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SEXUAL PHASES IN CREPIDULA

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EIGHT FIGURES

In both the pelecypod and gastropod mollusks there is a strong tendency toward protandry, although change of sex may take place in either direction and in one species it has been found that protandry or proterogyny may be controlled experimentally (Rosenwald, '26). In some forms an alternating sequence of male and female phases occurs more or less regularly throughout the life of the individual, while in others the initial sexual phase of the young animal appears to depend to some extent on environmental factors, with later changes of sex taking place in the interval between two breeding seasons (Coe, '34).

In some groups of gastropods functional hermaphroditism is common and self-fertilization sometimes occurs. In some forms an initial phase of separate sexes precedes the hermaphroditic adult condition. Among the gastropods the species of Crepidula are of interest because it has been thought that in one species the development of the male phase may be dependent upon the presence of larger individuals (Gould, '17 a), and that in another species the continuation of the male phase requires that the individual be associated with a female (Orton, '09).

The question as to the extent and generality of such environmental influences can be readily examined at the Marine Biological Laboratory at Woods Hole, Massachusetts, for three species of that genus (C. fornicata, C. plana, and C. convexa) can be obtained in abundance and individuals of all of these will live for months in the aquaria and live cars without special feeding.

The habits and relative sizes of the male and female phases of each of these three species were briefly described by Conklin (1897, 1898) many years ago and evidences of protandry were shown. Later Gould ('17, '17 a, '19) made a very complete histological and experimental study of the sequence of the sexual phases of C. plana and the influence of the environment on the control of the sexual condition. These species are essentially protandric and the initial male phase usually occurs while the individual is very young and before it has attained more than a small fraction of its normal definitive size. In each species the functional male phase is distinguished externally by the development of a coiled seminal vesicle filled with sperm and by a slender muscular penis which may become nearly as long as the body. The young males are motile and move about freely, while the older males are more or less sedentary and often remain for life in one position unless accidentally or forcibly removed. Conklin (1897) noted correctly the differences in the behavior of individuals of each species in this respect. In C. convexa even those in the female phase may change their position from time to time, while in C. fornicata the sedentary males as well as the females may remain immovably fastened together for a year or more.

Following the male phase is a transition period, often of rapid growth. At this time the residual spermatic tissue is absorbed, the penis atrophies and the seminal vesicles disappear. Later proliferation of previously inactive ovogonia replaces the original spermatic tissues, and the uterus with its seminal receptacles is formed at the base of the genital duct. During the rest of its life the animal functions as a female. All these stages have been fully and accurately described by Gould ('17) for C. plana.

Since there are some notable differences in the environmental responses of the three species in their successive aspects of sexuality, they will be briefly discussed separately.

SEXUAL PHASES IN CREPIDULA FORNICATA

In this species, as previously noted by Conklin (1898), Orton ('09) and the earlier zoölogists, there is a strong tendency for groups of individuals to pile up, one on top of another, in spiral, pyramidal form, with the largest at the bottom and successively smaller ones above (figs. 1, 2). Commonly five to eight individuals are thus grouped together,

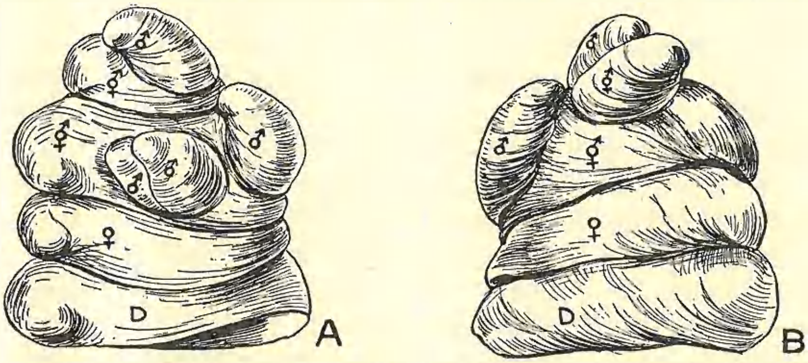

Fig. 1 Crepidula fornicata. A, small group consisting of basal female attached to dead shell (D), with two individuals in transition stages and one male above; three motile supplementary males are in mating positions on the lower transition individual. The female has presumably been fertilized previously both by the transition animal in an earlier male phase and by one or more motile males. The apical male is in position to fertilize the upper transitional individual as soon as the female phase is reached. B, same group from the left side. Natural size.

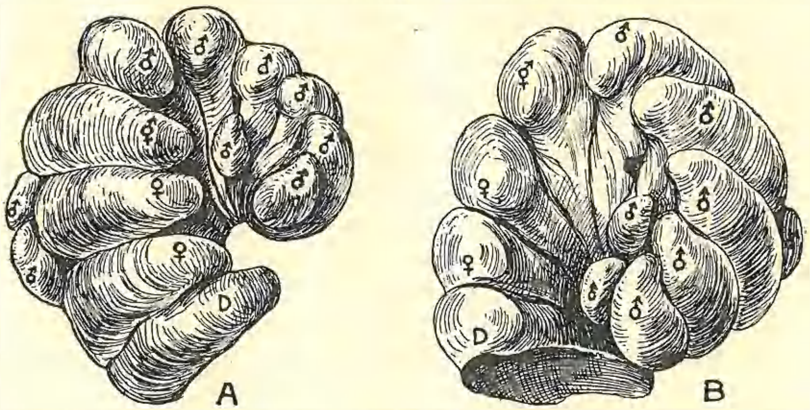

Fig. 2 Crepidula fornicata. A, large group consisting of two females, one transition individual and nine males; three of the latter are supplementary. B, same group from the right side; D, dead supporting shell.

but an irregular pyramid may contain more than a dozen. Such pyramids may be attached to stones, shells, or other solid objects, or they may lie free upon the pebbly or shelly bottom at a depth of several meters. In the latter case the bottom shell is empty or occupied by C. plana or some other mollusk.

Individuals of this species are also commonly found attached to both dorsal and ventral surfaces of Limulus. Others are carried about by Scycotypus and other gastropods. In these latter situations they are attached separately or in groups of two or three. The size reached at sexual maturity is correlated with these diversities of environment.

The basal individual in these groups is usually the oldest and hence likely to be found in the mature female phase. It is frequently also among the largest of the group, for large individuals are normally sedentary and if forcibly separated from the object to which they are attached they are usually unable to attach themselves to any other object in time to avoid destruction by their enemies. The top individual is the latest arrival and is usually either sexually immature or a young male. Between these two may be several functional males or transitional forms or females or one or more individuals representing each phase of sexuality (figs. 1, 2).

The following examples will illustrate a few of the hundreds of combinations of sexual phases found in groups examined in early summer. This will supplement a similar list given by Orton ('09) for the coast of England. The sequence, length of shell in millimeters and sexuality of each member of the group are in the order given, beginning with the base. F = functional female phase; T = transition stage; M = functional male phase; I = sexually immature; FE = female with egg cluster present when examined.

Group 1. 38 F, 35 M, 38 M, 31 M, 35 M, 27 M, 12 I.
Group 2. 43 FE, 43 FE, 45 F, 42 F, 44 T, 39 M, 24 M, 17 M, 12 I.
Group 3. 37 F, 37 T, 30 M, 26 M, 21 M, 18 M.
Group 4. 35 F, 37 F, 38 T, 37 M, 32 M, 23 M, 17 M.
Group 5. 39 FE, 40 T, 23 M, 18 M.
Group 6. 40 F, 40 T, 20 M, 15 I.
Group 7. 42 F, 21 M, 25 M, 20 M, 18 M.
Group 8. 33 FE, 35 FE, 37 FE, 33 M, 25 M, 14 I.
Group 9. 38 FE, 36 FE, 37 F, 30 F, 21 T, 16 M.

Nearly all groups have several supplementary males, 15 to 18 mm. in length, and immature individuals, 7 to 15 mm. in length, along the side.

It is obvious that the sexual condition of any group will change during the breeding season as additional males are added and as the sexuality of the component members changes with their advancing age.

Growth is normally rapid; young hatched in early summer may reach a length of 16 mm. and become functional males late in the autumn. Others mature more slowly and those hatching later do not reach the male phase until the following year. After functioning as males many of these transform into females during their first breeding season. Others remain in the male phase or in transition stages until their third or fourth summer when they breed as fully mature females.

Mating. If the seminal receptacle of the female has become fully developed, the male places his body along the anterior dextral surface of the female's shell and by means of the long, slender penis transfers the eupyrene spermatozoa from his seminal vesicle to her seminal receptacle. The mating position may be retained by motile males for several weeks and by sedentary males presumably for a year or more.

Several young males may participate in the insemination of a single female by placing their bodies in overlapping positions (figs. 1, 2), and these motile, supplementary males evidently do not interfere with the corresponding function of the one or more much larger, sedentary males with which the female may be associated. In small groups only young males may be present.

Primary gonad. Sections of very young individuals almost invariably show that the primary gonad is bisexual in nature, as Gould has described for C. plana. The nuclei of both spermatogonia and ovogonia are situated in a more or less continuous layer of cytoplasm without distinct cell boundaries. The more rapid proliferation of the spermatogonia and their encroachment on the lumen soon give the gonad a distinctly male appearance, although some typical ovogonia and ovocytes lie scattered along the walls in all or nearly all individuals.

Male phase. All the young animals are freely motile and with the exception of a few aberrant individuals, all of them reach the functionally male phase before the period of active

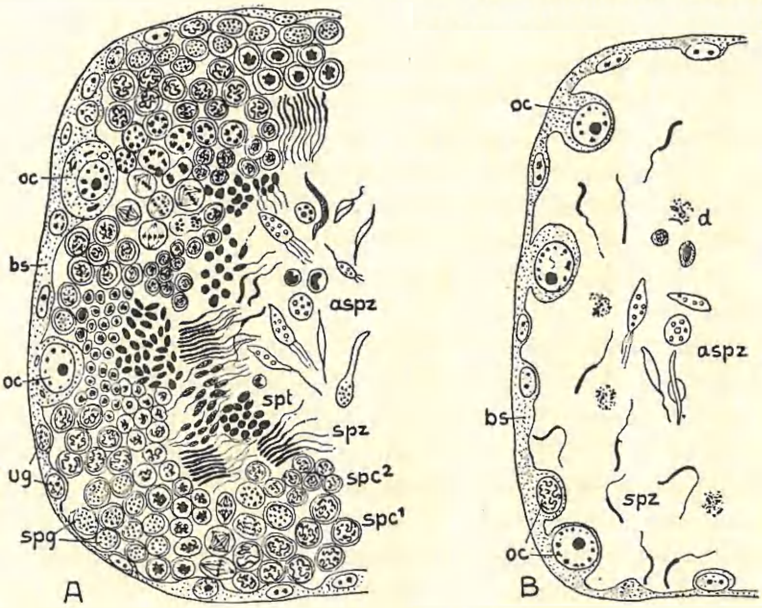

Fig. 3 Crepidula fornicata. A, portion of gonad of active male phase, showing development of eupyrene (spz) and apyrene (aspz) spermatozoa, with two ovocytes (oc) in basal syncytium (bs). B, gonad of similar individual in early transition stage (30 days after isolation), with only a few spermatozoa remaining; d, disintegrating spermatic tissues; ug, undifferentiated gonia; spg, spermatogonia; spc^1, spc^2, primary and secondary spermatocytes; spt, spermatids.

locomotion has passed. During this first sexual phase the slender, highly muscular and usually black penis grows out from the body wall immediately behind the right tentacle as soon as spermatogenesis commences. Both eupyrene and apyrene spermatozoa are formed and transferred to the seminal vesicles (fig. 3, A).

By their later behavior two more or less intergrading types of individuals may be distinguished. In one of these the male phase is comparatively brief, after which the animal settles down to undergo the transition to the female phase while the body is still relatively small (fig. 7). The gonads of such a feminine type of individual show more numerous and larger ovocytes than do those of the masculine type.

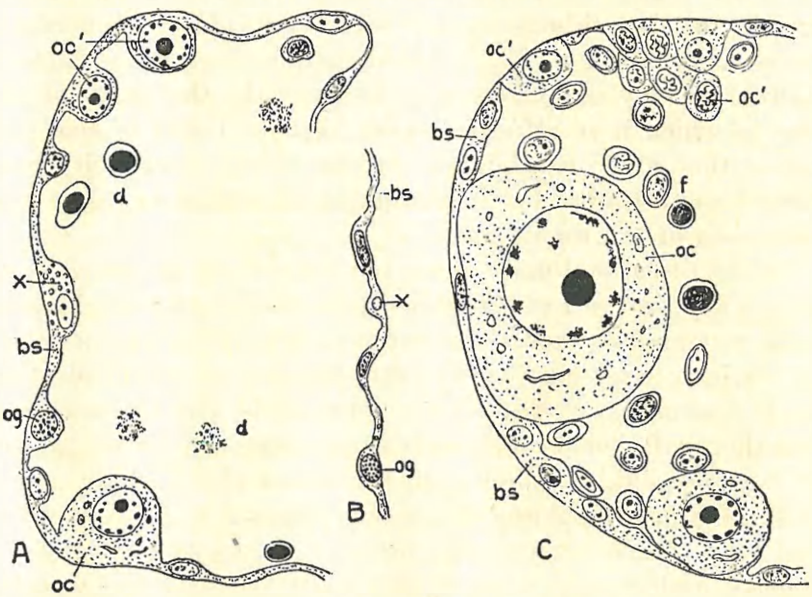

Fig. 4 Crepidula fornicata. A, portion of gonad of individual in late transition stage (63 days after isolation), showing thin follicular wall with scattered ovogonia and ovocytes and the remains of disintegrated cells (x); no spermatozoa remain but the lumen contains a few disintegrating spermatic cells (d). B, portion of wall of follicle devoid of ovocytes; C, portion of gonad of individual in early female phase (63 days after isolation) showing one large and one smaller ovocyte remaining from previous male phase and smaller ovocytes (oc') in early growth stages, with follicle cells (f) scattered in lumen. Other letters as in figure 3.

After a similar period of motility the young males of the masculine type establish themselves in mating positions if females are available and remain sedentary throughout the rest of their lives unless forcibly removed. Spermatogenesis may sometimes continue for a year or more, during which time the body has attained its fully adult size (figs. 5, 6, 7). Eventually, however, the transition to the female phase will normally occur. In groups in which no suitable females are present or in which the females are already covered by large males or transition forms, the young male of this sedentary type must take an available position at the top of the group of older males to await the sexual transformation of the underlying individuals before actual mating again becomes possible (figs. 1, 2). The transformation of such a sedentary individual may undoubtedly be hastened by the death of the one to which it is attached (Coe, '35 a). There is also evidence that after reaching a certain stage of maturity continued opportunity for insemination is conducive to a prolongation of the male phase.

In the functional male phase the wall of the gonad consists of a syncytial layer of follicle cells and undifferentiated gonia, with scattered ovogonia and more or less numerous ovocytes of various sizes projecting into the lumen. Proliferating spermatogonia, spermatocytes, spermatids and spermatozoa, together with numerous apyrene spermatozoa in all phases of development, completely fill the lumen (fig. 3, A).

Transition stages and the female phase. At the climax of the male phase the tubular, branching gonads are very extensive and occupy most of the space between the viscera, but as the male phase wanes and the spermatic follicles are emptied there is a rapid increase in the connective tissues, usually associated with an increased rate of growth of the whole body. In a short time the formerly active follicles are reduced to thin tubules filled with a fluid containing the disintegrating residual spermatic tissues (fig. 3, B). Spermatozoa often remain in large numbers. Some of these become cytolysed within the lumens of follicles and ducts, while others are ingested by the walls of the ducts and seminal vesicle to become assimilated by the syncytium of follicle cells. Only a few scattered cells along the walls indicate the progenitors of the future ova (fig. 4, A, B). Most of these cells are small ovogonia, but some have previously been transformed into ovocytes. The larger ovocytes frequently disintegrate with the spermatic tissues.

After further increase in the connective tissues groups of cells are found in active proliferation in some of the follicles and the characteristic synaptic chromosomes in the enlarged nuclei of the young ovocytes furnish unmistakable evidence that the female phase is inaugurated (fig. 4, C). When the body is removed from the shell it is noticed that the slender, branching tubules and follicles of the gonad have now become more deeply orange in color. The larger size of the body at this time allows the extension of new ovarian follicles into the newly formed tissues.

There is considerable variation in the extent of regression of the male characteristics before the ovocytes begin their rapid growth in the early female phase. Usually the two sexual phases are distinctly separated but occasionally some of the follicles have assumed unmistakably female peculiarities while normal spermatozoa remain in other parts of the gonad. Correspondingly, the uterus may become well developed and eggs may be laid before the penis has been reduced to half its former length.

During the transition stages the penis, which had previously been long and black, has been gradually depigmented and absorbed until it is represented by an orange colored tubercle only and even this may eventually disappear. The condition of the gonads is more or less closely correlated with the amount of regression of the penis, as has been previously reported by Orton ('09). A complete description of the formation and development of the ova of this species may be found in the classical study by Conklin (1897).

Effect of environment

The long period that the male phase may be retained when associated in groups with females was emphasized by Orton ('09, '22), who concluded that a properly mated male might remain in the functional male phase for several years. Isolated individuals soon complete the male phase and then transform to females.

The question arises whether this response is due to the removal of a specific influence exerted by the associated individuals or whether the inherent tendency toward a change to the female phase is merely hastened by any considerable alteration of the physical environment.

The problem was tested by segregating in an aquarium more than 200 functional males at the height of the breeding season toward the end of June. The maleness of each individual was determined by the presence of a normally developed penis, but in no case was an individual used unless the one next beneath it in a group was also in the functional male phase. No individual less than 14 mm. in length was included, because this is below the minimum size at which normal individuals in the population from which the samples were taken begin the sexual transformation. The morphological findings were checked, at the beginning of, during, and at the end of the experiment, by histological study of the gonads of samples from each group.

Such segregated males as were able to regain motility tended to form new groups similar in form to those from which they had been taken, with non-motile individuals at the base.

After the males had been separated from their associates and segregated for 30 days many of them had already entered upon the first transition phase. Examination of the gonads showed that spermatogenesis had ceased and that cytolysis of the spermatic tissues was well advanced (fig. 3, B).

At the end of 63 days (August 27th) 211 individuals were carefully examined after removal from their shells and the condition of sexuality of each, together with the size of the shell, recorded. It was found that only twenty-four, or 11.4 per cent of the total, had retained the functional penis and seminal vesicles. All the others had become transformed more or less completely toward the female phase, as indicated in table 1. The largest of the males was about 27 mm. in length at the end of the experimental period and the smallest 15 mm., the mean size being 21.4 mm. The maximum growth during the experiment did not exceed 6 mm., since only a little food was available.

In the first transition stage, with the penis about as long as a tentacle, were twenty-six individuals. The largest of these measured 26 mm. and the smallest 17, the mean being 21.6 mm.

Of the forty-seven individuals in the second transition stage, with the penis reduced to about half the length of a tentacle, the largest was 28 mm. long and the smallest 17, with a mean of 21.8 mm.

TABLE 1

Change of sexuality in segregated males of C. fornicata

SEXUALITY OF SEGREGATED MALES AFTER 63 DAYS, JUNE 23 TO AUGUST 25				CONTROL ANIMALS OF SIMILAR SIZES. PER CENT (ESTIMATED)
	Mean size	No.	Per cent	
	mm.			
Functional male	21.4	24	11.4	85
First transition stage	21.6	26	12.3	5
Second transition stage	21.8	47	22.4	3
Third transition stage	21.8	83	39.3	4
Functional female	21.1	31	14.7	3
Total		211		

The largest number, eighty-three, were in the third transition stage, with the penis reduced to a mere stub or tubercle, usually somewhat orange in color. Of these the largest individual measured 29 mm. and the smallest 15, the mean being 21.8 mm. In this stage the gonad has become reduced to thin-walled tubules with large, empty lumens (fig. 4, A, B).

Of the thirty-one individuals classed as functional females, with only a discolored spot to mark the position of the former penis the largest was 27 mm. long and the smallest 16, with a mean of 21.1 mm. The gonads show proliferating ovogonia and rapidly growing ovocytes (fig. 4, C).

Evidently the size of the individual after reaching the full expression of the male phase had little influence on the time which would normally be the first members of the group to begin transformation showed that some of them were more refractory to the changed environment than were the younger individuals. These are thought to represent individuals with a somewhat different combination of genetic factors, corresponding, perhaps, to the so-called 'true males' or to the masculine type of individuals of other protandric mollusks. They have fewer and smaller ovocytes in the gonads than do the more active males of the feminine type which respond to the environmental changes more quickly.

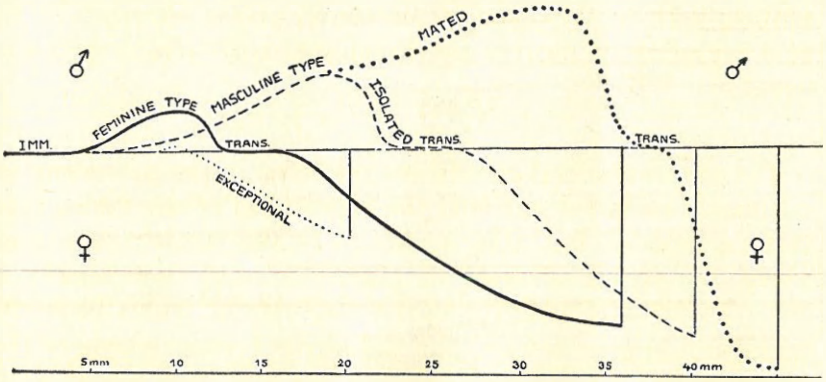

Fig. 7 Diagram indicating the successive phases of sexuality in typical individuals of the feminine and masculine types of C. fornicata as correlated with the lengths of the shells. The amount of deviation of the curves above and below the base line represents the relative volumes of male and female gonads.

A diagrammatic representation of the sexual phases of these two types of individuals is given in figure 7. This diagram also indicates the effects of isolation on individuals of the masculine type and the relative volumes of the active gonads in each sexual phase as correlated with the lengths of the shells.

Some individuals, however, appear to be intermediates between the feminine and masculine types and an occasional animal is found which approaches a truly hermaphroditic condition in that the ovocytes reach a large size before spermatogenesis is completed.

Aborted male phase

Immature animals kept in a refrigerator for some months occasionally transformed into the female phase without passing through the functional male phase. In such cases the penis is either not formed or developed to only a small fraction of its functional size and the usual orange stub is not found in the female phase. Spermatogenesis is completed only to the stage of spermatids. The spermary then disintegrates and the young ovocytes enter upon the synaptic and growth stages. Similar exceptional females are found in nature, both in this species and in C. plana, although in the vast majority of dwarfs the functional male phase occurs as usual.

Stability of sex in winter

The environmental conditions reported in the preceding experiments included not only the separation of the males from their associates but also a change in the temperature from about 15° to an average of about 22° and occasionally to 30°. Under such conditions the majority of males begin the transition stages toward the definitive female phase within a few weeks. Changes of temperature and food supply alone were shown to be less effective. In the autumn and winter, when the temperature of the water becomes lower, the sexual phase is evidently more stable, for less than 10 per cent of the segregated males kept at a fairly uniform temperature of about 7° showed any appreciable change in their sexual conditions from the end of October to the first of April. Transition stages promptly appeared after an increase in temperature.

In their natural habitat spermatogenesis is greatly retarded during the winter, although the seminal vesicles of the males remain distended with sperm. Following a mating period in early spring, there is a strong tendency for the gonads to remain inactive, terminating the male phase and leading to the transition stages.

SEXUAL PHASES IN CREPIDULA CONVEXA

This species commonly occurs attached to Littorina and other shells inhabited by the small hermit crab, but it is also found on a variety of solid objects. The early bisexual gonad has been described by Gould ('17).

The successive sexual phases are similar to those reported on the foregoing pages for C. fornicata, but in C. convexa the primary male phase is reached when the young animal is only 2 to 4 mm. in length (fig. 8). These males are likewise very active when young and move about on the female's shell and migrate from one female to another when both sexes are kept in the same aquarium.

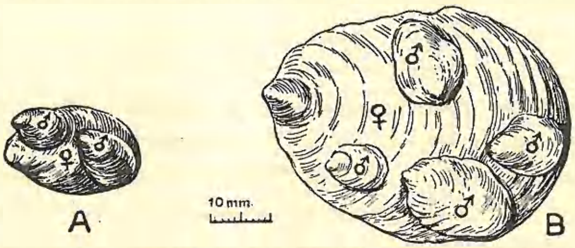

Fig. 8 A, Crepidula convexa; female with one mated (sedentary) and one supplementary (motile) male. B, C. plana; female with two of the four males in mating positions.

Transition stages from primary males to females may be found in animals only 4 to 6 mm. in length. In these there is a much stronger tendency toward hermaphroditism than occurs in C. fornicata, the ovocytes often beginning their growth long before spermatogenesis has been completed. Eggs may be laid before the penis has entirely disappeared. A 2-mm. functional male may be mated with a 6- to 7-mm. ripe female or the primary male may be attached to a sedentary male which is mated with a larger female.

In this species also one or two sedentary males may find a secure attachment on the female's shell until they become much larger than the motile primary males, often reaching 6 to 8 mm. in length. Further growth occurs during the transition stages, the female often reaching a length of 10 to 13 mm. (figs. 5, 6, 8).

This species is less favorable for an experimental study of the environmental influences on sex change, since the male phase is normally so brief.

SEXUAL PHASES IN CREPIDULA PLANA

Careful study of the development of the primary bisexual gonad and the changes which take place both in the gonad and in the copulatory apparatus during the successive phases of sexuality confirms in all important respects the admirable descriptions published by Gould ('17, '17 a, '19) and verifies the accuracy of his numerous illustrations. Gould's conclusions, however, that the immature animal must necessarily be associated with an individual of larger size, preferably a female, in order to realize the functional male phase is not supported by the evidence recently obtained. It is true that youngs males usually have such associations (fig. 8) when growing within the shells of large univalves, where colonies containing several to fifty or more individuals may be found. But the functional male phase is also realized when the young animal attaches itself as the only occupant of a dead shell of any other mollusk. Such isolated males are similar in all respects to those found in association with females, although the male phase is greatly abbreviated (fig. 6).

Gould has emphasized the great variation in the size which the young animal may attain before the functional male phase appears. Since these sizes seem to be grouped about two means (fig. 6) it is perhaps not unreasonable to surmise that two different combinations of hereditary factors may be involved, as is more fully discussed in the preceding account of C. fornicata. Gould has also correctly interpreted the long period of rapid growth which may take place between the two sexual phases, and has suggested that in an exceptional individual the male phase may be aborted, in which case the only functional phase would be female.

All these aspects of sexuality have been verified, but since they are indicated diagrammatically in figures 5 and 6 it seems unnecessary to add a further explanation.

It is sometimes difficult or impossible to determine in the living animal of medium size whether it is a sexually immature individual which has reached an unusual size before developing the male characteristics or whether it represents an animal which completed the male phase very early and is now in a late transition stage. But the response of the two will be very different if both are subjected to the same experimental procedure, such as changing the environment or placing them in contact with other individuals, for the one will soon enter the male phase of sexuality while the other will reveal its true status as a transition female.

It seems that any phase of the progressive cycle—immaturity, male phase, transition phase, female phase (figs. 5, 6)—can be abbreviated or prolonged by either genetic or environmental influences but there is no conclusive evidence that any part of the cycle can be reversed. Nevertheless, some of Gould's experiments were thought by him to indicate that the spermary may occasionally resume activity following a change of environment after a certain degree of disintegration of the spermatic tissue has occurred. The isolation of fully functional males usually results in the rapid absorption of the penis; if such individuals, before the transformation of the gonads begins, are replaced upon or beside large individuals of either sex the penis may sometimes be restored. Such association is not always necessary, however, for the development of the penis in the early male phase.

DISCUSSION

The three species of gastropods belonging to the family Calyptraeidae discussed in this report exhibit a series of sexual phases which closely parallel those of the pelecypods previously reported. In Crepidula, as in the viviparous and oviparous oysters and Teredo the primary gonad is essentially bisexual, both ovogonia and spermatogonia being differentiated at a very early period (Orton, '09, '26–'27; Coe, '33, '34, '34 a; Gould, '17). The more rapid proliferation of the spermatogonia soon gives the gonad a characteristic male appearance, with spermatogonia and spermatocytes filling the lumina of the follicles, but nearly always with some or many ovocytes in the cortical layer next the basement membrane.

In the protandric species, both of gastropods and pelecypods, the functional male phase usually occurs while the individual is very young and before it has become more than a small fraction of its definitive size.

Following the discharge of the spermatozoa is a transition stage, during which the residual spermatic tissues are cytolysed and their substances utilized for the general metabolism of the body. Such differentiations of the genital ducts as are peculiar to the male phase, including copulatory organs, if present, are likewise absorbed. This is usually a period of rapid growth.

The length of the transition period may be very brief, the proliferation and growth of such ovocytes as will be produced at the first ovulation in the female phase having been in progress during part or all of the male phase. In other cases, as in the oviparous oysters, a full year intervenes between the male phase and the next succeeding functional phase, which may be either male or female.

In the viviparous oysters there is an alternating sequence of male and female phases throughout the life of the individual. In the warmer regions three functional phases (male, female, male) may be completed during the first year of the animal's life, while in colder waters but a single phase is experienced each year and in some years the temperature of the water may remain so low that spawning does not take place at all. In warm areas there is a tendency for the normally succeeding sexual phases to overlap, producing a condition of true hermaphroditism. Self-fertilization is then possible if a portion of the gametes of one phase are retained until the discharge of those of the succeeding phase. A corresponding sexual rhythm occurs in Teredo, but the much shorter span of life usually limits the number of sexual phases to two, three or four (Coe, '34 a).

Similar conditions are found in the gastropods, but only a single change of sex occurs. In Crepidula convexa the transition phase is sometimes so brief that there is a distinct overlapping of male and female phases but not to such an extent as to render self-fertilization possible.

In none of the forms in which protandry is dominant does the initial male phase invariably become functional; critical examination of the gonads of large numbers of the population always reveals some individuals in which this phase is abortive, so that the first breeding phase is actually female.

Furthermore there are always some young males in which few, if any, ovogonial cells are formed, and which consequently do not experience the changes in sexuality characteristic of the rest of the group. These so-called true males, which may thus retain the male phase indefinitely, commonly comprise about 5 to 10 per cent of the young population in viviparous oysters (Coe, '34) and Teredo (Coe, '33, '34 a), and perhaps 35 per cent in Patella (Orton, '28). In Crepidula there is a small proportion of the large old males which resist longer the environmental influences which change the sex of the other males.

In all these forms, however, the term maleness is presumably relative and not absolute, since the 'true male' evidently represents merely an intensification of the protandric phase, with a corresponding postponement of the definitive female phase.

Between the extremes of the abortive and persistent males are many intermediate conditions. That these are to a considerable extent under environmental control is shown by the American oyster, in which the percentage of abortive young males may be as low as 3 per cent in one locality and more than 30 per cent in other situations which are more favorable for rapid growth during the animal's first year (Coe, '34). In older individuals, however, there is a close approach to equality in the sex ratio in all localities, indicating that such changes in sexuality as may occur are more directly influenced by the internal than by the external environment.

That some individuals have an inherent tendency toward one sex and others toward the opposite sex seems highly probable in all the forms studied, for not only do they differ materially as regards the proportion of male and female cells in the immature and male gonads but they also vary in their response to apparently identical environmental influences. In all these respects the observations recorded in this paper for the three species of Crepidula are in harmony.

There is no doubt but that in each of these species of Crepidula stable environmental conditions tend to prolong the male phase of those individuals that are suitably mated and sedentary. Forcibly removing such functional males from their natural associations causes most of them to undergo transition to the female phase several weeks or months earlier than similar males in their normal relations. Removing the animal from its sedentary position compels it to resume active motility for at least some hours and in many cases for several days. This unusual activity may be one of the factors which brings about some change in the animal's metabolism sufficient to initiate the first of the series of interdependent events which lead to a change of sexuality. Even slight changes in the metabolic conditions may conceivably tend to favor the proliferation of the resting ovogonia at the expense of the cells which have been participating in the male phase or may release other stimuli in the body. The animal is prematurely aged in the sense that the sexual phase normally characteristic of an older age appears long before the usual time and when the body may be only half as large as it would otherwise have been at the beginning of the female phase. Change of sex under these conditions is merely the premature realization of the animal's definitive genetic characteristics.

Other environmental changes, such as abnormal temperature or the accumulation of waste or toxic substances are also conducive to the termination of the male phase. The influence of associated individuals is manifest in all three species. The death of an underlying individual of C. fornicata or its removal from its shell often induces a prompt response by the superimposed male (Coe, '35 a). Sexual transformation in Crepidula, like metamorphosis in other animals, can be hastened or retarded experimentally but it cannot be reversed.

SUMMARY

1. In three species of the protandric gastropod Crepidula the initial male phase normally, but not invariably, becomes functional before the transition to the definitive female phase. In exceptional cases the male phase is aborted.

2. Preceding the male phase, association with females or other individuals is usual but is not necessary for the full development of the male characteristics in any of the three species.

3. Two intergrading types of males occur: predominantly feminine individuals which complete the male phase rapidly, and sedentary, masculine individuals which remain associated with or mated with females and which retain the male phase much longer.

4. Isolation or segregation of sedentary males causes most of them to transform to the female phase at a much younger age than that of similar individuals which are allowed to retain their normal associations. This response to a changed environment does not take place, at least in C. fornicata, in the autumn and winter at low temperatures. Removal of an underlying animal from its shell is equally efficacious in inaugurating the change of sexuality in a superimposed male.

5. It is suggested that the differences in the behavior of the two types of males may be due to differing genetic factors as well as to the diversity of environmental conditions, for these differences appear to be correlated with the relative abundance and size of the ovocytes present in the gonads during the functional male phase.

6. The change of sex which individuals in the male phase undergo upon isolation is not strictly 'sex reversal' but only a premature realization of the definitive (female) phase of sexuality which would otherwise have occurred at an older age.

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