

CONJUGATION IN THE CRAYFISH, CAMBARUS AFFINIS

E. A. ANDREWS

Professor of Zoölogy, Johns Hopkins University

EIGHT FIGURES

The devotion of the individual to the welfare of the race is especially patent in such complex animals as the Arthropods, in which many organs and actions relate directly to the processes of reproduction.

In many crustacea the most complex of all the interactions of the individual with the environment are the interactions with individuals of the opposite sex. A study of the conjugation phenomena of such crustacea should enable us to estimate the highest capabilities of these animals and to place them more accurately in the scale of being, than by study of organs and activities concerned merely with self-preservation. A comparative study of conjugation might aid in the understanding of the relationships of groups of crustacea.

Since the meeting of the sperm and egg will not take place, under usual conditions, without the preceding phases of conjugation, these become as necessary for the race as the fertilization of the egg.

The present paper is a description of the processes of conjugation in a common crayfish, *Cambarus affinis*, in which the sexes differ in many organs and actions.

The female not only lacks the male organs and instincts but possesses ovary and oviducts, a peculiar sperm receptacle in the shell, special glands used in connection with the care of the eggs, characteristic proportions of various parts of the body and character of first abdominal limbs. The female also possesses special

reactions and instincts used both in the processes of conjugation as well as in laying and caring for the eggs.

The male, while lacking the female organs and instincts, has testis and deferent ducts as well as three pairs of external organs directly concerned in the transfer of the sperm to the receptacle of the female. The male shows characteristic proportions of various parts of the body and has a pair of special clasping-hooks. The male also possesses a series of complex abilities that are shown in the processes of conjugation.

The phenomena of conjugation are, in brief outline, the following: The aggressive male seizes the comparatively inert female and mounting upon her ventral surface becomes firmly attached both by the large chelae and by special clasping-hooks on the bases of the legs. The sperm that issues from the deferent ducts through soft protrusions which we shall call the papillae, is conducted by the combined action of the first and second limbs of the abdomen into the special receptacle in the shell of the female,

The male has three sets of organs concerned in the transfer of sperm, the papillae and the two pairs of specialized limbs. These limbs we shall call the stylets, as they are firm, calcified, tapering organs that are thrust with force into the narrow slit of the calcified receptacle in the shell of the female. By means of these stylets the sperm is transferred some half-inch or so through the water from one animal to the other without being exposed to contact with the water, which would, it is believed, destroy the sperm.

During most all the time of conjugation the male is firmly locked to the female in the attitude shown in fig. 1, which is from a photograph of living crayfish under water.¹ The female is supine and relaxed except for the tightly rolled abdomen that is embraced by the tensely flexed abdomen of the male. The male is poised over the female and balanced right and left by the tip of but few legs, while the other legs hold the female, the great

¹ An excellent photograph of a different phase of conjugation in this same species was made from live crayfish under water by R. W. Shufelt and published by him in "Shooting and Fishing," 1898, and in "Natur und Haus," 1903, and by J. Arthur Harris in *Sc. Bull. Univ. Kansas*, 1903.

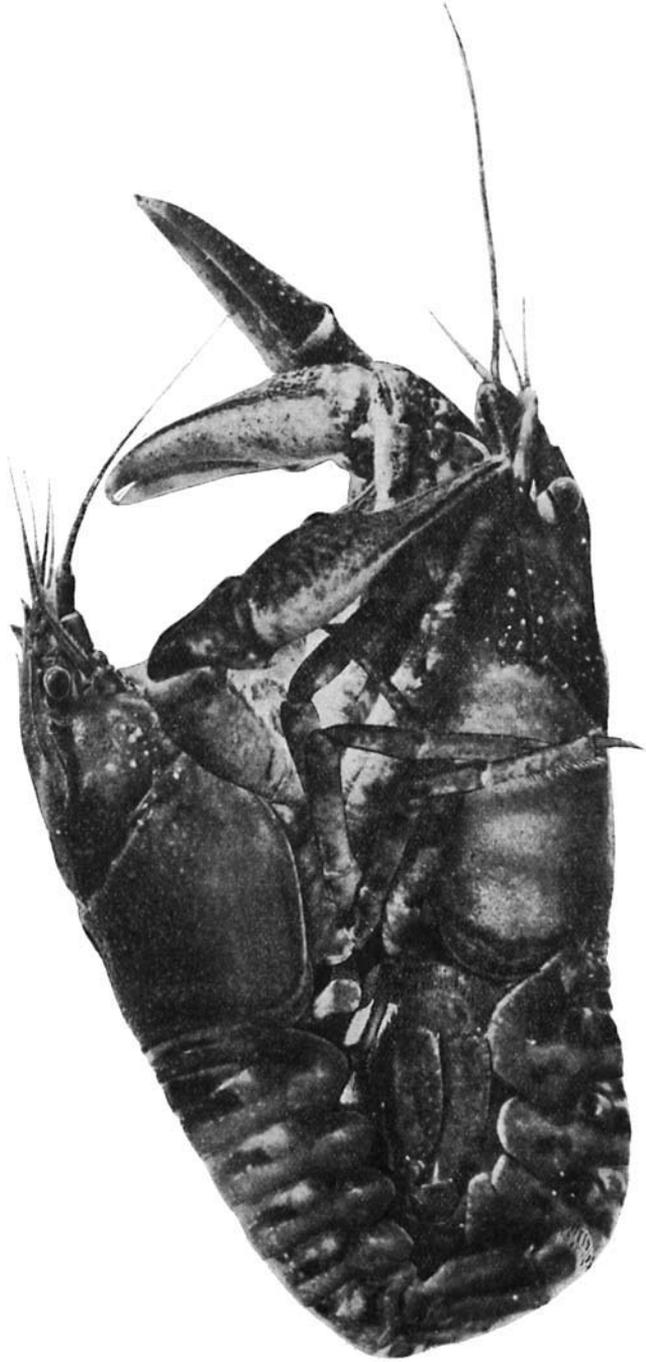


Fig. 1 Photograph in water of living male and female in late phase of conjugation.

claws grasping all the claw legs of the female. The apparent absence of the fifth leg of the male is due to its being turned abruptly across between the male and female. Here it supports the stylets, that are seen dimly, pointing forwards and downwards from the abdomen of the male to the thorax of the female.

Once the male is firmly fastened to the female the pair may be lifted out of the water without the male necessarily letting go or even showing any sign of being stimulated by the change in conditions. It is possible to separate the chelæ of the male from the female and to bind them shut without stimulating the male to let go with the other limbs. External environment seems for the time to be concentrated in the crayfish of the opposite sex, as far as evident responses indicate action of environment.

Before considering this process of conjugation in more detail, some consideration may be given the question as to how the male "recognizes" the female.

In *Cambarus affinis*, as studied in the laboratory, the males will unite with the females during all the fall, winter and spring months, and if a male be kept in a small vessel till accustomed to it he will generally conjugate with a female introduced into the same vessel, usually very soon, but sometimes not for twenty-four hours, so that it is not always the advent of a new crayfish that stimulates the male to action. Observation of males and females under these conditions gave the impression that the male has only a vague stimulus from any crayfish at a distance, without any recognition of the sex at all. But once the male had seized another crayfish the result depended upon the sex of the crayfish seized; so that, in a sense, the male might be said to recognize the difference between a male and a female after he had seized them. That is, the male seemed to act differently to males and females only after they had been seized. And even then there was no evidence of any recognition of sex, except in the sense that the mode of reaction of the female made possible the carrying out of a chain of reflexes on the part of the male, which could not be the case if a male were seized.

Incidentally to other observations, Dearborn observed that a specially vigorous male, when blindfolded by a tin helmet,

seized another male, which at once escaped. As he did not see male seize male on other occasions, he came to the conclusion that crayfish use their eyes as one mode of recognizing the sex of their fellows, though he also surmised the sense of smell might play some part in the sex recognition.

While carefully devised experiments would be necessary to disprove the use of the sense of sight and of smell in sex recognition in these crayfishes, there is as yet no evidence that the crayfish recognizes the sex of another with which he is not in actual contact. On the other hand the behavior of the sexes once they are seized is so different that this alone would account for the carrying out of the processes of conjugation between any members of the opposite sexes.

When the males are approached they brandish their claws and if there are two males one withdraws, after more or less deliberate holding and shoving of one another's claws. When a female is approached she generally retreats, but may show fight and more or less successfully keep off the male. In confined spaces, however, the female is almost always overcome by the male even though he be much smaller or lacking in one claw. A few females amidst many hundred males will always be found out and clasped by males; but when very many males were kept together only one case was seen in which a male had seized and held, as if in conjugation, a small male, but this one was dead. That this selection of females by the males may be merely a matter of adjustment of males to the different amount and character of resistance offered by the females and males when seized is, perhaps, supported by the fact that a difference in muscular tonus is rather distinctive of the sexes. In fact it was found possible to sort a lot of crayfishes into two sets, one male, one female, with one's eyes shut, merely by their differences in muscular contraction when taken hold of, the males have a much more pronounced habit of violently contracting their limbs and trunk muscles, so as to pass into a rigid state that may last a long time when the animal is out of the water. The females are, as a rule, notably more relaxed.

When the male acutely seizes a female or a male he seems to judge of the amount or the character of the resistance by pushing

his claws back and forth. That the male can thus be misled by the lack of male resistance and continue in the process of conjugation was shown by the above case of a male that was conjugating with a dead, inert male. The following experiment also shows that the male may be deceived, or at least be led to conjugate with a male, even if alive, provided the male acts passively, and does not resist like a male.

A male was given another male that had been operated on twenty-four hours before so that it could not walk but only move its legs, vaguely, as the result of destruction of the brain. This inactive male was at once seized, turned, mounted and treated just as if it were a female for more than hour and a half, during which the active male succeeded in carrying out the usual crossing of the fifth leg, though this was rendered difficult by the fact that the leg on the right was lacking and one on the left lacked two segments. Though the abdomen was contracted and thrusts of the stylets executed, there was no approach to the region where the sperm pocket would be in a female, owing to the fact that the hooks of the male did not engage in the joints of the assumed female. That this was due to some fault of the active male was proved by removing him and manipulating another male upon the paralyzed male, when the hooks were made to engage as if the paralyzed one was female. There is no special organ of the female that receives the hooks of the male, but the same joints in the legs of both sexes may be so used.

The above male after being separated from the paralyzed male was given a female with brain destroyed twenty-four hours and dead for some hours. The male paid no attention to the dead female till it was shoved towards him, when he instantly seized it, turned it over, mounted upon it, grasped its claws, crossed the left fifth leg, contracted the abdomen, and made thrusts of the stylets, all within one minute. But the female's left claw was not held in the male's right and, as if very purposely, he shoved the female's claw up with his second chelate leg then held it with the others in his right. The hooks, also, were not fixed, and five or six efforts were made before one of the hooks was engaged in the joint of the female's second leg. After two hours and a-half the male

was hooked so as to lie diagonally, that is, on the right his hook was in the female's third leg and on the left in the second leg. Half an hour later, however, the male had hooked straight and so continued. Thus at least all the early and possibly all the later stages of conjugation may be carried on with a dead female, which emphasises the passive nature of the female's behavior in normal union.

Again when the males were bound with chelae closed and limbs in the posture assumed by a female in conjugation, other males with bound chelae were excited by contact and, without the usual initiatory use of the chelae, even mounted upon the supine, bound males and endeavored to carry on conjugation.

It is thus possible that from the crayfish standpoint the only difference between the sexes is a difference in behavior and not a difference in form, and moreover a difference received by muscle and touch sense and not in effect upon any of the other sense organs.²

The crayfish is thus much like the amphipods studied by Holmes who found, "The different reactions of the two sexes to contact with other individuals is the factor which effects the union of the males with the females."³

Coming to a more detailed description of the processes of conjugation, we shall describe first the behavior of the female and then that of the male and divide his activities into the following groups: (1) Seizing; (2) Turning; (3) Mounting; (4) Claw clasping; (5) Erection and locking of the stylets; (6) Crossing of fifth

² The observations of Chidester upon the crayfish, *Cambarus bartonius*, confirm this point of view, for he observed that these males repeatedly grasped males and even, despite their struggles, turned them and attempted conjugation. The females when seized firmly ceased to struggle and lay passive. He inferred that the males did not recognize the females.

³ After this paper was ready for press appeared the account of the experiments of A. S. Pearse, in the *American Naturalist*, December 1909, in which the conclusion is independently reached that the crayfish has "little or no power of sex discrimination." His observations were made chiefly upon *Cambarus virilis*, and make it probable that all crayfish lack means of acting differently toward males and females till they are in contact with them and are influenced by their sexually different responses, in the fields of touch and pressure.

leg; (7) Hooking; (8) Advance; (9) Recession; (10) Palpation; (11) Contraction of abdomen and claws; (12) Entrance; (13) Thrusts of stylets; (14) Discharge of sperm; (15) Formation of plug; (16) Withdrawal; (17) Liberation of female. Some of these phases are very brief, some less essential, and pauses of longer or shorter duration come into the series of active states.

The part of the female during all these phases of conjugation, which may consume as many as nine hours, is chiefly a passive one, at least after the initial stages.

When the female is seized she generally struggles as if to escape and also defends herself with her claws more or less vigorously. The actual seizure by the male may well supply a strong stimulus to the female, since the male's chelae frequently close firmly upon the limbs of the female on one side while at the same time holding the rostrum, eyes and bases of the antennae. At this stage the female frequently makes violent leaping movements, backward, which however, may only facilitate the turning of the female over, since the female in these leaps becomes suspended in the water, while the male remains supported upon his legs and has the better leverage.

Once the female is turned over by the male she remains through the following states so passive that she appears dead and the above male that tried to conjugate with a dead female probably missed little reaction from the female. This passive state seems to be like the hypnotic condition which Dearborn says results from the holding of crayfish in any constrained position. In this state there is not a relaxation of all the muscles but a strong flexure of the abdomen, which remains coiled. This coiling of the abdomen is lacking in a dead crayfish and it may be of some aid in the process of conjugation, since the telson of the male is pushed against the coiled abdomen in a way that seems to aid in the leverage that makes possible the thrusting of the stylets, as will be described later.

A striking difference between the male and the female during conjugation is the fact that while the male carries on violent vibratory or fanning movements of the exopodites about the mouth (which may be in part a sign of excitement), the female remains

without these movements and probably has but a small respiratory exchange in comparison with the more active male during the conjugation.

But while the female is so inert the nervous system may be receiving stimuli of some sort. The two minute and apparently useless limbs of the first somite of the abdomen are seen to reach up in the water to the stylets of the male, and in one case to touch the endopodites that had sperm upon them. Possibly some general sensations are received through these palp-like limbs. Possibly study of these organs would show that they are of use as sense organs, though of no great importance. Herrick has suggested that in the female lobster these limbs have been reduced to prevent eggs being attached to them, as that would interfere with the closure of the abdomen over the other eggs; but even granting this, the limbs may have a sensory value, both in conjugation and in egg-laying, though they are not necessary, as I have proved by removing them. After conjugation these little organs reach to the sperm plug, but it seems improbable that the female is aware of the success of conjugation.

During conjugation there are sometimes twitchings of the muscles of the abdomen and when the stylets happen to be thrust against the soft membrane posterior to the annulus there are twitchings of the body of the female that indicate that the apparent hypnotic state is not one of paralysis of all the body. Again since the annulus is pushed dorsally by the stylets, which enter it so firmly that when the male is pulled away the annulus is drawn out as far as the cuticle will allow, it may be that the female has some sense of the change of pressure in that region.

Turning to the activities of the male, they may, as above stated, be resolved into many different phases, the first of which is the seizing of the female. Most of the males in the mating season seem ready to seize any other crayfish, and if they seize females the rest of the conjugation generally follows. The female is grasped by first one and then both of the chelae, though a mutilated male with only one chela can accomplish conjugation. One chela often seizes the head of the female, but here is much variety in the modes

of seizure. Once the chela has taken hold it does not often relax, but the second is added and henceforth the hold is maintained.

Immediately, or sometimes after an interval, the male that has seized a female enters upon a struggle, a sort of wrestling, that leads to the second act, the turning of the female. Viewing this process, it is hard to escape the impression that the male has a purpose in view to which instinct leads with even what looks like intelligence sometimes assisting.

At times one must admire the solution of the problem of turning over another body braced upon ten legs and actively resistant. But again the clumsy efforts of the female seem to bring about a happy chance position that the male utilizes.

Sooner or later the female is upside down and still held by both chelae of the male. The second phase of the activity, the mounting, now follows: It is not known how the male is aware of the inverted position of the female, but the complex actions that follow lead one to believe that the sense of touch and muscle sense give the male a means of quite accurate response to the form and position of the female. The male mounts upon the female so that the ventral surfaces of the two are near together. The male then brings the two into a position in which the median planes of both coincide and, their heads being in the same direction the right of the male is over the left of the female and vice versa, fig. 1.

After this mounting comes the difficult phase of claw-clasping, which seems to satisfy a strong instinct. The object attained is that most all the legs of the female are firmly held in the two chelae of the male. The old hold of the chelae is gradually changed, without letting the female at any time free from the grasp of one chela. Generally in a very few minutes all the left clawed legs of the female are held by the right chela of the male and all the right clawed legs by the left chela of the male.

This feat is facilitated by the habit of the female, when seized, of throwing all the legs forward and upward alongside of the head, so that they are much more readily taken hold of in a bunch than could otherwise be the case. Yet the male that has hold of but few legs generally tries till all are finally grasped, exhibiting what seems a strong desire to hold them all.

The greater size and especially length of the chelae of old males seems directly connected with this function and it is noteworthy that the chelae so nicely encompass all the ends of the claws and walking legs of the female.

However, it is not necessary for the completion of conjugation that all the claws be held. Some may escape the normal male and a male with but one chela can hold the legs of but one side. One male lacking claws on both first and second left legs held in conjugation a female lacking the same claws on the right, but with the one chela of the right the male held all the claws that remained on the left of the female.

Experiments would be necessary to determine the true nature of this habit of claws-clasping. When some female claws are left free they have been sometimes seen to pinch parts of the male.

Mounted thus upon the female, the male is held in position not only by the two chelae but by the contractions of other legs that are wrapped, as far as their rigidity permits, about the convex thorax of the female. There remain but few legs that stand out right and left from the male and prop the body from falling over, fig. 1. As the back of the female is rounded the conjugating crayfish tend to roll over onto the side, but this is resisted by the male legs that act as props. But while the conjugation is commonly carried on with the male in the normal position of locomotion and the female in the forced, inverted position that crayfish assume only under compulsion (except at the period of egg-laying), it frequently happens that the pair lie upon their sides. When the water is so shallow that it will not cover the male as above mounted, the pair may lie upon the side, and thus conjugation was carried out in water too shallow to cover even one animal.

But before the firmest clasping of the female the male must erect the stylets, cross the fifth leg and attach the hooks. These three acts take place as follows:

When the male has seized all the claw-legs of the female in his two chelae he moves back and up away from the female, still holding the claws, and then raises the organs that are to transfer the sperm. These are the first and second appendages of the

abdomen. Crayfishes have the habit of swinging back and forth the small appendages found under the abdomen; in the female this serves to aërate and clean the eggs and embryos when they are attached to these appendages, but at other times, and in the male at all times, it is not obvious what use the swinging motions of the "swimmerets" may be. However, when the peculiar male appendages are to be used the entire series is set swinging and the first and second partake of this motion enough to be raised from their usual position to the one of use in sperm transfer. These two appendages at rest are carried by the male forward, horizontally, in the deep groove under the thorax where they are concealed and protected. However, at this stage of conjugation they are lifted up by their muscles, or really swung downward and backwards as far as their stiff basal joints will allow, only about 45 degrees from the horizontal. A slight muscular movement serves to place the second appendage against the first so that it locks into it. The normal position of these organs is horizontal and they tend to return to it, but during the following hours of conjugation they are held up by a remarkable device that relieves the weak muscle that elevates the stylet from the task of holding the stylet erect. The male with difficulty and care crosses either the left or the right, or sometimes first one and then the other of the last or fifth legs, under his thorax, in spite of the near presence of the body of the female that makes it awkward to swing the limb across; and thrusts it over between himself and the female till it lies as flat as possible under his thorax with the terminal joints protruding beyond the opposite side of the body. Henceforth in conjugation the male seems, at first sight, to lack the fifth leg on one side (fig. 1), while careful observation shows its tip on the other side. Either first or later trial has placed this leg across anterior to the erected stylets, and in this position this leg holds the stylets mechanically firm and erected at 45 degrees till the end of conjugation, when the leg is moved back and the stylets allowed to fall into their usual horizontal position of rest. Often, however, during the early stages of conjugation the male will try first one and then the other of the fifth legs in the attempt to carry on the chain of reflexes to the consummation of the fitting of the stylet into the receptacle.

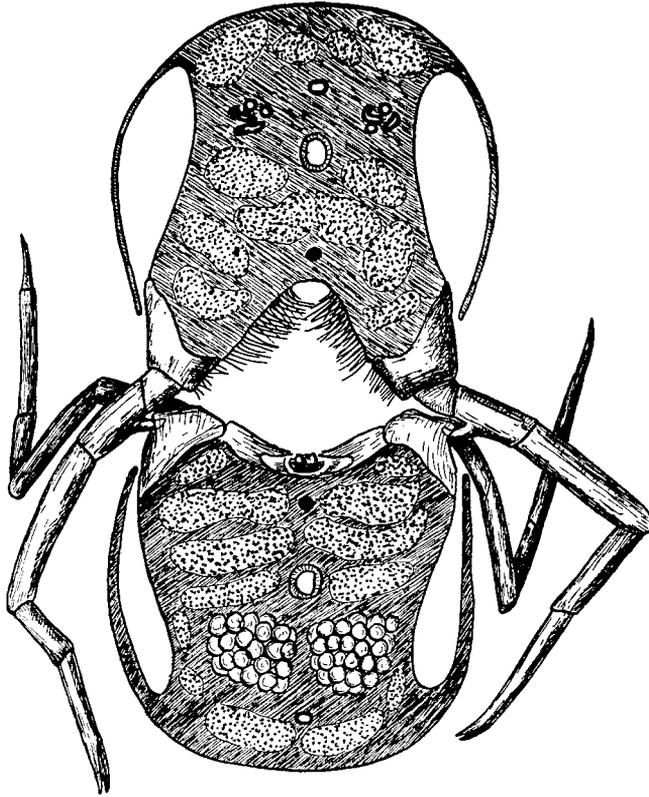


Fig. 2 Diagrammatic cross section of male and female in conjugation to show mode of attachment of hooks.

After the above preparation the male settles down against the female and tries to fasten the hooks to the bases of the female legs. Holding the left legs of the female in his right claw and her right legs in his left claw the male tries to make fast the hooks that are on the third joints of the third legs to the grooves at joint of first and second segments of the third legs of the female. These grooves are accentuated by the fact that the female legs are bent up dorsally. In accomplishing this the male no longer stands up high above the female but brings his ventral surface as near as possible to the thorax of the female. If the two animals are at

all nearly the same diameter it is finally possible for the male to catch the hooks into the joints of the female as indicated in the diagram of a section of the two conjugating animals (fig. 2). Really it is only the base of the legs that are in contact, since the ventral side of the males is hollowed out, as represented in the diagram by the groove lined with setae. And in this groove lie the two pairs of appendages of the male that are soon to be used.

The hooks hold so firmly that lifting the male out of the water lifts the female also, and indeed it is impossible to separate the two without unlocking the hooks.

Usually all the stages of preparation so far considered, including the hooking, require but a few minutes, but with great individual differences.

With the male and the female firmly locked together and the stylets held at an angle of about 45 degrees, the transfer of sperm can take place after the muscular efforts of the male have forced the tip of the first stylet into the firm orifice of the annulus of the female. To accomplish this the two animals must be quite accurately adjusted and this requires some trial. There is more or less advance and recession of the body of the male along the female till the tips of the stylets find the mouth of the sperm receptacle. In these trials there is sometimes seen a rapid play of the setose tips of the third maxillipeds, quick sidewise and lengthwise motions over the bases of the legs of the female. It seems that some information may be acquired by the male by this palpation.

To obtain actual entrance of the tip of a stylet into the receptacle, force is finally exerted by violent contractions of the muscles of the male. The posterior part of the abdomen of the male grasps the coiled-up end of the abdomen of the female as a hand about a ball (fig. 7) and tends to hold these ends of the animals together. And at the front end the chelae hold the claws of the female. Between these two points the hooks (fig. 2) bind the two animals immovably. When the abdomen contracts the long chelate legs also contract without losing their hold and they tend to straighten out. This elongation of the chelate legs tends to throw the head of the male up away from the female. As the

hooks do not yield and as the thorax and abdomen are held as one piece by the contraction of the powerful muscles that connect them, the result of the unbending of the chelate legs is to depress the base of the abdomen, to force it nearer to the female. This is resisted by the rigid stylets, but if their tips are at the mouth of the receptacle they must be driven into it.

This see-saw action of the whole animal that brings all the muscles into play to force the stylet to enter the annulus will be considered again after describing the anatomy of the hooks.

To bring the organs to the position in which the actual transfer of sperm can take place thus requires the exertion of much muscular force in carrying out the instincts of the male.

As will appear in another paper the mechanism of sperm transfer along the stylets is not completely understood. Besides the contractions of the sperm duct there are movements of advance and recession of the second stylet upon the first that have some significance and there is still another action, the movements of the abdomen that cause several successive short thrusts, or tamping movements of the stylets.

The last four phases—discharge of sperm, formation of plug withdrawal, liberation of female—were but little studied. Many hours are consumed before the sperm receptacle is quite filled, and the actual packing of it is removed from observation. It does not appear that the sperm leaves the male till the stylet has found entrance into the annulus, and it is rare that any of the sperm escapes into the water.

Normally all that is discharged goes into the receptacle, where sections show it arranged as if it had flowed in as a liquid mass. However, only the innermost part of the receptacle contains pure sperm and the outer parts are full of a secretion that is finally added in excess so that it protrudes from the mouth of the receptacle, as a sperm plug, that is evidence of conjugation having taken place.

After the receptacle is filled the male reverses some of the preliminary phases by first raising the bases of the legs so that the hooks are disengaged from the grooves of the female's legs, then rising up away from the female, then crossing the fifth leg back

into its usual position, then letting the stylets recede to their usual horizontal position and finally letting go of the claws of the female.

As soon as the claws of the female are released she returns to the customary position with the ventral side down and keeps this with great persistence except in the processes of egg-laying and turning, elsewhere described by me.

Though the stylets are the essential ducts for the transfer of sperm, it was found that when they had been cut off entirely the male would still carry on the preceding stages of conjugation and even contract the abdomen as if thrusting forward the stylets that were not there. While the series of events in conjugation may be thus carried on for a long time, though the end events will be impossible, it is also true that the series may go on for a while when the first part is lacking, or at least but dimly represented. Below it will be shown that the series may go on when a middle factor, the fastening of the hooks is omitted by the removal of the hooks, which prevents the success of the final acts, though they are attempted.

The experiments that show that the perfect expression of the first of the series is not necessary for the carrying on of subsequent parts were as follows: Ten males and females with chelae tied shut by elastic bands were kept separate for several days in water at 20 degrees C., though in December. When the females were put in with the males, each in his accustomed dish, the males acted individually, but most of them tried to seize and turn the females. The males generally remained quite inert till touched by the female crawling about in the strange dish, but a few males rushed at the females before being touched. That they tried to turn the females was seen in the pressure exerted by their claws and turning of their own bodies. The females and males fenced together, or the former sprang backward, as if their claws were not bound, except that there was not the usual open claw and pinching. Without this seizure the males did not succeed in turning the female, though one nearly did so and proceeded to mount upon and embrace the female, though the claw grasping stage was lacking. When the females were bound with the legs

in the conjugating attitude the males were little excited by them till moved about by a pair of forceps, but even then were less excited than by the normal moving of the female. One male mounted upon such a tied female that happened to lie supine and then struggled to carry on conjugation, advancing and receding over the female, grasping with the legs and abdomen and violently palpating with the third maxillipeds. Finally, when, in the pressure of the male's chela along the female's chela, the bands came off, the male used the chelae normally.

In another case, however, the male, though having lost one chela, used the single bound one so effectively that a female with chelae bound but otherwise free was conjugated with rather completely. That is, the female being found upside down was mounted and held. She lapsed into the normal inert state, however. The male mounted and grasped with legs and abdomen and crossed one fifth-leg above the erected stylets, then hooked to the female and made tamping movements with the abdomen. After that the body was elevated in front and force exerted to drive the stylets in. The male shoved the single bound chela against the face of the female and thus made a substitute for the usual grasp.

This attempt at complete conjugation without normal use of chelae lasted an hour and more and the fifth leg was changed to the other side. Eventually the male desisted and though sperm had been seen to issue from one of the first stylets there was no sperm plug in the annulus.

Evidently, however, the male with bound chelae neither is lacking in keen response to the female nor in instinct to carry on as many of the phases of conjugation as possible, though the first acts may be represented only by internal phenomena without complete external expression.

Coming to the anatomical side, we find three pairs of organs in the male absolutely necessary for the transfer of sperm in these complex processes of conjugation, and there are also in this crayfish two spurs or hooks on the legs, the importance of which has not been described. We will here describe only these hooks, reserving an account of the anatomy of the other organs more

directly concerned in sperm transfer for another publication. These hooks are mere hard tubercles, or blunt spines on the shell of the third segment of each third leg, (counting the chelate legs as the first of a series of five legs) fig. 3.

Many other kinds of crayfish have none, others have one on the second as well as on the third leg, while others have one on the fourth as well as the third leg.

The hook is thus not an organ absolutely necessary to crayfish conjugation, though we shall attempt to show that it has become necessary in *C. affinis*.

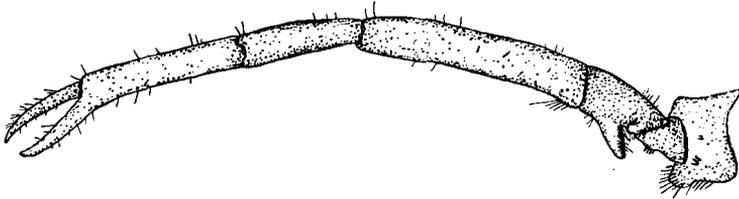


Fig. 3 Posterior face of left third leg of male 64 mm. long.

In the female there is no hook and in the male there is no similar protuberance on the other legs, so that in *C. affinis* the organ is not metamericly repeated, while the other cases referred to show that it may be repeated. Moreover the spine is not a specialization of something found on other legs in that region but is in each case a perfect spine or nothing. To be sure there are similar spines on the big claws and on the body, but not in the region corresponding to the hook.

In *C. affinis* the hook or spur (fig. 3) makes a very conspicuous projection from the proximal part of the third segment of the leg, forming a strong cone with its proximal face flattened, and sparsely set with setae. The whole is white and bony with the tip especially so and as if rolled over as a terminal ridge (fig. 4).

Springing from the underside the spur juts downward and also much toward the body and is so long that in old individuals (fig. 5) it runs far across the line of joint between the second and third segment and tends to become more parallel to the leg.

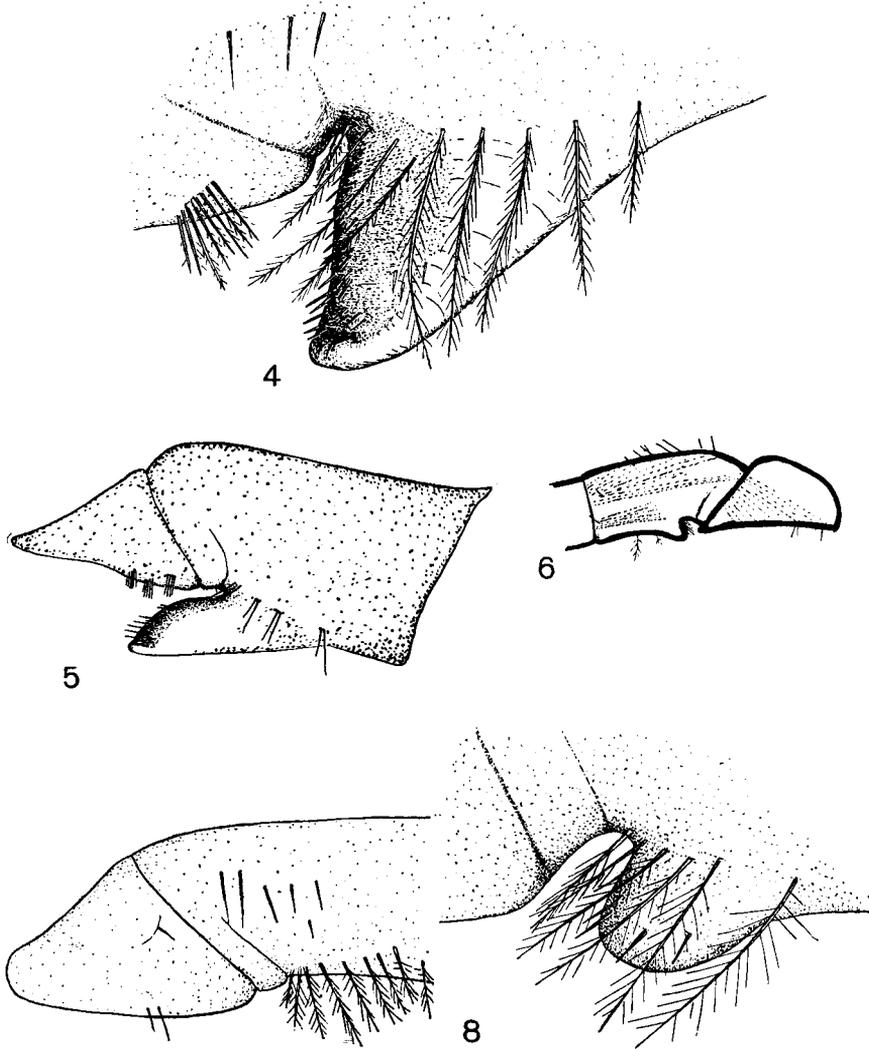


Fig. 4 Anterior face of the hook on the same leg.
Fig. 5 Anterior face of base of left third leg of male 195 mm. long.
Fig. 6 Section of base of third left leg of young male 48 mm. long.
Fig. 7 Anterior face of base of third left leg of male of 22 mm. Oct. 4th, 1904, showing no hooks as yet developed.
Fig. 8 Spur and setae upon base of leg shown in section in fig. 5.

There is thus with age an increase in the size and efficiency of the hook, for the more the tubercle becomes recurved the more is it fit to be a hook that will hold strongly to the female as will be seen presently. In old hooks, fig. 5, the base extends nearly the whole length of the third segment of the leg.

Sections, such as fig. 6, show that the hook is hollow; that is, it is an outgrowth of the shell, lined by epidermis and filled with connective tissue, as are the large tubercles elsewhere on the shell. The hook is thus a special employment of a local outgrowth just as the claw is a special use of the opposition of the terminal segment of the leg to a like tubercle, or outgrowth that opposes the last segment.

In the development of the male *C. affinis*, the hooks come quite late and are perfected after they have been used.

Crayfish of this species reared from eggs laid in the spring and measuring in October 22 mm., had no hooks (fig. 7), but merely the appearance of a false joint parallel to the real joint between segments two and three and a row of plumose setae in the region of the future hook.

But in males of 48 mm. of the same date (fig. 8) there is a large rounded lump with some of the above setae upon it. The section of this specimen (fig. 6) shows the thickness of the shell that continues over the spur and also the arrangement within the second and third segments of the muscles which thus have no connection with the region of the hook.

In the outgrowth of the spur the large setae are left about its base and some small ones are developed over its proximal face. These are well seen in living specimens with shells not too much worn, as in the specimen of 64 mm. shown in fig. 4. In many specimens, as in fig. 5, the setae are broken off more or less. These setae are long enough to suggest that they serve as sense hairs or as tactile setae, which may be of use in enabling the male to adjust the hook to its socket. The short ones along the proximal face may also serve pressure sense.

The above male (fig. 3 and fig. 4), being killed February 1st, had doubtless used its hooks in conjugation, as that has been found to take place in the first fall when the males are but 50–60 mm. long.

The way in which these strong hooks are used needs to be more clearly shown. The bases of the third legs are lifted up and away from one another by the male when mounted upon the female and then brought toward one another till, like spurs, the hooks fit into the joint between the second and third segments of the female's legs. This can be easily done, manually with dead or living crayfish, if only the legs of the female be held up against the sides of the thorax. The joint between the second and third segments of the legs is a soft membrane that can be displaced and the hard hook shoves it in. The face of the hook then comes against the hard rim of the second segment. The rim of the joint shows a small tubercle both on the first and the second segment. When the limb is extended these hinge tubercle are side by side, but when the limb is flexed up against the body the two tubercles together make a stiff, semi-circular rim about the soft membrane. It makes as it were the hollow of an elbow into which the hook enters and out of which it cannot come sidewise because the above hard rim opposes the flattened concave, or proximal, face of the hook. The overhang of the tip of the hook (fig. 4) seems advantageous. The semi-circular pit of the female's leg exists only when the leg is bent. Hence, when we hook two dead specimens together the weight of the female may be held up by the male hooks, mechanically, without muscular effort, as long as the limbs have the general positions shown in fig. 2; but if the leg of the male is raised, or if the leg of the female be straightened out the hook is loosened and the female drops off.

The mechanical nature of this use of the hooks is, poorly, indicated in the section, fig. 2. This shows, however, the inverted female containing ovarian eggs and the male with the coils of efferent ducts in like region of the body, poised upon the female. The bases of the limbs only come into contact, while the ventral surfaces of the bodies of the animals are separated by the space caused by the concavity of the ventral surface of the thorax of the male especially. It is across this space that the stylets will be extended to reach the annulus, which is indicated on the middle of the upturned surface of the female. The hooks on the legs of

the male are so thrust in, horizontally under the rim on the joint of the leg of the female, that any force that tends to push the ventral surfaces of the two animals apart will be resisted by the rim till it should break. If, however, the leg of the male be thrown out away from the side (raised up) the hook will be disengaged.

The female is held much as a piece of ice by tongs that tighten as the weight increases, or as a stone lifted by hooks in holes in its sides with converging chains.

That this use of the hooks is a necessary part of the conjugation in *C. affinis* seems demonstrated by the result of removing the hooks. As they are but protrusions of the connective tissue and epidermis covered with hard shell they can be cut off without serious injury to the animal and the animal retains all its usual eagerness for conjugation.

When normal males are kept with females there is usually a sign that conjugation has taken place in the presence of the sperm plug. But when eight males had one or both hooks removed and, after two to four days, were given females no sperm plugs resulted. The cause of this apparent failure in spite of the fact that many of the males were seen to seize the females was made out by observing several of those with one or with no spurs.

The male crayfish without any hooks seized the female eagerly, turned her, grasped her claws, raised and locked the stylets, crossed the fifth leg and made both the maximum contractions of the entire body and then six or seven quick thrusts of the stylets, just as if hooked to the female. That is, the phases of conjugation went on as usual; the hooking being absent, the next stage was carried on. But a halt came when it was found that the stylets had not entered the annulus. This entrance is a difficult act that usually requires repeated trials. In these crayfish with no hooks the maximum contraction resulted in the elevation of the male a half inch above the female. When the male had settled down close to the female as if to fasten the hooks he then passed to the state of violent contraction. The abdomen held by muscles formed one mass with the thorax, the tail was strongly applied against the coiled up tail of the female, the anterior part of the male abdomen was bent downward so that the stylets were

thrust down and forward, held stiff by the fifth leg, and at the same time the long chelate legs straightened themselves out. This last action, with the others, necessarily pushed the body of the male as one mass up away from the female. Had the body been hooked it would have acted as a lever about the hook as a fulcrum and thus the rising up of the anterior end of the body would have made the posterior end move down; this in turn would have thrust the tips of the stylets forward as well as downward and so have helped in the entrance.

Diagrammatically expressed, the normal use of the hooks is to hold the middle of the male firm so that the body of the male can move on the hook region as a see-saw. When the chelæ shove up the head end of the see-saw the other end goes down. The stylets being held firmly at an angle of 45 degrees tend to push against the ventral surface of the female both downward and forward. The strength of the chelæ is utilized as an aid to the entrance of the tips of the stylets into the sperm-pocket. All the most powerful muscles of the male seem necessary for sperm transfer.

When the hooks were not there and there seemed no way to hold the body of the male firmly to the female at the middle of the length of the see-saw, then the force of the chelæ raised the male away from any possibility of getting the stylets to the annulus.

The absence of the hooks thus led to a separation of the male and female thoracic surfaces, and nevertheless the stylets high in the water above the annulus were then seen to be quickly jerked forward several times, as if to enter the annulus. But the male next acted as if missing the usual response; he endeavored to hold the female tightly with his claws and legs that encompassed her thorax; and after some efforts turned the small antennae directly down and backward under his head as if in search of impressions. The third maxilliped was also used in rapid palpation of the bases of the female legs.

Another male with no hooks after such a first trial with the right leg crossed stood up an inch from the female and substituted the left leg and then went through the maximal contraction-phase

with like failure to bring the stylets to the annulus. The vain efforts continued more than an hour.

A male with only the left hook removed succeeded in attaching the right hook to the joint of the female's leg but, when the strong contraction followed, the hook came out, not being held by its opponent force on the opposite side of the body (see fig. 2), and then the male was thrown off as before. In this male with one hook the animal was seen to change from the right to the left and back again to the right leg after vainly trying to make entrance. This is taken to indicate that the normal male is in the habit of changing legs, as is seen, for the purpose of obtaining perfect entrance of the stylet, whether there is a right or a left annulus presented, and to indicate that but one of the stylets is used at a time.

After the male with one hook had made several quick thrusts of the stylet and encountered no resistance he always settled down close to the female and made exploratory palpations with the maxillipeds over the bases of the female legs. In one case after this examination of the bases of the second and third legs, a few rods of sperm were seen upon the telson of the female, showing that abnormal, premature discharge of sperm had taken place though the stylets had not been introduced.

In these males without hooks failure to gain entrance of the stylets was followed by advance of the body and palpation of the bases of the legs of the female before the next attempt. This raises the question whether in normal conjugation the male may have a complex knowledge of the form of the female, or may judge by sensing the surface of the female whether a change of position will lead to better results. At all events after failure there is the use of sense organs and then renewed trial.

Bearing upon the conjugation of this one species are the recorded facts as to conjugation in other species and the following facts as to attempts of one species to conjugate with another.

It will be shown elsewhere that the stylets in probably all Cambari are alike in use and essential structure despite remarkable differences in general proportions and external appearance.

We have observed conjugation in *C. affinis*, *C. virilis*, *C. clarkii*, *C. immunis*, *C. bartonii* and A. E. Ortman in *C. obscurus*;⁴ and as far as observed the process is like that in *C. affinis*, though the details have not been studied.

When some seven male and only one or two female *C. virilis* were kept with about two hundred *C. affinis*, the individuals of the former species found one another and the female *C. virilis* was conjugating with a male *C. virilis*.

While the males thus select the females of the same species, as far as these observations show anything, it is yet possible for the conjugating reflexes of the male to be brought into action toward a female of the other species. Thus when a male *C. affinis* from which the first and second stylets of the left side had been cut off was about to seize a female *C. affinis* in the corner of a dish, the female was removed and a female *C. virilis* put in its place. The male drew back, stood as if looking at the new female, went to the other end of dish, and in a few minutes returned to face the female and rest his long antennæ upon her thorax for a few minutes, then suddenly seizing her tried to turn her. In a few minutes the male was mounted upon the passive female and holding her chelæ, but not her other legs. The male then made attempts to get more and more of the claws of the female in his claws, at times holding most of them, and meanwhile made quick use of the third maxillipeds to feel over the ventral face of the thorax of the female. The claw of the second leg was also passed over the median part of the thorax of the female. But the male then desisted and the female remained passive, turned first on the side and then to the ventral face, but still held by the right claw of the male fast to her right claw and by the left claw of the male fast to her rostrum. Ten minutes later the male was mounted again and held all the chelate legs of the female in his claws, except one on the left. Half an hour later the male had crossed the left fifth leg anterior to the stylets which remained on the right side, having been cut off from the left side. Yet

⁴ Pearse has studied the conjugation in *C. blandingii*, *C. diogenes*, and *C. virilis*; *Am. Nat.*, Dec. 1909.

a half hour later the right leg was crossed anterior to these remaining right stylets. In this state the right second stylet was seen to swing back and forth by its own muscles through 70 degrees, quickly, before locking to the first. Two hours later the male had separated without leaving any sperm plug, but as this same result always followed when the males were thus mutilated the male had carried out the process of conjugation on the female of another species as far as would have been the case on the same species.

The possibility of crossing is thus good, and its failure would come from some mechanical difficulty rather than from the lack of the instinct to conjugate with any passive crayfish. Of course the fertilization of the egg may be ruled out by some inability of the egg and sperm to combine, but it seemed worth while to fill the annulus naturally or artificially with sperm of another species and await the result the following year.

The sternal plates of many females were cut off and fastened to females of another species, so that these now had their own sperm receptacle replaced by that of another species. But though some of these mutilated females lived to lay eggs, the eggs did not develop and the experiment failed. Attempts to have such transplanted receptacles filled by males also failed.

The male of *C. virilis* will also respond to the females of *C. affinis* as is shown by the following: A female *C. affinis* was put into the dish inhabited by a male *C. virilis*. The male in two hours had seized the female and was holding her chelate legs in normal manner and was trying to get the left fifth leg in advance of the stylets, which was difficult on account of the fact that the great length of the stylets of this species made them strike against the fifth leg, which was held with the elbow, or joint between the third and fourth segment from the tip, pressed against the thorax of the female. But finally the male rose up far enough away from the female to allow of the stylets being placed anterior to the fifth leg. While making these efforts the first and the second stylets were fastened and unlocked several times; when the second stylets were free they were swung back and forth quickly, while the first rested forward horizontally under the thorax. Then the first were raised a short way by their own muscles, but then be-

coming locked with the second both together were raised as far as possible to be at right angles to the body. The right and the left stylets acted together, yet one often moved a little ahead of the other for a short distance only.

These preliminary experiments show merely that conjugation between species may take place to some extent. Whether sperm transfer may be thus accomplished remains for future experiment to show.

This is of import here merely as showing the general nature of the instinct of the male and the similarity in the structure and use of the organs of sperm transfer in the different species of *Cambarus*.

CONCLUSIONS

The most complicated activities of crustaceans are those processes of conjugation that bring the sperm where it may ultimately meet the eggs.

In the crayfish, *Cambarus*, the sperm though injured by water is transferred from male to female while under water. The protection of the sperm involves many habits and organs of male and of female. In the female a peculiar receptacle stores the sperm till special habits and secretions bring it safely in contact with the eggs outside of the body. In the male three pairs of organs are devoted to the protection of the sperm while being transferred to the female.

The processes of conjugation in this crayfish involve the accurate adjustment of the entire body of the male to the body of the female when placed as a mirror image of the male.

The female plays a very inactive part while the male contracts most of the muscles of the entire body and limbs.

In *Cambarus affinis* the pair of hooks on the third legs are necessary for sperm transfer and hence for the continuation of the race. They become fastened so as to form a fulcrum about which the muscles of the claws and body act. In structure they are but elevations of the body-wall like other spines, but in this species they have no homologue on other legs.

They arise in the first year and become more developed after their first use.

The female has no special organ to receive the hooks, but the joint mechanism common to both sexes is used as a socket, which is efficient as long as the males and females keep their special postures in conjugation.

The fifth leg of the male is also used as an auxiliary organ as it furnished mechanical support for the stylets. Its use on the right and the left is guided by remarkable actions of the male.

The length of the claws of old males seems advantageous for carrying on conjugation.

In conjugation there is a series of events advancing in orderly sequence to the transfer of sperm and then, in part, reversing. In this series there may be considerable trial of various organs at various stages and this varies with individuals and cases.

The early stages of conjugation are carried out after the removal of the stylets has made the completion of the series impossible. Later stages are performed after removal of antecedent middle stages. After very imperfect expression of the first stages, later stages may follow as far as is mechanically possible.

The male acts as if receiving accurate local stimuli. In autumn and winter and early spring the males respond eagerly to other crayfish and conjugation is accomplished wherever possible. The entire series of conjugative actions seems preëxistent in the male to be awakened, at times, with explosive violence, on very slight stimulation. It is then carried out in its entirety whenever there is no mechanical obstacle.

Sex "recognition" exists, apparently, only in the sense that the male may carry out all the stages of conjugation if a female happens to be seized, but not if a male is seized. There is no sufficient evidence that the male recognizes the female as such, or as a whole. But the passive response of the female when seized makes the completion of the conjugation possible while the more effective resistance of the male when seized sooner breaks the series of conjugation acts.

The receptors of the male affected by male and female as objects seized appear to be only those that are stimulated by

actual mechanical contact. There is as yet no evidence that the light or chemical substances from the male and female act differently upon the males.

Crossing of species is not yet accomplished, though most of the phases of conjugation are carried on between species, in captivity.

The organs which are accurately adjusted during conjugation are of different values; some are ordinary and some specialized limbs; some are mere spines of the shell; one is a special invagination not represented in many other animals. Though some are old and some new they are all alike necessary at present for the meeting of sperm and egg.

It is by no means clear how any offered method of evolution can account for the assemblage of such constituents into their present working individuality.

BIBLIOGRAPHY

- DEARBORN, V. N. 1899 Notes on the individual psychology of the crayfish, *Am. Jour. Physiol.*, 3.
- CHIDESTER, F. E. 1906 Notes on the daily life and food of *Cambarus bartonius bartoni*, *Am. Nat.*, 42.
- HOLMES, S. J. 1903 Sex recognition among amphipods. *Biol. Bull.*, 5.
- HERRICK, F. H. 1895 The American lobster, *Bull. U. S. Fish. Comm.*
- ANDREWS, E. A. 1904 Breeding habits of crayfish. *Am. Nat.*, 38.
- 1906 Egg laying of crayfish, *Ibid.*, 40.
- 1911 Male sperm-transfer organs, *Jour. Morph.*