The Life-History of Sacculina.

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With 11 Text-figures.

INTRODUCTION.

THE Rhizocephalan parasite Sacculina was first described by Cavolini in 1787, but it was not until Delage's paper appeared in 1884 that a really complete account of the parasite's life-history and structure became available. In his magnificent memoir he gives a detailed description of the histology and development. Two years later (1886), Giard gave some account of the extraordinary effects of the parasite on the secondary sexual characters of the host crab. This subject formed the basis of a memoir by Smith (1906), who at the same time elucidated certain points on the development of the young parasite, and gave an account of the other members of the Rhizocephalidae.

Since Delage was concerned mainly with the embryology and larval development of the parasite, many points in the later life-history remained obscure. Later work on the effects of Sacculina has complicated rather than clarified the matter so that it now becomes necessary to review the life-history of Sacculina, to discuss any anomalies which exist in the literature and to put forward some tentative explanation of the questions which arise therefrom.

In order to do this successfully it is necessary to give first a résumé of Delage's work on the form and life-history of the parasite. This is supplemented by the work of Smith which deals mainly with the effect of the parasite on the host. The points of interest are then stated, and a revised version of the life-history is given stressing these points in the light of further research. Finally the bearing of this new work on the problem of 'parasitic castration' by Sacculina is discussed.

The Form of the Parasite and its Effects on the Host.

Sacculina is a Rhizocephalan parasite of the order Cirripedia, and attacks crabs. It consists of two portions, an internal root-system and a sac-like externa, which is connected to the former by a peduncle attached to the under-surface of the crab's abdomen. The externa thus occupies that space which in the berried female is filled with developing eggs.

The root-system consists of a hypha-like mass of tubes which ramify through the body of the crab and absorb nourishment. The roots are outgrowths from a basal membrane, the internal flattened end of the peduncle or stalk of the externa.

The externa is ovoid in form, yellow in colour, and about as broad as the abdomen of the crab. It is enclosed in a chitinous mantle in which there is a single opening, the mantle aperture, which allows the developing nauplii contained in the mantle cavity to escape. The mantle cavity surrounds a central visceral mass consisting of a nerve ganglion, colleteric glands, and the male and female reproductive organs.

Sacculina causes the degeneration of the gonads in both sexes of the host, and a modification of the secondary sexual characters in the male towards femaleness. Since other cases of parasitic castration among Arthropods have shown that these two results may be obtained separately, the dependence of the secondary sexual characters on the presence of the gonads is a matter of controversy (see R. de la Vaulx, 1922).

Further, G. W. Smith (1906) describes a specimen of Inachus mauritanicus which on recovery from infection possessed a perfect hermaphrodite gland containing ova and sperm. He further states that the secondary sexual characters of the mature female are not affected, but those of the immature female tend to assume the adult form.

Development.—The independent life of Sacculina begins with its emission from the mantle cavity of the externa as a nauplius. During the five or six days of its free existence the nauplius casts several times, the last cast revealing the cypris stage. After three days the cypris larva is ready to begin parasitic life. In all, the free-swimming period lasts about ten days.

During infection the cypris fixes itself by an antenna to a 'hair' on the crab's body. The abdomen and later the thorax of the larva are thrown off, and a mass of embryonic cells passes through the antennal tube and so into the body of the crab.

At this stage there is a break in the continuity of the observations. The tiny mass of cells is next seen on the mid-gut of the crab below the heart. A central tumour is differentiated and this carries the nucleus of the future externa back along the gut towards the abdomen. Meanwhile the young parasite sends out roots which spread over the organs of the host. When the nucleus has reached the definitive position on the abdominal part of the gut the externa is ready to evaginate.

According to Delage (1884), the parasite forces its way to the exterior by compression of the integuments of the crab, which finally necrose under the strain. Once evaginated the young externa grows rapidly, produces two or more batches of larvae, and then, on the approach of winter, falls off, leaving a blackened scar of necrosed tissue where the peduncle of the externa was attached to the under-surface of the crab's abdomen. The roots of the parasite remain alive long after the externa has dropped off. Occasionally the externa persists over the winter, and, if this be the case, the crab becomes feeble and its carapace often bears colonies of barnacles or other marine growths which it has been unable to cast off by ecdysis.

Delage summarizes the life-history according to the season: the nauplii appear in summer and within two weeks infection follows on crabs of 5 to 12 mm. (carapace length ?). Endoparasitic life lasts from twenty to twenty-one months so that the young externae are found in the spring two years later on crabs of 30 to 40 mm. These externae become mature in summer two or three months later and most of the parasites die on the approach of winter.

This, in brief, is the accepted life-history of Sacculina. In this account are several points which call for further discussion. However, it is when the life-history is studied in conjunction with later work on the effects of Sacculina on its host that the most interesting questions arise, and in this connexion the following important paragraph is quoted from Smith, 1906, p. 67:

'A preliminary point must be settled relatively to the time at which the transformation of the secondary sexual characters occurs. The parasite becomes external after establishing its root-system in the host by means of an ecdysis of the latter. It is at this ecdysis that the external transformation due to parasitism invariably occurs, and from this point onward, owing to inhibition of growth, the host does not moult again. In about thirty or forty cases I have observed that this moult has failed to bring the Sacculina to the exterior, so that the phenomenon was observed of the effects of parasitic castration being apparently brought about by a Sacculina interna. But these cases, all of them in large crabs with thick chitinous integuments, were only peculiar exceptions easy of recognition because the Sacculina interna was evidently at a period of development when it ought to have been evaginated. In normal cases, therefore, the effects of parasitic castration are revealed at the same period in the life-history of the parasite, and do not alter again after that period, and it is owing to this fact that the phenomenon of parasitic castration in these crabs affords us so secure and easily appreciable a means of analysing the sexual characters of infected animals, since the cause of castration is always equivalent and the same, so that the difference in effect must be chiefly due to differences inherent in the affected animals.'

The results of the present investigations are presented in the form of a revised life-history of Sacculina. The following important points are stressed.

1. The Size of the Crab when attacked by the Cypris Larva of the Parasite.—According to Delage only small crabs are attacked, but later work has shown that there is no correlation between the age of the parasite and the size of the host.

2. The Duration of Endoparasitic Development.—Delage assumed that the internal development of the parasite lasted twenty-one months, but he gives no records of the statistics that are necessary to confirm this.

3. The Evagination of the Parasite.—Smith's statement that the parasite appears at a moult of the host is generally accepted. Smith, however, gives no evidence in support of this statement, nor does he discuss the observations of Delage, that the parasite evaginates by breaking through the integuments of the host.

4. Modified Crabs without Externae.—A distinct group of these crabs appears at a season when other states of infection are scarce. Smith's account of their origin is not accepted as sufficient.

5. The Fate of Scarred Crabs.—By scarred crabs are meant those crabs which possess a scar of necrosed tissue on the under-surface of the abdomen; such a scar marks the position where an externa has dropped off. Both Smith and Delage agree that the roots of the parasite live long after the externa has fallen off. Smith suggests that the roots are reabsorbed, but records only three modified crabs without internal or external parasites out of 5,000 parasitized specimens.

6. The Degrees of Modification in the Secondary Sexual Characters of Parasitized Male Crabs.—The results of parasitism may vary from no modification to the assumption of complete hermaphrodite characters, but the only reason that is given in the literature to account for this variation is that given in the quotation from Smith above.

Outline of Investigations.

Work on Sacculina was undertaken on three main lines.

(1) Statistical. (2) Experimental. (3) Histological.

(1) Statistical.—The record of a year's infection was obtained by the examination of monthly samples of Portunus holsatus from the Mersey Estuary. The results are set down in Tables I, II, III, and IV.

The crabs were caught by means of a shrimp trawl, samples being obtained at more or less regular intervals throughout the year November 1931 to October 1932. Each sample was counted and measured, and then characters of each crab and external parasite were noted. Measurements were taken by means of a pair of callipers, using a millimetre scale. Finally, the crabs were dissected to find the state of the interna if such were present.

The headings of Table I are self-explanatory. On the left the actual numbers are given, on the right the corresponding

TABLE I.

Statistics of Portunus holsatus from the Mersey Estuary from November 1931 to October 1932.

	Records.						Percentages.				
Date of Sample.	*Total Crabs.*	*Total Crabs under 22 mm.*	*No. of Females Berried.*	*Internal Infection.*	*External Infection.*	*Total Infection.*	*Crabs under 22 mm.*	*Females Berried.*	*Internal Infection.*	*External Infection.*	*Total Infection.*
Nov. 12	143	20	0	48	12	60	14	0	34	8	42
Dec. 17	198	10	0	56	25	81	5	0	28	13	41
Jan. 20	116	43	0	13	7	20	36	0	11	6	17
Feb. 18	94	40	0	9	7	16	43	0	9	8	17
Mar. 20	57	6	3	2	16	18	11	20	4	28	32
Apr. 13	166	17	2	31	46	77	12	3	19	27	46
May 4	255	11	15	36	150	186	4	12	14	59	73
June 14	150	10	0	10	10	20	7	0	7	6	13
July 5	96	0	0	10	2	12	0	0	11	2	13
Aug. 9	128	6	0	8	11	19	5	0	7	8	15
Sept. 19	113	10	0	41	2	43	9	0	36	2	38
Oct. 10	34	5	0	14	1	15	15	0	41	3	44

percentages are worked out. The numbers of soft-shelled crabs are not included in the table because the softness of a shell is a difficult property to define and to compare, but it may be said that the greatest number of soft crabs were obtained in June, July, and August.

In Table II the percentages of infection are further analysed. The records given in Table I under 'Percentage of Internal Infection' are divided into small internae and large internae. The division is arbitrary, but in general 'Small Internae' refer to internae migrantes, a state of endoparasitic existence where the 'nucleus' is growing along the gut. 'Large Internae' refer to

TABLE II.

Analysis of the Percentage of Infection of Portunus holsatus from the Mersey Estuary during the year November 1931 to October 1932.

Date of Sample.	*Small Internae.*	*Large Internae.*	*Small Externae.*	*Large Externae.*	*Modified Males without Externae.*	*Scarred Crabs.*	*Total Infection.*
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Nov. 17	24	10	6	2	0	0	42
Dec. 17	18	10	10	3	0	0	41
Jan. 20	7	4	3	4	0	0	17
Feb. 18	7	3	6	1	0	0	17
Mar. 20	2	2	9	19	0	1	32
Apr. 13	8	9	8	17	1	3	46
May 4	7	6	17	39	1	3	73
June 14	1	2	3	3	3	1	13
July 5	8	1	0	1	2	1	13
Aug. 9	5	1	1	6	1	1	15
Sept. 19	30	5	2	0	1	0	38
Oct. 8	38	3	3	0	0	0	44

internae with the nucleus in the definitive position in the abdomen. In the same way, 'Percentage of External Infection' from Table I is divided into small externae and large externae. 'Small Externae' refer to immature parasites less than 12 mm. in length and 'Large Externae' to adult parasites of 12 mm. and over.

Two more columns have been added. 'Scarred crabs' refer to those that have lost the external portion of the parasite. In Table I these are included among external infections. Again under 'Modified Crabs without Externae' are placed those crabs which show modification due to Sacculina, but in which the parasite is in an internal stage of development. These were included under 'Internal Infections' in Table I.

Table III shows the different stages in the life-history of the parasite in relation to the size of the crab. The total number of crabs in the different millimetre-length groups are given and the percentage of infection is worked out for each group.

(2) Experimental.—The analysis of these statistics suggested certain experiments which were carried out at Plymouth.

TABLE III.

Distribution of the Developmental Stages of Sacculina. in Crabs of Various Sizes.

Carapace Length (mm.).	*Small Internae.*	*Large Internae.*	*Small Externae.*	*Large Externae.*	*Scarred Crabs.*	*Highly Modified Males (no Externa).*	*Total Crabs.*	*Total Infection.*	*Per cent. Total Infection.*
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
13	..	..	1	..	..	..	4	1	25
14	..	..	..	..	..	..	3	..	0
15	..	..	..	..	..	..	4	..	0
16	..	1	..	1	..	..	7	2	29
17	1	..	..	..	..	..	11	1	9
18	1	..	2	..	..	..	20	3	15
19	3	..	..	1	..	..	30	4	13
20	2	1	1	1	..	..	45	5	11
21	4	3	3	2	..	..	56	12	21
22	3	1	3	2	..	..	35	9	26
23	6	2	7	4	..	..	68	19	28
24	7	4	6	6	..	..	88	23	26
25	8	3	6	8	1	..	88	26	30
26	8	6	6	7	1	..	99	28	29
27	8	6	8	10	1	..	120	33	28
28	15	6	11	17	1	..	124	50	40
29	16	14	15	12	3	..	140	60	43
30	11	12	13	16	0	1	130	51	39
31	13	12	8	9	1	1	93	44	47
32	14	9	9	2	2	1	67	37	55
33	15	4	4	6	2	1	66	32	49
34	15	5	9	5	1	3	70	38	54
35	8	4	3	6	2	..	50	23	46
36	9	3	4	5	1	1	46	23	50
37	6	8	4	1	..	..	29	19	66
38	5	3	1	2	..	..	26	11	42
39	4	1	..	1	..	1	10	7	70
40	1	..	2	1	..	..	6	4	67

(3) Histological.—Various stages in the life-history of the parasite were examined by means of serial sections.

The Revised Life-History of Sacculina.

The life-history of the parasite may be divided into three stages:

(a) The free-swimming stage.
(b) Endoparasitic development.
(c) The Sacculina externa.

The Free-Swimming Stage and Infection.

Since many references will be made to Delage's valuable memoir on the life-history and development of Sacculina, it is necessary to point out in the beginning that the parasite which Delage worked on was Sacculina carcini Thompson, infecting Carcinus moenas Pennant. His material was obtained from Roscoff. The present work deals with Sacculina carcini parasitic on Portunus holsatus Leach. The material was obtained from the Mersey Estuary. In the classification of the parasite I am following Boschma (1928) with whose findings mine are in complete accordance.

It is important to notice that Delage considered that the events in the life-history were correlated with the seasons. In a summary he shows that ripe externae and nauplii are numerous in June, July, and August; very young internae are numerous in September, large internae in March and April, small externae in May and June, large externae again in June, July, and August, and scarred crabs in September and October. In the case of Portunus holsatus from the Mersey area it has been found that these sequences take place three months earlier.

Some other correlation must therefore be sought. As is to be expected it was found that the life-history of the parasite is closely correlated with the life-history of its host. Thus Delage's seasonal table which is true for Carcinus moenas from Roscoff does not hold for Portunus holsatus from the Mersey, but in both cases the nauplii of the parasite appear a little earlier than the zoëa, and young internae become evident when the ovaries of uninfected female crabs are beginning to ripen. The growing parasite in the female is thus able to absorb nourishment that would normally be stored as yolk.

Smith (1912) found that infected male crabs produce a yolky substance which he supposed is absorbed by the parasites.

As shown in Table II the majority of ripe externae are found in March, April, and May. The period of free-swimming existence of the larvae has been shown by Delage to be ten days, after which the cypris must find a host or die.

The first question that must be considered is the size of the crab when attacked by the parasite. Since it is impossible to trace the mass of embryonic cells from the cypris till it comes to rest on the mid-gut of the crab, it is impossible to ascertain whether a parasite in such a stage is present or not. Once the young interna starts to grow it becomes visible by fine dissection. According to Delage, infection occurs when the crab is very young. He says, p. 594: 'Les plus jeunes Sacculines internes se trouvent toujours sur les plus petits Crabes.' Again on p. 677 he states that in general young internae are found in Carcinus of 5 to 12 mm., large internae in crabs of 20 to 25 mm., and externae on crabs of 30 to 40 mm. He previously admits, however, that he kept no record of the number of internae found or of the size of the infected crabs. In the present investigation this has been done, and Table III shows that small internae may be present on Portunus of all sizes and that there is no correlation between the size of the crab and the state of development of the parasite.

The question of infection may be approached from another standpoint. The larvae appear at a time when the uninfected female crabs are in berry (see Table I). Delage has proved that the cypris larva of the parasite does not attack the crab in its larval stages. Thus the larval Sacculina produced at this time must attack the crabs produced in some previous year. It is inferred from the samples examined that Portunus holsatus has attained a mean carapace length of 22 mm. at the end of its first year, and since the percentage of infection in crabs under this size is small and suddenly increases at this point, it is concluded that the larvae of the parasite, produced each year in spring, attack the crabs that were hatched in the previous summer.

Table III (column 10) also shows that the percentage of infection increases among larger and larger crabs. It would thus seem that crabs which escape infection at the end of their first year may be infected during their second or third years, thus augmenting the number of parasitized individuals. The second increase in the percentage of infected crabs of 28 mm. carapace length seems to indicate that crabs of this size and over have had a second chance of being infected.

The Sacculina Interna.

As shown in Table II, column 1, the majority of young internae do not become evident to dissection till September or October, when they form a large proportion of the infected population. During the winter the interna migrans grows along the gut and establishes the nucleus in the definitive position. By the end of the winter the number of young externae begins to increase. (The sudden drop in the percentage of infection to 17 per cent. in January and February is due to the large proportion of immature crabs in these samples [see Table I]. More than half of the total number of crabs under 22 mm. carapace length were obtained at this time, and among these, the percentage of infection is less than 9 per cent.; the infection among the total population being 35 per cent. The minimum percentage of infection in mid-summer cannot be ascribed to the same cause as very few immature crabs were captured at that time.) The percentage of external infection reaches a maximum in May and the majority of externae are then mature and are producing nauplii. Most of the adult externae then die and by July the percentage of infection has dropped to a minimum which remains stable throughout the summer months till the next series of young internae become visible in September or October.

From these records it appears that the endoparasitic life of Sacculina lasts only nine months and not one year and nine months as postulated by Delage, who considered this the length of time necessary to account for the supposed correlation between the development of the parasite and the size of the host.

The endoparasitic development of Sacculina infecting Portunus holsatus from the Mersey area may be summarized thus: infection takes place in spring, the interna remains invisible within the crab during summer, becomes evident in autumn, grows rapidly throughout the winter, and the externa appears early in the following spring. It is noteworthy that the time of maximum growth (winter) coincides with the time when quantities of yolk are being stored in the ovaries of uninfected females. Smith (1912) found that infected male crabs also produce a yolky substance and suggests that as the parasite is continually absorbing this, the metabolism of its host is changed to the female type.

Evagination of the Parasite.

It has been shown in Tables I and II that the majority of parasites are evaginated between February and May, and according to the accepted theory of Smith, this evagination must be preceded by a moult of the host. But it has also been found that the majority of large uninfected crabs moult in summer. Again, if the externa appears at a moult, the youngest external parasites should be found on soft-shelled crabs. Such is not the case. On the contrary, it is a striking fact that very young externae have never been found on soft-shelled crabs though at least a dozen soft-shelled crabs with large internae have been noted. Delage's memoir may here be quoted: 'Le 29 avril 1883 je capture un Crabe de 31 millimètres prêt à muer. A travers ses intéguments j'aperçois une Sacculine interne. J'enlève la carapace et sous la nouvelle bien formée et molle, la Sacculine se voit encore interne. Elle ne sortira que plus tard, sans utiliser le moment où la carapace encore molle, offre peu de résistance.'

The parasite then does not depend on a moult of its host to evaginate. To test this an experiment was undertaken at the Plymouth Marine Laboratory. The crabs used were Carcinus moenas, first because the infected specimens of this species are easily obtainable at Plymouth and secondly because Carcinus is the best suited to withstand laboratory conditions.

Experiment 1.—From a sample of Carcinus collected in spring ten hard-shelled crabs without externae were selected and kept in large glass jars under sea-water taps. For a week they were fed and examined daily. On the sixth day a pale circular area appeared on the under-surface of the abdomen of a crab of 27 mm. carapace length. By the following morning the chitinous integuments covering this area had fallen off, and a pimple of white tissue filled the space. Within six hours the young externa was fully evaginated, and within four days had attained a breadth of 4 mm. As none of the other crabs showed signs of producing an externa the experiment was discontinued. Unfortunately I neglected to dissect the remaining nine crabs to find whether internae were present or not, but this does not affect the results of the experiment, which are confirmed by experiment 2 (see p. 564).

It would seem, then, that the evagination of the parasite does not take place at an ecdysis of the host. This striking fact may be explained in the following way. Delage observed that the parasite becomes external by compressing the integuments of the crab's abdomen, which then atrophy and finally necrose, allowing the externa to appear. It has also been shown by Elmhirst (1923) that at ecdysis the lobster absorbs a large amount of water, and it is the pressure of this water which causes the new shell to swell out before the lime salts are laid down and the chitin hardens. Any pressure exerted by the Sacculina at this time would probably have the same effect and the soft integuments would merely stretch before it.

The Externa.

It has been shown by Delage (1884) and Smith (1906) that soon after the externa appears it is surrounded by cypris larvae which attach themselves to the mantle opening. These are the so-called complemental males, though as Smith has shown, they take no part in fertilization; the parasite is completely self-fertile and the sperm presumably reach the oviducts by means of the mantle cavity while the chitin in the vasa deferentia and atrium is still in a semi-fluid state.

The externa grows rapidly; within six weeks or two months it has reached a breadth of 12 mm. and may produce its first batch of nauplii. The larvae are expelled by rhythmical contractions of the mantle, which alternately expands with the intake of water and then contracts, forcing out water and larvae through the mantle aperture. While this process is going on, new chitin is formed below the old lining of the mantle cavity, and when the last of the nauplii have been extruded, the old lining is moulted and forced through the mantle aperture. Self-fertilization again takes place and a little later the lining of the colleteric glands is everted to form the branching ovigerous tubules which fill the mantle cavity and are themselves filled with developing ova.

Experiments were initiated to find out the length of time between the expulsion of one batch of nauplii and the next, and though these experiments were unfortunately not completed, it was evident that under laboratory conditions the interval is greater than three weeks. Delage suggests that the period is about six weeks and the examination of the samples from the Mersey area indicated that the externa is able to retain the larvae for some considerable time should conditions not be suitable for the survival of the larvae.

After the last batch of larvae have been extruded, the mantle wrinkles up, the externa falls off, and finally the peduncle alone remains as a blackened scar. The method whereby the externa is caused to fall off is not known, but that it does so is certain. In a few cases the externa does not drop off and the crabs concerned are easily identifiable. The parasite is wrinkled, the crab is sluggish, and marine growths such as barnacles, algae, hydroids, or serpulid worms are often attached to the shell which the crab cannot cast because of the rivet-like effect of the Sacculina externa which attaches the tissues of the abdomen to the shell.

The falling off of the externa is apparently the end of the parasite, but a number of very interesting questions now arise. What is the fate of these scarred crabs? Many of them undoubtedly die. Smith suggests that many of them finally recover, so that the only indication of the parasite's past existence is the modification of the host's shell which may also disappear at a future ecdysis. Smith noted only three such recovered crabs in a collection of 5,000 parasitized specimens of Inachus mauritanicus from Naples. In the present collection of 2,000 specimens of Portunus holsatus from the Mersey area, not one modified crab without a living parasite was found.

As has been quoted on p. 552 Smith also found modified crabs without externae but with abnormal internae. He describes these as being large crabs with thick chitinous integuments and the parasites as being at a stage when they should normally have been evaginated. He suggests that these cases are due to the parasites not availing themselves of the moult at which they normally evaginate. While my observations on Portunus holsatus differ from those of Smith on Inachus mauritanicus, a number of parasitized male crabs have been found all showing a maximum degree of modification without an externa. As a more complete description of these crabs and their parasites will be given later, it is sufficient to mention here that most of the crabs were soft and in all a large root-system was present with a nucleus in some stage of internal development. Moreover, in two of these crabs degenerate roots were found as well as living ones. Reference to Table II shows that these crabs occur in mid-summer after the majority of externae have disappeared and before the next series of young internae are large enough to be found by dissection.

These facts may be a clue to a further stage in the life-history of the parasite. Smith has already expressed the opinion that individuals of a colony of the Rhizocephalan parasite Thylacoplethus may be derived from a single cypris larva by a process of budding at an early stage of endoparasitic existence. Thompsonia shows further development on the same lines. It has been shown by both Smith and Delage that Sacculina exhibits polyembryony, a process whereby two externae are formed from a single young interna. Smith (1906, fig. 10, Pl. 6) shows two 'nuclei' developing in a single tumour, and Pérez and Eliane Basse (1928, text-fig. 1), show an incomplete twin externa derived from such a tumour. Smith's observations of the internal stage have been confirmed in the present work, but although two or more separate externae are often found on one host, it is difficult to prove that they arise from the same root-system.

From a consideration of these facts it seems possible to explain the presence of living roots in scarred crabs, the anomalous group of highly modified crabs without externae, the absence of 'recovered' modified crabs and the difference in the degrees of modification due to parasitism by assuming that when the externa has dropped off, the living roots begin to regenerate a new interna. The scarred crab freed from the externa would be able to cast, and the new shell formed at this moult would show a maximum degree of modification due to parasitism. Within would be the new interna which emerges as a normal externa in due course.

To test this assumption, a second experiment was undertaken at Plymouth. The crabs used were again Carcinus moenas.

Experiment 2.—Four parasitized male crabs were selected and put into separate large glass jars under sea-water taps. The experiment was begun on September 28, 1932, and when the writer left on October 8, the experiment was left under the care of Mr. D. P. Wilson, to whom I am much indebted. The crabs were fed each day on mussel or squid, and were examined from time to time. The condition may be formulated thus:

Scarred Crab A.—Carapace length 32 mm. The normally fused third, fourth, and fifth abdominal segments were divided. Carapace brown, bearing a serpulid tube.

Scarred Crab B.—Carapace length 33 mm. Third, fourth, and fifth abdominal segments divided. Carapace brown, bearing a barnacle.

Modified Crab C.—Carapace length 31 mm. Third, fourth, and fifth abdominal segments divided. Carapace hard, green, and fresh.

Modified Crab D.—Carapace length 38 mm. Third, fourth, and fifth abdominal segments divided. Carapace hard, green, and fresh.

The experiment was discontinued on March 21, 1933, and the results are formulated below.

Scarred Crab A.—Cast on November 28 after two months in captivity. New shell showed a broadened and fully divided abdomen. Sections of the abdomen showed that the necrosed tissue of the externa had not been completely removed and that many of the roots were still alive. Shrunken roots which seemed to be in a process of absorption by the crab were present. The crab grew very little at the moult, the increase being from 32 to 34 mm. in carapace length.

Scarred Crab B.—Died on March 21, 1933, after six months in captivity. Sections showed that a considerable portion of the parasite's roots had necrosed and turned black. There was no evidence of regeneration in this case.

Modified Crab C.—An externa appeared during February of 1933 after the crab had been five months in captivity, during which time it had not moulted. The young externa grew slowly. Sections showed the roots normal.

Modified Crab D.—An externa appeared on October 6 after the crab had been a week in captivity. The externa grew slowly and died about the beginning of January. Sections showed that necrosis was slight, and had been cut off from the healthy tissue by a layer of chitin.

Critique of the Experiment.

Neither the crabs nor the parasites grew well under laboratory conditions, but the results from the modified crabs were satisfactory. The very slight increase in size of the new carapace of Crab A may indicate either that the crab was very feeble or that Sacculina retards normal growth, since the increase in carapace length of a crab of 32 mm. should be from 5 to 7 mm. Again, the fact that both the externae grew very slowly and that one died indicates that the environment was not suitable for the parasites. The results from the scarred crabs are not conclusive, due, possibly, to the age of the crabs used and the effect of laboratory conditions. Unfortunately the fact that crabs bearing barnacles and serpulid tubes have not much vitality was not realized at the time when the experiment was undertaken. It was thought that such crabs would cast within a short time as their shells were obviously old.

Nevertheless five points are clear from the experiment.

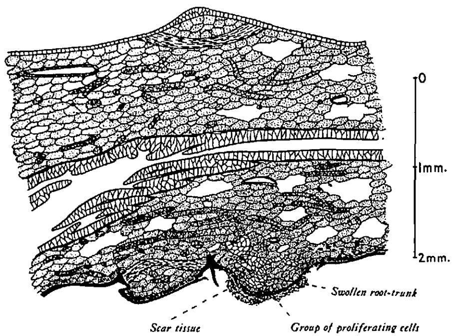
(1) The roots of a parasite may live for six months after the parasite has fallen off. (Scarred Crab A.)

(2) A parasitized crab once freed from the externa is able to cast. (Scarred Crab A.)

(3) The parasite may die at this stage of its life-history. (Scarred Crab B.)

(4) The results of the previous experiment are confirmed, i.e. the evagination of the parasite does not depend on the ecdysis of the host. (Modified Crabs C and D.)

(5) Geoffrey Smith's explanation of the existence of the group



TEXT-FIG. 1.

Longitudinal section through the abdomen of a 'scarred' Portunus holsatus showing necrosed tissue cut off by chitin and a highly vacuolated root of the parasite with budding cells. The tissues of the crab are represented diagramatically.

of modified crabs may now be questioned. He says in his monograph, p. 67: 'In about thirty or forty cases I have observed that this moult (the critical moult) has failed to bring the Sacculina to the exterior, so that the phenomenon was observed of the effects of parasitic castration being apparently brought about by a Sacculina interna.' If he means that the modification has been caused by a parasite which will never evaginate he is wrong, for as has been shown by modified crabs C and D, these crabs produce externae in due course.

Since the question of regeneration remained unsettled, and further experiments at Plymouth were unfortunately impossible, the work was continued in another direction. Specimens of

Portunus holsatus from the monthly samples already recorded were used for obtaining serial sections. First the scarred crabs were examined and then the group of modified crabs without externae.

The Examination of Scarred Crabs by means of Serial Sections.

The material available was not plentiful. The abdomens of six were sectioned. From these it is possible to state the following results without going into histological detail.

(*a*) In two cases the necrosis was not entirely confined to the basal membrane, i.e. to the flattened internal end of the peduncle which gives rise to the root-system. Even in these cases the necrosed tissue was partly separated from the living by a layer of chitin.

(*b*) In the other cases the necrosed tissue was completely cut off by chitin and but little remained. The main trunk of the root-system was swollen and highly vacuolated.

(*c*) A later stage showed that several cells had been budded off from the cellular lining of the enlarged root. A longitudinal section of the abdomen of this crab is given in Text-fig. 1, and an enlarged view of the budding cells within the swollen root is given in Text-fig. 2.

The Examination of Modified Crabs without Externae by Means of Serial Sections.

A larger amount of this material was available, and when investigated it was found that:

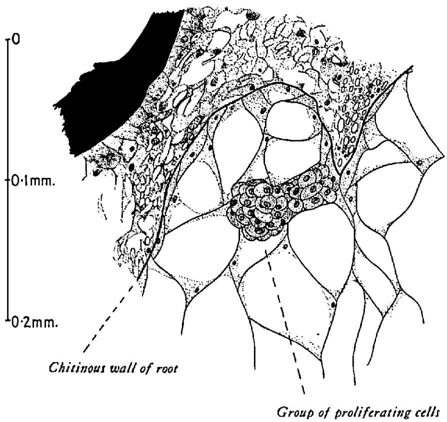
(1) In all cases a parasite is present.

(2) The central tumour or enlarged part of the root-trunk which contains the nucleus of the future externa is always in the definitive position and the root-system is always fully formed.

(3) Though a tumour is always present a nucleus is not always identifiable within it. When a nucleus is not present one or more groups of cells are found, such as have been

remarked in the swollen root-trunk of a healthy scarred crab (see Text-fig. 3).

(4) The nucleus may be in a stage of development which is normally found in tumours of internae migrantes, i.e. in tumours which are growing along the gut but have not yet reached the definitive position. Thus the perisomatic cavity may not be

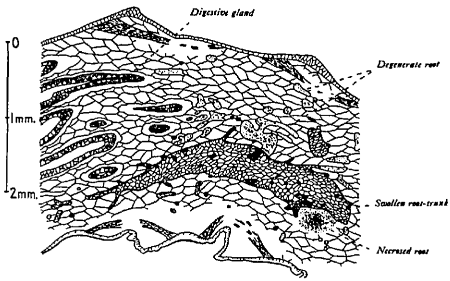


TEXT-FIG. 2.

Portion of Text-fig. 1 enlarged, showing the structure of the vacuolated root and the rapidly growing mass of cells within it.

present, and the organs of the interna may not be established (see Text-figs. 4 and 5). In normal internae migrantes the tumour is small. In normal tumours in the definitive position, large and conspicuous 'nuclei' are present and the external epithelium of the tumour has been folded in to form part of the epithelial lining of the perisomatic cavity (see Text-fig. 6). The tumours from this group of modified crabs may combine the characters of both. The tumour is large, the nucleus is small and lies close to the epithelium. There is as yet no indication of the folding in of the epithelium to form the perisomatic cavity (see Text-fig. 5). This abnormal development of the nucleus, considered in conjunction with the complexity of the root-system, would seem to indicate that the nucleus had not been formed within the tumour of an interna migrans; it is being formed by a tumour already established in the definitive position and fed by an adult root-system.

(5) Later stages were found in which the nucleus had reached



TEXT-FIG. 3.

Longitudinal section through the abdomen of a modified and soft-shelled male Portunus holsatus showing a swollen root with necrosed roots, degenerate roots, and living roots.

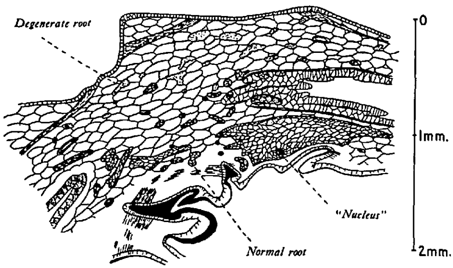
a stage of development normal to tumours in the definitive position. They were thus indistinguishable from normal large internae. These form the majority of the specimens.

The Process of Regeneration.

If it be admitted that the regeneration of the externa does take place, and the evidence seems to indicate that it does, the observations may best be explained as follows.

After the externa has dropped off, necrosis of the exposed surface begins. If the parasite is old or its roots are unhealthy, necrosis proceeds and finally that part of the root-system which lies within the carapace of the crab degenerates and is absorbed by the host. If the crab is also enfeebled by age or long-continued parasitism, necrosis proceeds so rapidly that both host and parasite die. Many parasitized crabs, however, are quite as vigorous as unparasitized ones, and it is in such crabs that regeneration is most likely to occur.

The necrosed tissue is separated off by a layer of chitin, and the tissue below remains healthy (Text-fig. 1). Presumably the roots of the parasite are still absorbing some nourishment and this collects in the blind end of the root-trunk. As there is no



TEXT-FIG. 4.

Longitudinal section through the abdomen of a modified male Portunus holsatus showing a regenerate interna with living roots and a few degenerate roots.

outlet this part becomes turgid, the vacuoles in the spider-cells become enlarged and the result is a highly vacuolated root, a condition similar to that in the central tumour. Since the root-system grows without symmetry and throughout the life of the individual, it is probable that all the cells lining the cavity have potentialities of growth. One or more begin to divide and the resulting groups are budded off into the cavity.

The groups of cells within the cavity of this new tumour grow rapidly until one dominates the others and becomes the new nucleus. This, being developed in situ, would appear peculiar in the early stages by reason of its small size relative to the position of the tumour, and its undeveloped state relative to the position of the tumour on the gut. In later stages it would be indistinguishable from a normal or primary interna.

The host, meanwhile, freed from the attachment of its tissues to the abdominal part of its shell by the riveting action of the externa and from the drain which the parasite causes, is able to cast. The scar of necrosed tissue is removed at ecdysis, and at first the maximum effect of parasitism will be seen on a crab that has not an externa. This latter is developed shortly afterwards.

Subsequent to the completion of this work, evidence was obtained showing that regeneration does actually take place in two other members of the Rhizocephala.

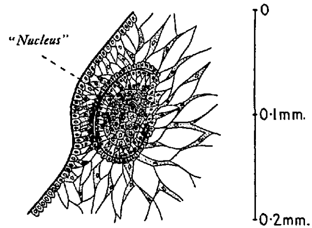
I am indebted to Professor E. S. Goodrich, the editor of this journal, for bringing to my notice the work of Pérez on Peltogaster (Chlorogaster) sulcatus. By careful dissections, Pérez has shown that this gregarious parasite undergoes periodic regenerations of the externae. In his earlier paper (Pérez, 1928) he pointed out that the numerous externae on a single host are all of the same age, and that they each produce a single batch of nauplii and then drop off. The host then moults and another set of externae appears in the same positions. These new externae are formed as buds from a deeply seated part of the original root-system. The exact formation of these buds is to be found in Pérez's later papers (Pérez, 1931 and 1931 *a*).

An independent confirmation of Pérez's work must be noted. Mr. J. D. Macdonald, working at Plymouth on the development of the Paguridae, collected several specimens of Anapagurus laevis infected with Peltogaster (Chlorogaster?) socialis. The writer, being at that time unfamiliar with Pérez's work, suggested that these be kept in aquaria to see whether any regeneration took place similar to that in Sacculina. From preliminary observations (for the use of which the writer is indebted to Mr. Macdonald) it is obvious that a process of regeneration takes place similar to that described by Pérez.

The Effects of Parasitism on Portunus holsatus.

In this paper the effects of the parasite on male crabs only are dealt with. In his monograph, Smith (1906) notes also the effects on mature and immature females. No change was found in the secondary sexual characters of mature females of Portunus holsatus. Smith's observations on immature females have not been repeated as no externa was found on an immature female, and only a very few have been found on immature males. The reason for the comparative absence of parasites on immature crabs has already been discussed (p. 558).

The results of infection on Portunus are in no case as great as those on Inachus. This lesser degree of modification



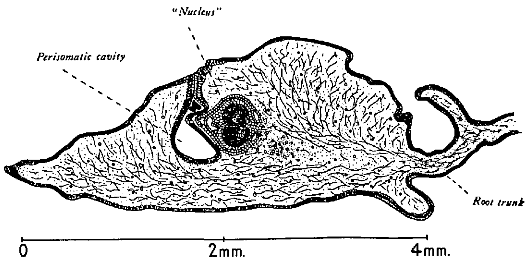
TEXT-FIG. 5.

Portion of Text-fig. 4 enlarged, showing the structure of the nucleus.

also holds in the case of Carcinus as has been shown by Potts (1909), and is probably correlated with the smaller differences between the secondary sexual characters of the two sexes of both Carcinus and Portunus. The amount of change effected by the parasite would thus seem to be in proportion to the differences existing in the normal males and females.

The obvious changes in infected males of both Portunus and Carcinus are the redivision of the fused third, fourth, and fifth abdominal segments, the broadening of the abdomen and the lengthening of the hairs fringing the sides of the abdomen. Measurements of the chelae of male and female specimens of both species show that the right chela of the male is slightly larger than that of the female, and that in both sexes the right chela is slightly larger than the left. The differences between the sexes proved too small to show whether the parasite causes any modification in the male towards femaleness in this character.

Potts (1909) states that parasitized male Carcinus exhibit modifications of the abdomen that may arbitrarily be divided into categories. He measures the degree of modification by



TEXT-FIG. 6.

Longitudinal section through an interna migrans which has almost reached the 'definitive position'. The tumour has been dissected out of the connective tissue on the dorsal surface of the posterior gut. × 28.

estimating the ratio: $\frac{\text{Breadth of fourth abdominal segment.}}{\text{Length of abdomen.}}$

Quite independently a somewhat similar method of measuring the degree of modification was developed in the present work. The ratio is: $\frac{\text{Breadth of fourth abdominal segment.}}{\text{Breadth of carapace.}}$ Later work on Carcinus moenas indicates that the different abdominal segments are modified to different amounts. Sufficient has been done to show that the increase in breadth of the sixth segment is greater than that of the fourth. Since it has been shown by Smith that the length of the abdomen in the case of infected males of Inachus mauritanicus is affected by Sacculina, the use of the carapace breadth as a standard is more liable to give comparable results.

It was found that the increase in breadth of the abdomen takes place in co-ordination with the redivision of the third, fourth, and fifth abdominal segments. This provides an easy

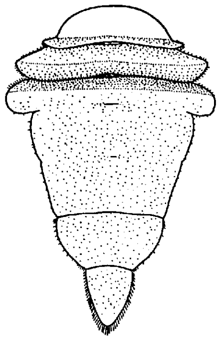


Fig. 7.

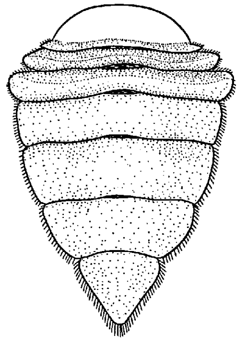


Fig. 9.

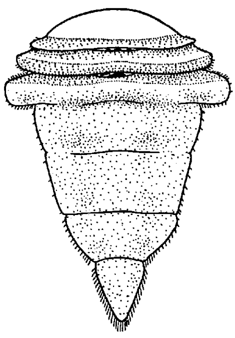


Fig. 8.

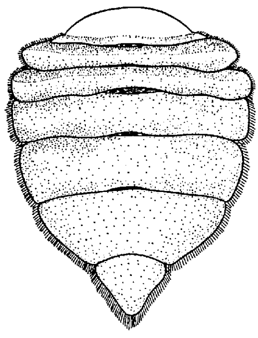


Fig. 10.

TEXT-FIGS. 7–10.

Dorsal view of the abdomina of normal and parasitized specimens of Portunus holsatus. × 2½.
Fig. 7.—Normal male.
Fig. 8.—Parasitized male, Group II.
Fig. 9.—Parasitized male, Group III.
Fig. 10.—Normal female.

and natural method of grouping parasitized males according to the degree of modification.

Group I.—Males showing no redivision of the third, fourth, and fifth abdominal segments.

Group II.—Males showing partial redivision of these segments.

Group III.—Males showing complete redivision of these segments so that the abdomens of this group of parasitized males resemble, in segmentation at least, the condition found in normal females.

Specimens of male and female abdomens as well as the three groups of parasitized males are shown in Text-figs. 7, 8, 9, and 10.

Explanation of Table IV.

Table IV is a statistical record of the results of infection of male Portunus holsatus from the Mersey Estuary by Sacculina. All measurements were taken by means of a pair of callipers using a millimetre scale.

Column 1 gives the carapace length in millimetres. The measurement is taken from the tip of the central tooth on the anterior margin of the carapace to the middle of the posterior margin of the carapace.

Column 2 gives the average carapace breadth corresponding to each carapace-length group. The carapace breadth is the distance in millimetres between the tips of the two posterior spines on the lateral margins of the carapace.

Column 3 gives the average abdominal breadth of normal males, for each millimetre-length group of crabs. The male abdomen is broadest at the third segment, but from inspection it was suspected that the fourth segment shows greater increase in size due to parasitism. Accordingly the breadth of this segment was measured, and since the sides of this segment taper distally, the broadest portion was always measured.

Columns 4, 5, and 6.—In these columns the externally parasitized males are divided into three groups according to the extent of redivision of the abdominal segments. In sub-column A of each column, the number of individuals is given for each carapace-length group. In sub-column B the average

TABLE IV.

The Modification of the abdomen of male Portunus holsatus.

1	2	3	4A	4B	5A	5B	6A	6B	7
			Group I (No Division).		*Group II (Partial Division).*		*Group III (Complete. Division).*		
Carapace Length (mm.).	*Average Carapace Breadth (mm.).*	*Average Abdominal Breadth. Normal Males (mm.).*	*Number.*	*Average of B_4 (mm.).*	*Number.*	*Average of B_4 (mm.).*	*Number.*	*Average of B_4 (mm.).*	*Average Abdominal Breadth. Normal Females (mm.).*
13	16·0	..	..	..	..	..	1	5·5	..
14	17·0	..	..	..	..	..	..	..	..
15	18·5	..	..	..	..	..	..	..	..
16	19·5	..	..	..	..	..	1	6·7	..
17	20·7	..	..	..	..	..	..	..	7·7
18	22·0	6·5	..	..	1	7·0	1	8·0	8·8
19	23·6	6·9	..	..	..	..	1	9·0	9·3
20	24·9	7·1	1	7·0	1	8·0	1	8·7	10·1
21	26·1	7·7	..	..	..	..	5.	9·1	10·6
22	27·4	7·9	..	..	..	..	2	9·0	11·0
23	29·0	8·2	3	8·2	1	8·9	5	10·4	11·9
24	30·4	8·5	3	8·8	3	9·7	2	9·6	12·4
25	31·7	8·9	3	9·2	2	9·5	2	11·5	13·2
26	32·8	9·4	..	..	1	10·0	3	11·6	14·0
27	34·2	9·8	4	9·9	3	10·5	5	11·5	14·6
28	35·3	9·9	3	10·3	5	11·0	2	12·0	15·0
29	36·9	10·4	1	10·3	4	11·2	4	11·9	15·6
30	38·2	10·6	5	10·9	3	11·3	3	11·9	16·5
31	39·5	10·9	4	11·0	1	12·0	2	12·4	17·2
32	41·0	11·1	6	11·5	2	12·7	..	..	17·4
33	42·2	11·8	6	12·0	2	13·0	1	13·0	17·8
34	43·6	12·1	10	12·1	..	..	5	13·6	..
35	45·3	12·3	8	12·5	3	13·3	1	14·0	..
36	47·1	12·5	7	12·9	4	13·2	..	..	..
37	47·6	13·1	3	13·1	1	14·0	..	..	..
38	48·6	13·5	1	13·5	..	..	..	..	..
39	50·1	14·0	1	14·0	..	..	..	..	..
40	52·2	..	2	14·0	1	15·0	..	..	..

breadth of the fourth abdominal segment—B_4—is given in each case. Thus, in column 4, three crabs were obtained which measured 23 mm. in carapace length; the average abdominal breadth of these three crabs is 8·2 mm.

Column 7 gives the average abdominal breadth of normal females for comparison. The segment measured was the fourth.

The Percentage of Infection.

From Table III p. 556, it is evident that the percentage of infection increases among larger (or older) crabs. As there is no reason to suppose that older crabs are more liable to attack by cypris larvae than are young ones, this increase in the percentage of infection with increase of age must be due to the possibility of infection being repeated each year of the crab's life. This has already been pointed out (p. 559), but it is not the whole story, for if infected crabs die a year after they have been attacked by cypris larvae, the percentage of infection should remain the same in all crabs over a year old. To explain these records it is necessary to assume that the parasite lives in the body of the crab for more than one year, and in a form that is visible to dissection. Whether the period between attack by the cypris larva and the first appearance of the young interna lasts three months, or a year and three months, will not affect the record of the year's infection.

It has already been shown that the life-history up to the production of the nauplii by the adult externa is almost certainly complete within a year, and that thereafter most of the externae disappear. The most satisfactory manner to account for the prolongation of the parasite's life and the consequent increase of infection among older crabs is by the theory of regeneration.

The Number of Individuals in Groups I, II, and III.

The numbers in each group are too small to graph satisfactorily, but it has been pointed out to me by Dr. R. J. Daniel that in such cases the approximate position of the modal points is obtainable by finding what he calls the shortest half range, i.e. by finding the minimum number of size groups which contain 50 per cent. of the population. In Group I the shortest half range lies between 31 and 37 mm. carapace length; in

Group II, between 26 and 31 mm. carapace length, and in Group III between 21 and 27 mm. carapace length.

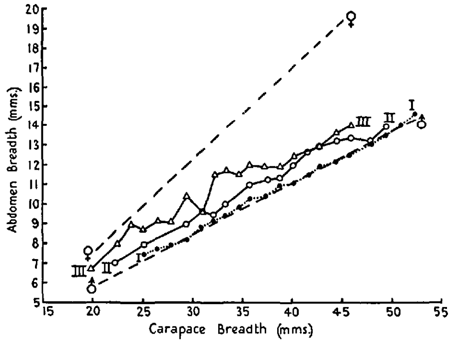
This shows quite clearly that among externally parasitized crabs (these being the only ones dealt with in columns 4, 5, and 6) the smaller the crab the greater the liability to be modified to a maximum extent. This is not the same as saying that the smaller the crab the greater the degree of modification, for it has been shown in Table IV that a few large parasitized crabs also fall into Group III. It will be shown later (p. 580) that the maximum amount of modification possible decreases among older (or larger) crabs.

These two points, and the unsuspected difference between them, has been a fruitful basis for controversy. Giard (1886) found that in general smaller crabs were the more modified. The work of Guérin-Ganivet (1911) confirmed this. Potts (1909) did not find this phenomenon in either Carcinus or Eupagurus. Nilsson-Cantell (1927) found no correlation between the size of the host and the extent of modification, for he says, and rightly, that maximum effects are found in large as well as in small hosts.

This liability of small crabs to be modified to a maximum extent may be due to the following cause. Young crabs cast more frequently than old ones (Pearson, 1908). If a crab is infected when young, it will be able to cast more often during the nine months prior to the appearance of the externa than will an older crab during the same period. At the time when the externa appears and prevents further casting, it is probable that the recently acquired shell of its young host will be modified. In the case of an older crab it is possible that no ecdysis may have taken place during the period following infection, and there will be no external modification at all. The case has been exaggerated for the sake of clarity, but so long as it is agreed that young crabs cast more frequently than old ones, the argument holds. A definite case of this may be cited. A crab was found casting; the old shell showed no evidence of modification, but the new shell had the abdominal segments partially redivided. The parasite was a large interna in the definitive position. It is noteworthy that the examination of the old shell would have placed the crab in Group I and the examination of the new shell would have placed the crab in Group II.

The Breadth of the Fourth Abdominal Segment in each Group.

In sub-column B of columns 4, 5, and 6 the average breadth of the fourth abdominal segment of Groups I, II, and III are



TEXT-FIG. 11.

Graph of abdomen breadth / carapace breadth for normal males and females and for parasitized males of Groups I, II, and III.

given, and in column 3 and 7 the breadth of the same segment is given for uninfected males and females. In Text-fig. 11, this value B_4 has been plotted against the carapace breadth. Each graph will be considered separately.

The Graphs of Normal Males and Females.

The divergence of the graphs of B_4 for males and females shows that the rate of growth of the abdomen as measured by the breadth of the fourth abdominal segment differs in the two cases. When the ratio $\frac{\text{Abdomen breadth}}{\text{Carapace breadth}}$ is plotted as a percentage against carapace breadth as suggested by Huxley (1924) and used by him for Fiddler Crabs, it is found that the growth-rate of the male abdomen is negatively heterogonic and the growth-rate of the female abdomen is positively heterogonic. When the ratio $\frac{\text{Abdomen breadth}}{\text{Carapace length}}$ is plotted as a percentage against carapace length as used by Huxley (1931) for Carcinus, it is found that the growth-rate of the male abdomen is isogonic and that the female abdomen is still positively heterogonic. The difference in the results is of course due to the standard of body-growth chosen. The carapace of the crab grows more rapidly in breadth than in length and the result is influenced accordingly.

The Graph of Group I.

This graph coincides with that of normal males, showing that infected crabs in which the third, fourth, and fifth abdominal segments remain fused, do not have broadened abdomens. In other words this group of externally parasitized crabs shows no change in secondary sexual characters.

The Graphs of Groups II and III.

These two graphs may best be considered together. Reference to Text-fig. 11 shows that the graph of Group II is roughly parallel with the graph of normal males, but lies between it and the graph of normal females. Graph III representing the maximum amount of modification is above Graph II but approaches it in the larger size groups of crabs. It is hence concluded that the maximum degree of modification of the male abdomen decreases among the older (or larger) crabs.

The effect of Sacculina has been described as 'parasitic castration'. Though it is still a matter of controversy among invertebrates, it is known that among vertebrates at least, castration has the greatest effect when the operation is performed on the young animal. Not only is the young individual more plastic, but it has not progressed so far from the undifferentiated condition as the mature male.

The Degrees of Modification.

After these preliminary matters have been discussed, we may consider the reason why degrees of modification in the secondary sexual characters are found. Possible causes are given below.

(1) The liability of small crabs to be modified to a maximum extent. This has already been discussed.

(2) The decrease in the maximum degree of modification possible, among the large crabs. This has also been discussed.

(3) The difference in the resistance of individual crabs. This has been put forward by Smith (1906) and Nilsson-Cantell (1927). Smith says, p. 67: '. . . the difference in effect must be chiefly due to a difference inherent in the infected animals.' There is no evidence either in support of this argument or against it, but it does not seem sufficient to explain why some crabs should show no external modification and others of the same size show an almost complete change in secondary sexual characters.

(4) If one suggests a difference in individual hosts, one may equally suggest a difference in individual parasites. Again, this argument does not seem sufficient to cover all the observed facts.

(5) Re-infection by a second cypris larva after the first externa has dropped off, the second parasite causing a further modification of its host. There is nothing impossible about this but re-infection must depend on chance, so that a certain number would escape and modified crabs would then be found without parasites. Not one of these has been found among the 2,000 Portunus examined and in Smith's collection of 5,000 parasitized Inachus only three are recorded.

(6) Regeneration of the original parasite, the second phase causing a further increase in modification which would appear at the ecdysis of the scarred crab.

In summarizing the possible causes for the presence of degrees of modification, it would seem probable that all the points given with the possible exception of no. 5 have some influence, but stress must be laid on the liability of small crabs to be modified to a maximum extent, the decrease in the maximum degree of modification among large crabs, and the theory of regeneration.

SUMMARY.

This paper contains a revised version of the life-history of Sacculina stressing certain points which have arisen as a result of work on the effects of the parasite on its host.

From the statistical analysis of a year's samples of an infected crab population it is concluded; first, that crabs may be infected at any age; secondly, that the developmental sequences in the life-history of the parasite are dependent not on the season but possibly on the phases of the host; thirdly, that the internal development of the parasite lasts nine months, and that the first batches of larvae appear three months later.

Two separate experiments showed that the evagination of the parasite does not take place at an ecdysis of its host.

It is suggested that an anomalous group of highly modified male crabs without externae but containing living parasitic roots are 'scarred crabs' that have moulted; further, that such modified crabs will in time regenerate externae. Experimental and histological evidence supports this suggestion, but it is not held that the theory of regeneration is completely proved.

An account is given of the effects of Sacculina on the secondary sexual characters of Portunus holsatus. Only male crabs are dealt with. It is shown: (*a*) that various degrees of modification are possible; (*b*) that the percentage of infection increases among large crabs; (*c*) that the smaller the crab the greater the liability to maximum modification; (*d*) that the maximum amount of modification which is possible decreases among larger crabs.

REFERENCES.

Boschma, H. (1928).—"Über europäische Formen der Gattung Sacculina", 'Zool. Jahrb. Jena', liv.

Delage, Y. (1884).—"Évolution de la Sacculine", 'Arch. Zool. exp. gén.', tom. ii, sér. 2.

Elmhirst, R. (1923).—"On the moulting of the Lobster", 'Proc. R. Phys. Soc. Edinburgh', 20, pp. 271–6.

Giard, A. (1886).—"De l'Influence de Certains Parasites Rhizocéphales sur les Caractères sexuels extérieurs de leur Hôte", 'C.R. Acad. Sc. Paris', vol. 103.

——(1887).—"La 'Castration Parasitaire' et son Influence sur les Caractères extérieurs du Sexe Mâle chez les Crustacés", 'Bull. Soc. Nord France et Belg.', (2), 10me année.

Guérin-Ganivet, J. (1911).—"Contribution à l'Étude systématique et biologique des Rhizocéphales", 'Trav. Sc. Lab. Zool. Physiol. Maritimes de Concarneau', tom. 3 (7).

Huxley, J. S. (1924).—"The variation in the width of the abdomen in immature Fiddler Crabs considered in relation to their relative growth rate", 'Amer. Nat.', New York, 58.

Huxley, J. S., and Richards, O. W. (1931).—"Relative growth of the abdomen and the carapace of the Shore Crab, Carcinus maenas", 'Journ. Mar. Biol. Ass. Plymouth', 17.

Lebour, M. V. (1928).—"The Larval Stages of the Plymouth Brachyura", 'Proc. Zool. Soc. London.' Part 2, 1928.

Nilsson-Cantell, C. A. (1926).—"Über Veränderungen der sekundären Geschlechtsmerkmale bei Paguriden durch Einwirkung von Rhizocephalen", 'Ark. Zool. Stockholm', Bd. 18 A (13).

Pearson, J. (1908).—'Memoir on Cancer pagurus, the Edible Crab', no. xvi, Liverpool.

Pérez, C. (1928).—"Sur le cycle évolutif des Rhizocéphales du genre Chlorogaster", 'C.R. Acad. Sc. Paris', 187.

—— (1931).—"Remplacement successif des sacs viscéreaux chez les Chlorogaster", ibid. 192.

—— (1931).—"Remplacement successif des sacs viscéreaux chez les Chlorogaster Rhizocéphales parasites des Pagures", ibid. 192.

—— (1931).—"Organogénèse des bourgeons de remplacement chez les Chlorogaster", ibid. 193.

Pérez, C., and Basse, E. (1928).—"Sur un monstre double de Sacculine", 'Bull. Soc. Zool. France', 53.

Perkins, M. (1925).—"Further abnormal chelae of Carcinus moenas, &c., in a parasitized specimen", 'Ann. Mag. Nat. Hist.', ser. 9, xvi.

—— (1929).—"Growth gradients and the axial relations of the animal body", 'Nature', London, 124.

Potts, F. A. (1909).—"Observations on the changes in the Common Shore Crab caused by Sacculina", 'Proc. Camb. Phil. Soc.', vol. xv.

Smith, G. (1906).—"Rhizocephala", Mon. 29, 'Fauna und Flora des Golfes von Neapel', Berlin.

—— (1912).—"Studies on the Experimental Analysis of Sex. Part 7. Sexual changes in the Blood and Liver of Carcinus moenas", 'Quart. Journ. Micr. Sc.', vol. lvii.

Vaulx, R. de la (1922).—"La 'castration parasitaire' des Arthropodes", 'Rév. gén. sc.', Paris, 33.