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THE METHOD OF SEX RECOGNITION IN THE WOOD-FROG, *RANA SYLVATICA* LE CONTE

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In the preceding number of Novitates, one of us has presented evidence that the secretion of a distinctive type of integumental gland, restricted to the males of most plethodontid salamanders, functions in stimulating the female to sexual activity. It has been recorded (Wright and Allen, 1909) that the mere presence of the female *Ambystoma maculatum* in the tank with males was sufficient to stimulate the sexual interest of the latter. Whether or not the stimulating factor of *Ambystoma* is a chemical substance is not known. It seems certain from the work of Hilton (1902) and of Rogoff (1927) on the newt and from the observations on *Eurycea bislineata* reported in the previous paper, that a secretion from certain skin glands of the male of these salamanders plays an important rôle in their courtship. We have been interested in attempting to determine if similar secretions function in the courtship of frogs or toads.

Among the Salientia no hedonic glands have been described, and there is only one convincing account of a chemical sense as playing a part in sex recognition. Banta (1914) studied the mating of the wood-frog, *Rana sylvatica*, in the field and tested the reactions of males in a breeding colony to other individuals, male and female, attached to a fish-line. From these observations and experiments Banta concluded:

The readiness with which the attempt of a male to pair with another male is given up on near approach, the keenness of the male's pursuit after once approaching very near or touching a female, the discrimination between a dead male and a dead female particularly in cases in which, to the human eye, the latter is indistinguishable in size, color and general features from a male, suggest that a chemical sense is involved in final sex recognition though one experiment designed to test this hypothesis was unsuccessful.

There is considerable difficulty in testing the reactions of frogs and toads in their native ponds. First, the animals under observation are always in danger of escaping. Secondly, other individuals may appear and be readily confused with the animal previously tested. Thirdly, one frequently is unable to approach close enough to observe details of behavior. We, therefore, decided to study the mating of the wood-frog under more controlled conditions with the view of determining the

mechanism of sex recognition in this species. The study was undertaken as part of a more extensive investigation of the significance of secondary sexual characters in Amphibia.

METHODS

Breeding wood-frogs were collected in the local field and held in large tanks for further observations. The experiments were conducted in a dark room illuminated by a feeble red light. A tank 76.2 cm. long, 30.5 cm. wide, and 30.5 cm. high was half filled with water. Freshly captured specimens were tested in this tank and only the most vigorous used in the experiments. The injecting of the frogs was done with a Luer syringe provided with a long needle; and water was withdrawn from the body cavity with the same instrument. Care was taken to inject through the thigh muscles into the peritoneal cavity in order to avoid leakage when the needle was withdrawn.

PRELIMINARY OBSERVATIONS

Banta's account of the mating of the wood-frog has been critically reviewed by Cummins (1920) who, from observations of his own, fails to obtain any evidence of a precopulatory recognition. Miller (1909) had previously pointed out that toads are unable to distinguish the sexes at sight. The males grasp any individual within reach. If the individual seized is a male it chirps and is released; if it is a female it remains silent and is retained. Cummins concludes that sex recognition in the wood-frog results from a similar differential behavior of the sexes: "Males were found to clasp other males as well as females. Clasped normal males struggle, inflate the vocal sacs and croak, and are always released. Clasped females show usually brief and weak resistance and the clasp is nearly always retained."

In a series of preliminary experiments we definitely established that factors other than the warning croak play a very important part in sex recognition in the wood-frog. When a chloretonized male and female were offered alternately to active breeding males, both were clutched but only the female retained. As neither of the introduced frogs was capable of producing sound or movement it was clear that the breeding males were capable of distinguishing the sex in quiescent individuals as Banta had previously noted.

Hinsche (1926) has recently made a detailed study of sex recognition in the European toad, *Bufo vulgaris*. He found that males at the begin-

ning of the breeding season change their mode of locomotion to more of a hopping gait. Males tend to seize companions walking or creeping near them more quickly than individuals hopping by. Hinsche stresses the importance of mutual excitement in a colony of breeding toads in calling forth the clasping reflex and, indeed, anyone who has witnessed a breeding colony of American toads or wood-frogs would conclude that this factor is operative in these species as well. Hinsche found that voice apparently plays little or no part in sex recognition in *Bufo vulgaris*. The shape and size of the female is of importance in permitting the male to maintain a firm hold; the roughness of the skin has a significance, but it is chiefly the vibrations of her flanks and the jolting movements of locomotion which stimulate the male to grip more firmly. This was demonstrated in *B. vulgaris*, by experiments, not only with living specimens, but also with models and inanimate objects. Male European toads will grip an agitated model longer than a stationary one.

In our preliminary examination of the problem of sex recognition of the wood-frog it became obvious that several factors influenced the rate at which the process was accomplished. There was evidence that the warning croak stressed by Cummins as well as the agitation factor emphasized by Hinsche both modified the length of the clutching reaction in the male frog. Fatigue was also found to hinder or even prevent the reaction entirely. Our experiments were, nevertheless, directed toward determining unknown factors in sex recognition rather than to working out the extent to which these additional factors might affect the result.

Our observations on the wood-frog were made from March 16 to 25, 1929. The first specimens were collected the night of March 16. We noted, as Banta had observed, that the females remain usually near the bottom of the pond; all the floating frogs were males. This difference of position in the water was very marked in the laboratory. Males introduced into the dark-room tank usually rose at once to the surface, and after a few swimming strokes sprawled out in the same characteristic floating attitude observed in the field. Females never assumed this spread-out attitude on the surface of the water. When rising for air they came up almost vertically. This inability of the female to float seemed to a large extent due to the weight of the eggs, or to the different extent of lung inflation. There was no marked difference in the size of their lungs.

Males are usually very active and frequently croak while bobbing up and down on the surface of the water. When two males strike each other one or both usually croak. Females are able to croak but their voices are not so loud as those of the males. When pinched in the leg

the female will sometimes open its mouth and give a different sound which may be written, ye-ow.

In a series of experiments it was found that the male will hold tighter and longer to an active than to a chloretonized female. The active female never croaked when seized by a male, while a male clutched by one of his own sex usually croaked within a few seconds. Males released active males on many occasions when neither croaked, indicating clearly that males are able to recognize their own sex whether or not the warning croak is given. Of the several hundred cases of embrace witnessed in the laboratory, only twice did one male cling to another uninjected male over a minute. These may be considered cases of excessive excitement comparable to instances where a male will cling to the observer's finger after being separated from a female.

Throughout the series of experiments we could obtain no evidence of the male being able to distinguish between the sexes at a distance. On several occasions a female in the observation tank thrust her head above the surface near one or more active males without being perceived. Males varied enormously in the intensity of their ardor, but we could find no consistent difference in the rapidity with which they approached and embraced one of their own sex as compared with similar reaction toward a female.

EXPERIMENTS WITH WOOD-FROGS

We noted in our preliminary observations that active males could readily distinguish between chloretonized male and female. That voice frequently played no part in sex recognition was obvious in those cases where a male rejected an active male without the latter croaking. In several of these cases the rejecting male was tested with a normal female and found to embrace in the usual vigorous way. Hence, the rejecting of the clutched male was not due to fatigue. In order to put the question of voice to an adequate test we performed a series of experiments in which the larynx of a male was incised in such a way as to prevent his making any sound. The reaction of the males toward this male did not differ from their reaction toward those still equipped with a voice.

Females of *Rana sylvatica* are usually larger than males and when burdened with eggs are usually distinctly stouter. This suggested that girth and particularly the difference in resistance to pressure between the bodies of the two sexes might be an important factor in sex recognition. We have tested this hypothesis by offering a chloretonized male to an active male, both before and after injecting the body cavity of the former

with water in sufficient amounts to make the body resistant to compression. The experiment was repeated with different frogs on different days and always with fairly uniform results. The following experiment may be considered as typical of the series. It is more extensive than some of the experiments in that we began with a male and a female and later replaced the female by a male.

EXPERIMENT 1, MARCH 23

5:41 P. M.—Eggs were stripped from a female in embrace. After filling peritoneal cavity with approximately 7 cc. of water the female was offered to an active male which immediately seized her.

5:51—The pair which had been in active embrace, swimming about the aquarium for ten minutes, were forcibly separated.

5:58—Water withdrawn from peritoneal cavity of female. When latter was offered to male he seized her about head and let go.

5:59—Same female offered to same male in normal position. She was seized feebly and, as both sank to bottom of tank, was released within minute.

5:59:45—Chloretonized male offered to same male was seized and immediately released.

6:00—Same chloretonized male was injected with 7 cc. of water and offered to same male. The latter seized the former violently and attempted to slip forward to pectoral region. His efforts drove the pair forward along bottom of tank. In struggling for a pectoral grip the active male lost his hold but instead of rising to surface turned abruptly and swam to injected male lying quietly on bottom. The new grip was made again in the pelvic region and the struggle for a more anterior position continued.

6:06—Active male lost hold for second time in endeavoring to reach pectoral position. This time the struggling male rose to surface.

6:06:30—Injected male offered head foremost to the same active male was immediately seized and carried under the surface. While under water the active male turned abruptly around to face the same way as the injected male but in his efforts to grip abdomen of latter, he turned the injected partner over and secured a position with ventral surfaces opposed.

6:11—The same pair was still in embrace, with ventral surfaces together; the active male attempted several times to slide forward to the pectoral region of the injected specimen. Apparently the chloretonized male was too fully injected to make this possible.

6:13—Pair was swished about tank by hand, the active male still maintaining his ventral position. A vigorous push caused latter to lose position; he swam after other but came to surface before reaching goal. During this whole struggle the injected male was fully anesthetized and hence sound or movement could have played no part in arousing the activity of the embracing male.

6:17—The water was withdrawn from anesthetized male and the latter offered to the same active male. The now normal proportioned but inactive male was seized at once and carried to the bottom. On slipping forward to the pectoral region the active male made a few weak clutches and released his grip entirely one minute, thirty

seconds after securing the first hold. It was clear that the chloretonized male in this condition did not interest the active male.

6:19—Same chloretonized male offered active male again with quicker results. The latter released grip in less than a minute.

6:20—Trial repeated; a grip in the pectoral region resulted in immediate release.

6:26—Same chloretonized male injected with 7 cc. of water. The same male which had just showed no interest in his impassive companion, seized the latter violently in the pelvic region and attempted to slide forward to the pectoral region. During this struggle on the bottom of the tank, the active male lost grip twice and each time swam back to companion. On the last struggle, the chloretonized male turned over and the active male gripped with ventral surfaces opposed.

6:29—While endeavoring to struggle forward to pectoral region, the active male lost grip but returned to the ventral surface.

6:30—Active male lost grip again while working forward to pectoral region and came to surface.

6:42—Same injected male offered to other in normal position. The latter gripped strongly.

6:43—Active male finally reached pectoral region in normal position but was still not satisfied, for he continued to make clutching movements.

6:52—After struggling for ten minutes under water to better his position, the active male lost grip and came to surface.

6:53—The water was withdrawn from the same chloretonized male, and the latter offered to the same active male. The chloretonized individual was seized several times but in each case released within a few seconds. Three offers were made head foremost with similar results.

6:55—The same chloretonized male was injected for the third time and again offered to the same male. The latter made two feeble attempts to seize the former but the third effort was with the same force as previously displayed towards the injected specimen. The gripping male paddled the pair half-way along the bottom of the tank endeavoring, as usual, to reach the pectoral region.

7:00—Active male reached about mid-region of chloretonized companion and still struggled.

7:01—In struggling the active male turned the chloretonized one over and gripped with ventral surfaces opposed.

7:02—Active male lost grip and rose to surface. The chloretonized male was found to be soft, indicating that the frequent needling had caused a leak and consequently uniform results could no longer be expected.

It is clear from this experiment and a series of others in which a chloretonized male was offered to an active one of the same sex that the girth and the resistance of the body embraced plays a very important rôle in maintaining the grip of the male. In these experiments we used the same pair for a series of trials during which the body of the chloretonized male was successively inflated with water, offered to the active male, deflated and offered again. Thus, we removed the voice factor, could control the agitation factor, and could show the importance of the size

and resistance factor without any modifying influences other than fatigue. When using freshly captured males we found that fatigue did not seriously affect the results. The following experiment illustrates the extent to which a male wood-frog will retain its hold on one of its own sex if this should be of approximately the same girth and rigidity of abdomen as a female.

EXPERIMENT 2, MARCH 23-24

8:10 P. M.—Chloretonized male was offered to active male. Latter seized former and immediately released his grip.

8:14—Same chloretonized male injected with 7 cc. of water; again offered to the other male. The latter gripped and held firmly to the chloretonized specimen.

8:26—The pair which had been in embrace for twelve minutes were forcibly separated, the active male struggled vigorously to maintain his hold on his chloretonized companion.

8:30—Water was withdrawn from same chloretonized male and the latter offered to the same active male. It was held loosely for a few seconds and then released.

8:31—Four attempts made to induce the active male to seize the chloretonized one. In each case the active male grasped the other and released his hold within thirty seconds.

8:34—Same chloretonized male injected with 7 cc. of water and again offered to the same active male. The latter clutched the swollen male, and while uttering a number of low croaks, swam actively about the tank, maintaining a firm grip while doing so. The pair were lifted from the water several times by seizing the legs of the chloretonized male.

9:10—Same active male continued to embrace the chloretonized injected one for over half an hour without showing any tendency to release his grip. The pair were allowed to remain in embrace over night.

11 A. M.—(The following morning.) The pair apparently had been in embrace all night for the active male still held the injected one.

11:12—Water was withdrawn from same chloretonized male and the latter offered to the male which had been clutching him all night. The active male seized the chloretonized one at once but released his grip within thirty seconds.

11:13—Trial repeated with same result. The active male clutched the chloretonized one but maintained a loose grip for only a few seconds.

11:13:30—Trial repeated with the same results.

11:17—Same chloretonized male inflated by injection of 7 cc. of water intraperitoneally. When offered to the same active male it was seized at once and held firmly. With much struggling the active male attempted to work his way forward to the pectoral region of the injected one.

11:27—During the preceding ten minutes the active male reached approximately the mid-point of the injected one's body. The pair were lifted up from the water by holding the legs of the chloretonized male. The pair were then swished about the tank without the active male losing his grip.

Experiment then discontinued.

Records were made of experiments with nineteen different male wood-frogs in addition to those given above. If the embracing males were active we obtained very uniform results. Variations were obviously due in some cases to one or more of the modifying factors observed in our preliminary experiments. The following experiment is typical of a case in which the embracing males became fatigued or possibly frightened during some of the trials. Nevertheless, the experiment taken as a whole is convincing evidence of the importance of body size and firmness in sex recognition.

EXPERIMENT 3, MARCH 23

3:15 P. M.—Three active males offered a chloretonized male and female. They distinguished between them at the first trial, maintaining grip on the latter but not on the former.

3:21—The chloretonized male which had been refused was injected with 7 cc. of water and offered to first active male head foremost. It was seized at once and upon being allowed to float in the tank, was released and quickly seized in the normal direction but in the pelvic region. With a series of short clutches the embracing male attempted to slide forward to the pectoral region. In making one of these clutches the active male lost his grip and released the injected male.

3:26—Injected male was offered head foremost to second active male and was seized at once but released a minute later.

3:27—Same injected male was offered this active male in the normal position and was seized. Latter maintained his grip while swished about tank but released grip within a minute.

3:28—Trial repeated with same individuals but injected male, after being seized, allowed to float on surface of water.

3:30—Pair still in embrace were pushed about tank and frequently lifted from water.

3:35—Pair broken loose by a jerk.

3:35:30—The injected and chloretonized male was offered to the first active male again. It was seized but released a minute later.

3:37—Trial repeated but again first active male lost grip.

3:37:30—Trial repeated but injected male brought quickly against active male's body. The latter gripped and both sank to bottom of tank. The grip strengthened while the pair rose and sank in the tank.

3:42—Embracing male forcibly removed from injected male.

3:43—Injected male offered to the second active male again was seized at once about lower limbs. In attempting to change position two minutes later it lost grip.

3:56—The water was withdrawn from the peritoneal cavity of the chloretonized male, and the latter offered the two active males experimented with above. In three trials each, in both normal and reverse position, the active male seized and immediately released the chloretonized male. In one further trial the active male carried the chloretonized male to the bottom but released grip as both gradually rose to surface.

4:08—Chloretonized male injected again with 7 cc. of water. When offered to first active male it was seized and pushed to the bottom. The pair rose to the surface twice while the active male attempted to shift its grip to the pectoral region. Pair pushed about tank.

4:12—Male released grip while being pushed about tank.

4:15—Trial repeated but injected male offered head forward. It was seized but released a moment later.

4:16—Injected male offered to second active male in normal position. The latter seized the former violently and carried it about bottom of the tank, making some effort apparently to lift it to the surface.

4:21—Active male forcibly released from injected male and experiment discontinued.

It was pointed out above that females usually swim and remain near the bottom, coming up only at irregular intervals for air. We noticed in our experiments that the males usually held a firmer and longer embrace if they were kept below the surface. If the pair in embrace floats, the male, if fatigued, will frequently open his eyes, look around, weaken his grip, and finally release his hold entirely. The following experiment is typical of those in which the modifying influence of floating seemed to play a part.

EXPERIMENT 4, MARCH 22

7:02 P. M.—Three active males which exhibited the normal embrace towards females were offered a chloretonized male and a chloretonized female held in the hand with heads directed toward them. Neither chloretonized animals showed any activity and of course did not croak, yet in every case the female was seized and held, the male clutched and released on the first trial.

7:10—The chloretonized male was injected with 5 cc. of water which made it puffy but not taut across the abdomen. Two of the active males seized the injected male and released it while the third gripped it tightly, was lifted clear of the water and later washed about tank without releasing his grip. Two minutes later a quick jerk caused this male to lose his grip.

7:15—Two additional cubic centimeters of water were injected intraperitoneally making the male fairly taut.

7:16—Injected male now offered to first active male was seized at once and lifted clear of water. The pair in embrace were then released and allowed to settle to bottom of tank.

7:26—Male which had held the injected male for nearly ten minutes at bottom of tank, was forcibly removed.

7:27—Injected male offered to second active male. The latter seized and maintained a hold for over a minute.

7:27:30—The same active male was then tested with another chloretonized male and a chloretonized female. It gripped both but maintained a hold only on the female.

7:30—The same active male was then offered the first injected male. It gripped and maintained a hold for over three minutes although the injected male was inactive and floated.

7:34—The same injected male offered to same active male and again he seized and swam with injected male to bottom of tank. The pair swished about tank, the embracing male strengthening his hold.

7:36—Pair being allowed to rest on surface for a few moments the embracing male's grip weakened and finally loosened.

7:37—Injected male offered to this second active male for the third time, but in this case with head foremost was immediately embraced. Pair swished about tank and finally anchored by one of the legs of the injected male to the bottom of the tank.

8:23—Male still maintained reverse position on back of injected male anchored to bottom of tank. For over forty minutes the male held this one position on his inactive companion.

8:24—Pair forcibly parted.

8:30—Same male which had been so long under water offered an uninjected chloretonized male and a similar female. It gripped and released the male, gripped and held tightly to the female. It was lifted out of water while maintaining grip on the latter.

8:31—Same male offered the injected male which it had previously held. It seized and held tightly to the latter while lifted free of water.

8:32—Grip broken loose and the chloretonized male and female as well as the injected male offered again. Reaction the same but weaker.

Experiment discontinued.

It was noticed in our preliminary observations that a male which has been clutching a female if forcibly separated will cling tenaciously to the observer's finger while an active male floating in the tank would never do this. It thus seemed that a prolonged embrace modified the reactions of one male toward another. We tested this proposition by a series of observations. The following experiment is one of those in which the effect of prolonged embrace apparently modified the expected result.

EXPERIMENT 5, MARCH 22

2:10 P. M.—Two males placed in tank and their reaction to a female containing eggs found normal. When female was held with head toward male she was quickly seized and held firmly, even though lifted free of water and shaken several times.

2:12—The pair were separated and female offered to second male, head on. She was quickly seized and together they swam about the tank, the female making efforts to escape.

2:14—Female succeeded in freeing herself of the male. Male held in hand and offered to other male induced a clutching reaction in the latter but the grip was immediately released. A second attempt also failed.

3:01—One of the males was injected intraperitoneally with 8 cc. of water, making it plump and nearly helpless in the water. It was offered head forward to the second male and was instantly seized. The pair were lifted out of the water by holding to the hind legs of the injected male. The pair were washed back and forth in the tank, the embracing male maintaining his grip and frequently croaking.

3:10—Pair lifted from water and embracing male forcibly removed from other.

3:14—The larynx of injected male incised after removing 5 cc. of water from the peritoneal cavity.

3:16—Voiceless male offered to other. It was seized and immediately released. Four more times the voiceless male was offered and each time seized and released.

3:21—Voiceless male with 3 cc. of water still in its peritoneal cavity was offered to a second male which had been grasping a normal female for over half an hour previously. Voiceless male seized and held until 3:48 P. M., when the experiment was discontinued.

THE RELEASE OF THE FEMALE AFTER EGG LAYING

It seemed to us highly probable that if the presence of eggs within the body of the female played such an important part in the process of sex recognition in *Rana sylvatica*, as we have demonstrated above, the absence of eggs in the body of the spent female was the immediate cause for the male releasing his embrace after egg laying. Spent females would not be distinguished from males by the active individuals of the latter sex and hence would not be mobbed while they were attempting to leave the pond. Wright (1914, p. 7) records a case of a wood-frog maintaining an amplexus after ovulation. This abnormal behavior was possibly due to excessive ardor. The male was not tested in its reaction to other individuals. There is no doubt that most male Salientia release the female a short time after egg laying. We have tested the question of the importance of body size in the release after egg laying by a series of experiments which gave very similar results. The following experiment may be considered typical of the series.

EXPERIMENT 6, MARCH 22

1:10 P. M.—Eggs removed by stripping from a breeding female. The latter then offered to an active male which seized her and released her immediately. Same female offered to a second active male which slipped with half-closed arms to the waist of the female. Within a minute the female had freed herself, the male making no effort to hold her. The same female offered to a third active male which gripped and released hold at once.

1:20—Female injected with 7 cc. of water was offered to the first male which seized her vigorously and maintained a grip while the female swam actively about the tank. The second male attacked the pair but was driven off by the first male which croaked loudly and struck out vigorously with his legs.

1:30—After ten minutes of embrace the pair was forcibly separated. During most of this period the pair had been swimming actively below the surface, the male maintaining a strong grip.

1:30:30—Water withdrawn from peritoneal cavity of the female with a syringe. When the female was now offered to the first male she was seized but released at once. Three consecutive attempts to induce the male to maintain a hold on the female in her present condition was without success.

1:37—Female injected again with 7 cc. of water. She was then offered to the same male which clutched and held firmly to her body.

1:52—The pair which had been actively swimming in the tank for nearly fifteen minutes were forcibly separated.

1:55—Water withdrawn from the same female and the latter offered to the same male. Each time the female's body was brought under him he seized her but released grip within a few seconds.

1:57—Same female injected for the third time and offered to the same male. Again he actively clutched her and maintained a vigorous hold.

2:07—Water withdrawn from female for the third time. When the female was offered to the same male he again made no attempt to maintain a grip. Female offered in various positions but without success.

2:11—Female injected with water for the fourth time was again grasped by the active male and held firmly.

2:22—The pair which had been in embrace for ten minutes were forcibly separated and the water withdrawn. When the deflated female was offered to the same male he behaved as on previous occasions. In no trial did he hold the female more than a few seconds.

2:26—Same female inflated with water for the fifth time. Same male vigorously clutched and maintained hold.

2:31—For some unknown reason the male suddenly dropped the inflated female.

2:32—When the inflated female was offered again to this male he seized her and maintained the usual vigorous grip.

2:40—Pair separated and water withdrawn from the female for the fifth time and again when the female was offered to the same male he gripped weakly and released hold at once.

2:44—Same female inflated with water for the sixth time, and same male seized her vigorously.

Experiment was discontinued at this point, the male having seized the female inflated with water six different times and refused her when deflated even a greater number of times because each attempt to make the male seize the deflated female consisted of several trials.

In some of the experiments it was clear that modifying influences could enormously affect the uniformity of the results. The following experiment is typical of those in which one of the males soon failed to respond, probably because of fatigue.

EXPERIMENT 7, MARCH 22

3:15 P. M.—A female which had just laid its eggs was offered to two males which had been embracing females with eggs for a half hour previously. The female was offered four times to each male. In every case the male gripped but released his hold within a few seconds.

4:08—The female was injected intraperitoneally with 8 cc. of water. When offered to one of the same males it was seized at once. The pair were washed about the tank and frequently lifted out of the tank, the male maintaining his hold continuously.

4:18—An especially vigorous swing of the pair in mid-air over the tank dislodged the male.

4:19—Injected female offered to other male previously tested. Male seized the female which now showed no activity.

4:21—Injected female now held as a dead weight in male's arms was released.

4:22—Injected female offered to same male was held as a dead weight for two minutes more and then released.

4:25—A normal female containing eggs was offered to this male but the latter made no attempt to seize it.

4:25:30—A second attempt and the male seized the active female which swam quickly about the tank, the male maintaining his grip.

4:26—Injected and quiescent female was offered to the first of the two males and was seized. Pair pushed gently around tank, the male strengthening his grip.

4:30—The pair allowed to remain without movement in the tank.

5:05—The male still maintained his grip on the injected and quiescent female.

5:25—Male forcibly removed from injected female and the water withdrawn from peritoneal cavity of latter.

5:28—Six times the same female was offered to the same male; every time the latter gripped and released hold either at once or within a few seconds.

5:30—Same female offered to a male which had been gripping a normal female containing eggs for over half an hour. Male seized and maintained a feeble hold for nearly two minutes when the female was dropped.

5:32—Same female offered to this male. The latter made a few feeble grips and finally refused to react at all.

EXPERIMENTS WITH EXTRASPECIFIC AMPLEXUS

It is well known that male wood-frogs will embrace other species of frogs. Wright (1914) mentions several cases which came under his observation. Cummins (1920) reports wood-frogs embracing a female leopard-frog and a female *Ambystoma maculatum*. He concludes: "In the light of extraspecific pairing (No. 5 and No. 10) it seems absurd to attribute any rôle of sight in sex recognition either on a basis of color or behavior." In view of the preceding experiments the question arose: Were these cases of prolonged extraspecific embrace reported by Wright and by Cummins due to the fact that the object clutched had the proper girth and firmness of body or were they not due merely to excessive ardor on the part of the embracing male? We have tested this question by a series of experiments.

(a) EXPERIMENTS WITH *Ambystoma*.—Two active specimens of *Ambystoma maculatum* were offered to a series of active male wood-frogs. One of the *Ambystomas* was fresh from the field and was swollen with eggs. The other was a female of the same length but it had been kept in an aquarium all winter and was slimmer than the other. The first female

was held half an hour on the first test and was finally removed forcibly from the wood-frog. Tests with the other males were not as effective but we recorded three fifteen and one twenty minute embrace for these other males. The slim *Ambystoma* was as active as the plumper one but none of the male wood-frogs would retain a grip on this individual for over a few seconds.

(b) EXPERIMENTS WITH MECHANICAL DEVICES.—A series of objects were offered March 21, to the active males after they had been tested with chloretonized male and a female wood-frog to make sure that no modifying factors were influencing their ability to discriminate. A thin rubber finger cover when blown up with water until it was approximately the size of a female wood-frog, was seized and held for nine minutes. During this period the male clutching to the dummy was lifted several times from the water. It was found in the preliminary experiments that lifting the object embraced, out of water, was a critical test, for males never held to chloretonized uninjected males lifted out of water while they almost invariably maintained their grip on females similarly manipulated.

Attempts to repeat this experiment with rubber balls, pieces of rubber tubing and other devices gave no consistent results. Apparently the shape of the object was of some significance in sex recognition.

The importance of shape was brought to light many times in the other experiments. If a female is offered to the male head foremost he will seize her at once but eventually turn abruptly around under water to the normal position. Again we succeeded in one case of injecting 12 cc. of water intraperitoneally and subcutaneously into a male wood-frog. Although this bloated creature was seized by several males none would retain a grip more than a few minutes.

(c) EXPERIMENTS WITH LEOPARD-FROG AND GREEN-FROG.—It seemed probable from the above experiments that a wood-frog might not be able to distinguish a male of a larger species from a female of its own kind. In a series of preliminary observations this suggestion gained considerable support. Thus we witnessed a male wood-frog clinging to the pelvic region of a male leopard-frog, *Rana pipiens*, for over an hour. During this period the leopard-frog croaked regularly every few seconds with very few interruptions. Males of both the leopard- and pond-frogs were very active and usually freed themselves of any wood-frog which chanced to embrace them. Females of these species when swimming in a tank, were also usually successful in avoiding the attack of a male wood-frog. Nevertheless, the firmness of the body of the male green-frog was

apparently not adequate for the wood-frog's maintaining his grip in the pectoral region. The following experiment is one of several which gave similar results.

EXPERIMENT 8, MARCH 25

12:10 P. M.—A male green-frog was etherized and offered to two active male wood-frogs. Both clutched the green-frog and released hold immediately.

12:19—Three successive attempts to induce wood-frogs to maintain grip on the anesthetized male green-frog resulted in failure.

12:25—Same male green-frog injected with water until plump. When offered to the first of the males it was immediately seized and retained.

12:35—During the previous ten minutes the male wood-frog maintained a firm grip on the injected male green-frog, while the pair rested on the bottom of the tank. At this moment the pair were forcibly separated.

12:37—Water withdrawn from body of male green-frog and the latter offered to the same wood-frog again. He gripped green-frog and released hold within a minute.

12:40—Same male green-frog offered again to the same male wood-frog was seized vigorously. After a few struggles the wood-frog slipped to pelvic region and assumed a feeble grip. The green-frog was gently touched with a view to stimulating the reflex in the wood-frog but the latter immediately released grip entirely.

12:44—Trial repeated but the male wood-frog merely gripped and released hold.

12:45—Same male green-frog injected with water and offered to the same male was immediately seized and held firmly.

12:55—Male wood-frog continued to maintain firm embrace on the injected green-frog. The pair were pushed vigorously about the tank and finally allowed to rest quietly.

1:00—Male wood-frog still maintained grip on injected green-frog.

Experiment discontinued.

DISCUSSION

The above experiments demonstrate that a chemical sense plays no part in sex recognition in the wood-frog. Males clasp any individuals in their vicinity and sex recognition results from the different size and resistance to pressure afforded by the bodies of the two sexes before egg laying. Various factors may modify the result. The warning croak of the male when touched by another male seems to act as a secondary factor hastening the speed of recognition. Females in locomotion, particularly below the surface of the water are held usually more firmly than quiescent or floating ones. In repeating experiments with the same individuals, fatigue may intervene to prevent a duplication of the results. The observations made by Banta (1914) differ so widely from our results that we are forced to conclude that the modifying factors discussed above were responsible for this difference. We performed many more experi-

ments than Banta and obtained fairly consistent results. When the modifying factors are eliminated as far as possible, the importance of body size and resistance in sex recognition becomes very evident. Our experiments show that the difference between the sexes in body size and firmness affords the most important basis for sex recognition.

It does not follow from these experiments that sex recognition throughout the Salientia is accomplished by use of the same sensory data. Long ago Goltz offered stuffed males of a European frog to other males but failed to obtain any clear demonstration of the importance of the size and resistance factor in sex recognition (Holmes, 1916). Very recently Lullies (1926) has shown that in *Rana esculenta* and *Rana temporaria* weak action currents of the embrace reflex are induced by the breathing movements of the female. When these impulses cease, following the death of the female, the clasp, if no other stimulus excites it, is soon loosened. Thus in these frogs and in the toad studied by Hinsche, the agitation factor recognized in various parts of our own experiments, seems to become of primary importance. Hinsche did not consider the size factor independent of other factors in *B. vulgaris* and it may be more important in this species than he assumes.

The male wood-frog is readily distinguished from the female during the breeding season by the convex margins to the webbing between the toes (Wright, 1914, Pl. III, fig. 1a). These hypertrophied webs might function in assisting their owner, floating on the surface, to make a quick plunge toward an approaching frog. In both our field and laboratory experiments we failed to note that the male thus equipped was more agile than the female, but as the female does not float, the relative speed of get-away from the normal sprawled position of the male could not be compared.

SUMMARY

1.—Sex recognition in the wood-frog, *Rana sylvatica*, is first accomplished after the embrace, the males retaining their grip on individuals having a certain girth and degree of firmness of body.

2.—A chemical sense plays no part in sex recognition but the warning croak of an embraced male may shorten, and the normal movements of an embraced individual may increase the time period of the embrace. Other modifying factors are fatigue, degree of sexual ardor, and retreat of pair below surface of the water.

3.—Males release females after egg laying for the same reason they release males which they happen to embrace. The body of both groups of individuals does not have the required girth or firmness to permit their continuing the embrace.

REFERENCES

- BANTA, A. M. 1914. 'Sex Recognition and Mating Behavior of the Wood-frog, *Rana sylvatica*.' Biol Bull., XXVI, pp. 171-183.
- CUMMINS, HAROLD. 1920. 'The Rôle of Voice and Coloration in Spring Migration and Sex Recognition in Frogs.' Journ. Exp. Zool., XXX, pp. 325-343.
- HILTON, W. A. 1902. 'A Structural Feature Connected with the Mating of *Diemitylus viridescens*.' Amer. Nat., XXXVI, pp. 643-651.
- HINSCHÉ, G. 1926. 'Über Brunst- und Kopulationsreaktionen des *Bufo vulgaris*.' Zeitschr. Vergl. Physiol., IV, pp. 564-606, 7 Figs.
- HOLMES, S. J. 1916. 'Studies in Animal Behavior.' Boston.
- LULLIES, H. 1926. 'Der Mechanismus des Umklammerungsreflexes.' Pflug. Arch. Ges. Physiol., XXIV, pp. 416-420.
- MILLER, NEWTON. 1909. 'The American Toad (*Bufo lentiginosus americanus* Le Conte). A Study in Dynamic Biology.' Amer. Nat., XLIII, pp. 641-668.
- ROGOFF, J. L. 1927. 'The Hedonic Glands of *Triturus viridescens*; a Structural and Functional Study.' Anat Rec., XXXIV, pp. 132-133.
- WRIGHT, A. H. 1914. 'North American *Anura*, Life Histories of the *Anura* of Ithaca, New York.' Publ. Carnegie Inst. Washington, No. 197.
- WRIGHT, A. H., AND ARTHUR A. ALLEN. 1909. 'The Early Breeding Habits of *Amblystoma punctatum*.' Amer. Nat. XLIII, pp. 687-692.

