deposited the same night, and the larvae hatched during the same period, and were reared under identical conditions. Of the fifty larvae from each form of female reared to determine the sex of the offspring, forty-three from the light female and forty-two from the dark female transformed successfully and were sectioned. In order to determine the appearance of the offspring, whether light or dark, other larvae from these females were reared to maturity.

The results of the matings explain the conditions found in nature. The forty-three offspring of the light female were of female sex. Of the forty-two larvae from the dark female, seventeen were males and twenty-five were females. The conclusion is, therefore, that dark males are produced only by dark females, and that light females produce offspring which resemble only the female parent in appearance and in sex.

The significance of such matings is important. Since in all localities studied, light females are at least as numerous as the dark, the mathematical chances for mating of a light female are as great or greater than for a dark female. Since the number of males does not exceed the number of dark females, and since the spermatophores produced by each male are few, it follows that in localities where light females are greatly in excess, as at Lodi and Pittsfield, the chances for dark females to mate are small. In other words, in a locality where the sex ratio is 2 ♀: 1 ♂ (1 light ♀: 1 dark ♀: 1 dark ♂) there is an equal chance for light and dark females to mate. In localities where the ratio is 50 ♀: 1 ♂ (49 light ♀: 1 dark ♀: 1 dark ♂), the chance for a dark female to mate is only one in fifty. In the former instance males are produced in half the matings, and in the latter, only once in fifty matings. This means that

as the proportion of light females increases, the chances for the production of males decreases. In such places the species must in the course of time become extinct through the failure to produce males.

Evidence of extinction has been found near Ann Arbor. The species is most numerous at Scio, where over a thousand adults have been taken in one habitat. The ratio here is 2 ♀: 1 ♂. At Pittsfield and Lodi, where the ratio is over 50 ♀: 1 ♂, adults are not common. At another habitat an adult female was found, but no eggs nor larvae were discovered in three years of observation. There is no marked difference in the habitats, all of which have other species of *Ambystoma* well represented.

EFFECT OF SEX RATIO ON LIFE HISTORY.—Although both abnormal sex ratio and low vitality of the eggs have been reported there has been no mention of any connection between the two. It seems probable that an excess of females would affect the breeding. That the disproportion of females is really an excess of this sex is evident when it is known that a male can mate with one or possibly two females a season.

In order to determine the number of times a male mates, it was first necessary to know how many spermatophores were deposited, and over how long a period breeding takes place. This was done by placing an unmated male with an excess of unmated females for several nights. Six males were tested in this manner. One male produced eight spermatophores, two produced six, and three produced three each, an average of about five from each male. Four males produced all their spermatophores the first night; one produced spermatophores the first and second nights; the other, the first and third. It is to be seen, as mentioned by Mohr (1930), that the number of spermatophores is small and that the period over which they are produced is relatively short.

The fact that males deposit about five spermatophores does not mean that each male mates with five females, for there is considerable waste. It is only occasionally that the female secures the first spermatophore deposited; quite often a male

deposits all his spermatophores without the female securing any of them. Even at Scio, where the ratio is only about two females to one male, not all the females are able to mate.

In a future paper it will be shown that *Ambystoma jeffersonianum* winters in the woods adjacent to the breeding ponds. With the first night rain after the snow has disappeared migration is started to the breeding pond. If the weather turns cold or the rain ceases the salamanders stop and wait for favorable conditions. The first migration brings a considerable number of salamanders to the water. A second migration usually occurs a few days later. Subsequent migrations take place until all the breeding adults have reached the pond.

NON-MATING.—Since the males are not able to mate with all the females present when they enter the water, many females do not mate at the proper time. These females may mate with males from the next migration, or they may not mate at all. Delayed mating, discussed under the next topic, results in an over ripeness of the eggs, which affects their vitality.

The effect of non-mating is less noticeable than delayed mating, for the eggs are absorbed in the ovary with apparently no harmful effect. The first indication that non-mating occurs was found in the springs of 1929 and 1930 when a considerable proportion of the females dissected exhibited black pigment spots in their ovaries. It was also noticed that females kept in the laboratory without mating had spotted ovaries. In a dry season, 1931, as many ponds had no water, few females mated. Frequent dissections of females which could not find water showed that the eggs were absorbed. The first sign of absorption is the formation of a capillary network around each egg. The eggs become soft and the yolk material soon disappears. The pigment remains as black spots in the ovary for as much as a year after the time the eggs should have been deposited. The absorption apparently does not affect the development of next year's eggs.

LOW VITALITY OF THE EGGS.—Piersol (1910) in reporting the low vitality of the eggs at Toronto, Canada, estimated that three-fourths of the eggs died before gastrulation. In some years the proportion was greater, in others less. Smith (1911) did not find low vitality at Syracuse, New York, nor did Mohr (1930) at Woodward, Pennsylvania; this shows that the condition does not occur throughout the range of the species. The conditions near Ann Arbor are so similar to those reported by Piersol that it is probable that they are comparable.

Piersol (1929) published a second paper in which he presented his conclusions on the subject. He found that some of the eggs die because of polyspermy, and that a larger number are not fertilized. In regard to polyspermy he states:

The known causes of polyspermy are of two kinds; first, things that increase the frequency with which sperms will come into contact with eggs, such as great concentration of the sperm; and second, things which cause slight injury to the egg, and so retard its metabolism. . . . Such causes are mere over ripeness of the eggs, narcotics and fertilization at temperatures somewhat below those normal for the eggs at that time.

He does not exclude the possibility of excessive concentration of the sperm or over ripeness of the eggs as causes for low vitality, but finds sufficient cause in the low temperatures occurring at the time of fertilization.

In regard to unfertilized eggs, he states:

There are two possibilities here: first, the females may not have mated . . . and second, in some parts of the pool the water may have been so cold as to prevent fertilization.

He concludes that coldness of the water causes the low vitality of the eggs of *Ambystoma jeffersonianum*, yet he states:

While an external condition—cold—is thus a deciding factor in the production of pathologically polyspermic eggs in *A. jeffersonianum*, the same season (1924) showed that there is another factor residing in the eggs themselves, or in the sperm, a factor that is commonly absent in the case of *A. maculatum*.

I do not believe that coldness of the water is the cause of the low vitality of the spawn of *A. jeffersonianum*, but that the "factor residing in the eggs themselves" is the cause. This factor is over ripeness, brought about by the delayed mating resulting from the scarcity of males. The first migration brings a varying number of individuals to the pond. In some years all the salamanders enter the water within a relatively short period, and the breeding season is short. In other years, cold periods stop the migrations for a time, and the breeding season is longer. In years with a short breeding season, the percentage of abnormal eggs is smaller; in years with a long breeding season, the percentage is higher. The females must mate soon after they enter the water if normal eggs are to be deposited. Since the males are outnumbered and cannot mate many times, many females of the first migration go unmated. These mate, if at all, with males of the next migration and produce abnormal eggs.

With the above idea in mind, observations were made to check the correlation between abnormal spawn and the progression of the season. Work was carried on at two places. At Pittsfield, supposedly mated females were collected from the water at various dates (see Table I), and placed in small cages to deposit their spawn. At Scio, females were mated in breeding cages and transferred to small cages, where the spawn was deposited. The work was commenced about a week after the first migration, so does not represent an entire season.

In both cases, the percentage of abnormal eggs was lowest in the earliest period studied, that is, the date closest to the first migration. At Scio, the breeding period was short, for the salamanders had to wait under logs exposed to the sun. The high temperature, as well as the delay, undoubtedly shortened the breeding period. At Pittsfield, the percentage of abnormal eggs in the first period was about the same as at Scio. In the second and third periods, the percentages of abnormal eggs were much greater; in the fourth period, less than in the second and third.

It is noticeable that different females under the same conditions gave different percentages of abnormal eggs. This was not to be expected if low temperature were the cause of the low vitality. If over ripeness were the cause, it is more un-

TABLE I
RELATION OF ABNORMAL EGGS TO PROGRESSION OF SEASON
PITTSFIELD 1931

| Collection date | Normal eggs | Abnormal eggs | Examination of ovaries | Summary |
|-----------------|-------------|---------------|------------------------|-------------------------------------|
| March 30 | 2 | 1 | No eggs | 165 Normal eggs |
| | 66 | 6 | " " | 141 Abnormal eggs |
| | 0 | 1 | " " | 46% Abnormal eggs |
| | 0 | 0 | Eggs | 1 Female did not deposit eggs |
| | 45 | 58 | No eggs | No females through laying |
| | 36 | 10 | " " | 2 Females laying only abnormal eggs |
| | 16 | 11 | " " | |
| | 0 | 54 | " " | |
| April 2 | 10 | 4 | No eggs | 26 Normal eggs |
| | 0 | 90 | " " | 208 Abnormal eggs |
| | 15 | 12 | " " | 89% Abnormal eggs |
| | 0 | 95 | " " | 2 Females did not deposit eggs |
| | 0 | 0 | " " | 1 Female through laying |
| | 1 | 3 | No eggs | 2 Females laying only abnormal eggs |
| | 0 | 0 | Eggs | |
| April 6 | 18 | 2 | No eggs | 18 Normal eggs |
| | 0 | 32 | " " | 102 Abnormal eggs |
| | 0 | 68 | " " | 85% Abnormal eggs |
| | 0 | 0 | " " | 2 Females did not deposit eggs |
| | 0 | 0 | Eggs | 1 Female through laying |
| | 0 | 0 | " " | 2 Females laying only abnormal eggs |
| April 11 | 0 | 0 | Eggs | 68 Normal eggs |
| | 45 | 50 | No eggs | 134 Abnormal eggs |
| | 13 | 11 | " " | 66% Abnormal eggs |
| | 0 | 0 | Eggs | 3 Females did not deposit eggs |
| | 0 | 0 | " " | 1 Female laying only abnormal eggs |
| | 0 | 73 | No eggs | |

TABLE I—(Continued)
Scioto 1931

| Mated | | Normal eggs | Abnormal eggs | Examination of ovaries | Summary |
|---------|---------|----------------|------------------|---------------------------|--|
| April 2 | Light | 76 | 33 | No eggs | 413 Normal eggs 275 Abnormal eggs 40% Abnormal 2 Females did not de- posit eggs |
| | Dark | 97 | 83 | “ “ | |
| | Light | 79 | 49 | “ “ | |
| | Light | 0 | 0 | Eggs | |
| | Light | 44 | 64 | No eggs | |
| | Light | 117 | 46 | “ “ | |
| | Light | 0 | 0 | Eggs | |
| April 4 | Light | 0 | 0 | Eggs | 190 Normal eggs 699 Abnormal eggs 78% Abnormal eggs 4 Females did not de- posit eggs |
| | Dark | 9 | 141 | No eggs | |
| | Light | 15 | 120 | “ “ | |
| | Light | 8 | 41 | “ “ | |
| | Light | 31 | 76 | “ “ | |
| | Light | 14 | 80 | “ “ | |
| | Dark | 20 | 88 | “ “ | |
| | Dark | 0 | 0 | Eggs | |
| | Dark | 93 | 153 | No eggs | |
| | Light | 0 | 0 | Eggs | |
| | Light | 0 | 0 | “ | |
| | April 6 | Light | 0 | 0 | |
| Light | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |
| Dark | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |
| Dark | | 0 | 0 | “ | |
| Dark | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |
| Light | | 0 | 0 | “ | |

derstandable, as the interval between maturation and mating differs with individual females.

Piersol (1929) accounts for unfertilized eggs as being laid by unmated females, and although these probably do deposit a portion of the unfertilized eggs, some of the eggs laid by mated females are unfertilized, and some are polyspermic. It is certain, moreover, that not all unmated females deposit spawn; often the eggs are retained in the ovaries. When unmated females are confined in aquaria, they behave in differ-

ent ways in regard to spawning; some deposit their eggs, some do not. This variation occurs in the field as well as in the laboratory.

INTERPRETATION.—From the work of Piersol, Smith, and especially that of Mohr in central Pennsylvania, it is evident that in certain regions there is a difference in habits of Jefferson's salamander.

The spermatophores deposited in central Pennsylvania differ in size and shape from those deposited in southern Michigan by males of the dark form. Comparative measurements of the spermatophores are as follows:

| | Total height | Diameter at base of stalk | Diameter at neck of stalk | Greatest width of sperm sac |
|--------------------|--------------|---------------------------|---------------------------|-----------------------------|
| Central Penn. | 5.5 | 7.0 | 1.5 | 2.0 |
| So. Michigan | 2.9 | 3.1 | 1.1 | 1.3 |

Several descriptions of the spawn have been given stating that the eggs are deposited in small bunches. In southern Michigan many of the eggs are deposited singly. By isolating gravid females in small cages it is possible to observe individual variation. Some deposit most of their eggs in small bunches, others as single eggs. The tendency toward the deposition of single eggs was greatest in dark females.

The differences in spermatophores and manner of deposition of eggs led to an examination of museum specimens from various parts of the range of the species. In the eastern part of the range the specimens are unlike either form in southern Michigan. In the northern part of the range they are of the dark form. The female group, intermediate between the eastern and northern specimens, is found in the south central part.

The situation may be explained in this way. At some time in the history of the species there has been a separation into two groups. After some differentiation had taken place the

two groups were reunited. Interbreeding produced an all female group of hybrids. The hybrid group, able to maintain itself by mating with males of the parent group, has moved out of the region of contact and spread over a considerable area. Judging from the condition of the species (sex ratio, and low vitality of the eggs), the hybrids are found at Buffalo and Rochester, New York; Toronto, Canada; and Ann Arbor, Michigan. It appears that the hybrids are not found at Syracuse, New York, and at Woodward, Pennsylvania.

SUMMARY.—In southern Michigan the salamander *Ambystoma jeffersonianum* is represented by two forms. One, designated as dark, is found in equal numbers of each sex; the other, designated as light, is composed almost entirely of females. In all habitats studied the females outnumber the males, ranging in ratio from two to one to fifty-two to one. Matings of both kinds of females, under similar conditions, show that dark females produce both male and female offspring while light females produce offspring like themselves in appearance and sex.

The scarcity of males brings about certain irregularities in the life history. Many females do not mate; most of these absorb their eggs, others deposit unfertilized eggs. Some females, mating late, have over ripe eggs, a condition which causes some of the eggs to be polyspermic.

It is believed that the light females are hybrids resulting from the interbreeding of two forms or races. The two forms are found in the original state in certain parts of the range, one in the northern, the other in the eastern mountainous region. The hybrid group occupies the central southwestern part of the range.

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PLATE I

Representative specimens of the two forms of *Ambystoma jeffersonianum* in southern Michigan. The lower figure is the light form.

