SEX DIFFERENTIATION AND DEVELOPMENT

EDITED BY
C. R. AUSTIN

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Edited on behalf of the Society for Endocrinology by
C. R. AUSTIN

PREFACE

The Symposium on Sex Differentiation and Development, the Proceedings of which constitute this Memoir, was arranged by the Society for Endocrinology and was so designed as to bring together authorities in several different disciplines.

To advance the frontiers of knowledge, an effective strategy should first establish communications between distantly separated salients and in this way yield new perspectives on the intervening ground that remains to be won. Accordingly, it was decided that the subject-matter of the Symposium should range as widely as possible, should deal with single cells as well as complex organisms, with structure and function, including behaviour, and with both endogenous and exogenous influences. Such an ambitious plan of campaign was clearly not possible of execution in detail in the time available, but its adoption seemed justified because it offered unusual possibilities of stimulating and informative reconnaissance.

The broad plan of the Symposium was developed in committee, with Professor F. T. G. Prunty, Dr J. A. Loraine and Dr W. Klyne, and the details were filled in with the very welcome help of discussions with Dr M. W. H. Bishop and advice from several people, especially Dr A. S. Parkes, Dr A. U. Smith, Dr G. E. W. Wolstenholme and Dr B. Lacey. For their skilful conduct of the Symposium, grateful acknowledgment is made to the four Chairmen: Dr A. S. Parkes, Professor J. D. Boyd, Professor F. W. Rogers Brambell, and Professor E. C. Amoroso. Sincere thanks are due also to Dr P. Eckstein for aid and guidance in the editing of this Memoir.

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A. S. PARKES, C.B.E., F.R.S.

Chairman's introduction

The Society for Endocrinology organizes each year a symposium on some subject immediately related to the interests of the Society. In the past, these symposia have dealt with strictly endocrinological subjects, such as hormones in relation to obesity, and methods of administering hormones. This year, a much wider field has been selected, and the papers to be presented cover ground ranging from bacteria to mammals and biophysics to anatomy. The thread binding together these varied topics can readily be seen from the programme; it is sex, in many guises and many contexts.

This symposium, indicative of the wide interests of the Society, will take us far outside the boundaries of endocrinology, even on the widest definition of that word. Nevertheless, we shall no doubt hear that hormones are a dominating factor in sexuality, not only in vertebrates but in more than one phylum of invertebrates. The sexual endocrinology of the Crustacea, for instance, always of interest since the work on parasitic castration, seems to become more fascinating and even more fantastic every day, and it is salutary to consider how our knowledge of endocrine effects would have developed had it been based on crustaceans rather than on mammals and birds. We may indeed congratulate ourselves that, in the animal world, the complexities of sexuality seem to be inversely rather than directly proportional to the size of the organism. The crustaceans, however, have no monopoly of the curiosities of inverte-brate crinology. Among the insects, an entirely novel principle appears, the use of a secretion not for the integration of the individual but for the integration of a population of individuals. Whether this type of organization should properly be regarded as endocrinological or exocrinological is not important. The concept is awesome, and it is fortunate that the use of queen substance to maintain all other females in subjection as sexless workers appears at present to be restricted to insects.

I have already indicated that endocrinology does not exhaust the subject-matter of this symposium. We shall also hear of such diverse matters as sex in bacteria, the possibilities of separating X- and Y-spermatozoa in mammals, recent work on mam-malian oocytes, and not least, of sex chromatin in man and of the curious diagnoses made with its aid.

The selection of this theme we owe to Dr Austin, the Programme Secretary of the Society, who rightly regards sex as an important matter. Moreover, Dr Austin has been responsible for the hard work and careful planning inevitably associated with the organization of a successful symposium, and the success of the present meeting is not, I think, in doubt.

In the past, the Society has been fortunate in securing the participation in its symposia

of distinguished endocrinologists from abroad. This occasion is no exception, and we have with us today Dr M. Klein of Strasbourg, Professor A. Jost of Paris, and Dr W. P. U. Jackson of Cape Town, to all of whom we extend a most hearty welcome to this country and to the symposium.

SEX DETERMINATION AND CASTE DIFFERENTIATION IN THE HONEYBEE (*APIS MELLIFERA*)

By C. G. BUTLER

Over 100 years ago, Dzierzon [1845] stated his well-known theory that unfertilized eggs of the honeybee always give rise to males—drones—whereas fertilized eggs always give rise to females, which may become either workers or queens, depending upon the manner in which the larvae concerned are fed by the worker members of their colony. Dzierzon suggested that a normal, mated queen can lay either a fertilized or an unfertilized egg at will, and that the kind of egg she lays depends on the kind of cell—worker, queen or drone—in which she is about to lay.

The Dzierzon theory has, of course, become well established, although it is now known that females can sometimes develop from unfertilized honeybee eggs. In fact, in two races of honeybees, the South African Cape bee (*Apis mellifera capensis*) and the Tunisian bee (*A. mellifera intermissa*), it has been reported that both queens and workers are frequently reared from unfertilized eggs [Jack, 1916; Gough, 1928]. An American worker, Mackensen [1943], has also demonstrated that such impaternate females occasionally occur in several races of European honeybees, and he succeeded in rearing queens as well as workers from such unfertilized eggs. The mechanism by which such impaternate females are produced in the honeybee is unknown, but Ruttner & Mackensen [1952] have suggested that perhaps the mechanism is similar to that which is believed to occur in the parasitic wasp *Habrobracon*, in which Speicher & Speicher [1938] have obtained cytological evidence indicating that they probably arise from tetraploid tissue in the ovaries. Perhaps the Cape and Tunisian honeybees tend to have such tissue in their ovaries and, as a result of reduction, the egg becomes diploid and consequently develops into a female without fertilization.

According to Nachtsheim [1913], and various other workers, the honeybee sperm has sixteen chromosomes and the fertilized egg thirty-two.

Two theories of sex determination in the honeybee have been put forward. In the first, Manning [1949-50] has claimed that sex determination is dependent upon the presence of a sex chromosome that is lost in the development of the sperm. According to his theory, both male and female possess only a single sex chromosome, although the female has twice as many autosomes as the male. He supposes that, during the formation of the ovum, differential maturation results in the sex chromosome always remaining in the nucleus of the ovum, with the result that a mature ovum contains fifteen autosomes together with a single sex chromosome and, if unfertilized, gives rise to a male. On the other hand, fertilized eggs contain thirty autosomes and a sex chromosome and develop into females. However, Sanderson & Hall [1951] have subsequently confirmed their previous finding of sixteen chromosomes in the male and

thirty-two in the female; and Ris & Kerr [unpublished observations quoted by Ruttner & Mackensen, 1952] have shown that Manning's sex chromosome does not stain with Feulgen, although it does so with haematoxylin, and they have, therefore, concluded that it is a nucleolus and not a sex chromosome at all.

Some further evidence against Manning's scheme is provided by the gynandromorph honeybees studied by Rothenbuhler, Gowen & Park [1951], who claim to have, shown that the male tissue of these gynandromorphs was produced by the entry into the ovum of supernumerary sperm. Since, on Manning's hypothesis, maleness is due to a sex chromosome, which is not contained in the sperm, and the effect of the autosomes in the sperm is to make the egg female, supernumerary sperms could only make the zygote still more female.

The other theory of sex determination in the honeybee has been put forward by Mackensen [1951]. Its theoretical mechanism resembles that shown by Whiting [1940, 1943] to exist in *Habrobracon*. Mackensen suggests that in the honeybee a series of sex alleles exist that give female characteristics when heterozygous and are lethal in the homozygous conditions, a viable male only being produced from the unfertilized haploid egg. His evidence in favour of this theory lies in the rapidity with which, on close inbreeding, the viability of the eggs laid diminishes towards 50%.

Whilst, in our own bee breeding at Rothamsted, some reduction in viability has been observed on close inbreeding, we have at present inadequate data on which to decide for or against Mackensen's theory.

The ability of worker honeybees to rear both queens and workers from fertilized eggs has long formed the basis of practical queen rearing by beekeepers, and there is no possible reason to suppose that differentiation of the queen and worker castes is determined genetically, as it has been shown to be in some of the so-called stingless bees of the genus *Melipona* [Kerr, 1946]. Caste determination in the honeybee is determined in the feeding of the female larvae concerned.

It has been shown experimentally that it is essential for differential feeding to begin before the larva is 3 days old if a perfect queen is to result, although queens with a number of worker-like characteristics can be produced from older larvae.

Two principal theories to explain caste determination have been put forward. In the first, it is suggested that the quality of the food received by a larva acts as the determining factor, and in the second, that it is the quantity of the food received that matters.

According to the qualitative theory, differences occur in the brood food (which consists partly of a secretion, rich in protein, of the pharyngeal glands of the worker bees) fed to the larvae of queens and workers after the first 24 hr or so of larval life [von Rhein, 1933]. But analyses that have been carried out by various workers give very contradictory results, and this has led Haydak [1943] and others to conclude that brood food varies in composition quite fortuitously, and that such variations as occur cannot be responsible for caste determination.

Having decided that the quality of the food was unlikely to be the responsible factor in caste determination, Haydak considered that it must be attributable to differences in

the quantities of the essential nutrients supplied to and consumed by worker and queen larvae, respectively. He pointed out that, until about the 3rd day of life, all larval honeybees in prosperous colonies receive an excess of food, but that, subsequently, while the queen larvae in their large cells continue to be supplied with more food than they can eat, the worker larvae in much smaller cells only receive relatively small quantities of food from time to time. The result is that the queen and worker larvae grow at approximately the same rate for the first day or two, but subsequently, whereas the queen larva, surrounded by an abundance of food, continues to grow rapidly, the much more frugally fed worker larva grows more slowly. Haydak [1943] pointed out that, even after her cell has been sealed, a queen larva continues to feed upon the surplus of brood food in the bottom of her cell, whereas a worker larva, having no such source of food, cannot feed any more once her cell has been sealed, and actually loses weight.

In order to test his hypothesis, Haydak removed the larvae from queen cells that were either just about to be sealed or had just been sealed, so that they could not eat any more food. Most of the larvae that he treated in this way died in the pupal stage, but he reported that seven of these pupae possessed worker rather than queen characteristics, while several others were intermediate in form between queens and workers. Nine adults were obtained, all except one of which were normal queens, the exceptional individual being intermediate between a queen and a worker. Haydak reported that the average initial weight of those of the larvae that developed into queens was 14% greater than that of larvae developing into individuals with worker or intermediate characteristics.

From Haydak's results, it certainly appears likely that continuous liberal feeding of those larvae that are destined to become queens plays an important role in their differentiation. However, von Rhein [1933] who, in the laboratory, fed female larvae very liberally with brood food taken from queen cells, failed to obtain the queens one would have expected if Haydak's hypothesis were correct. Indeed, his failure to produce queens in this way led von Rhein to suppose that some unstable, differentiating substance is fed by worker bees to those larvae that are destined to become queens, and that this fugitive substance had either been destroyed (or, perhaps, lost during storage) in the brood food he took from queen cells and fed to his larvae.

Simpson [1957] has also shown that abundant feeding alone, even when it is carried out by worker bees themselves, is insufficient to cause a female larva to develop into a queen.

Recently, Weaver [1955, 1957] has reported the results of some experiments similar to those of von Rhein [1933], in which he fed young female larvae, taken from worker cells, on abundant brood food freshly collected from queen cells containing larvae of approximately the same ages as the experimental ones. In these circumstances, queens were produced. On the other hand, when similar larvae were fed abundantly with brood food collected from queen cells and stored for some time, only workers were produced. These results clearly fail to support Haydak's conclusion, as the experimental larvae were continuously supplied with a superfluity of food so that quantitative starvation could not have been the determining mechanism. On the other hand, they

strongly support the conclusion of von Rhein [1933] that some substance (or substances) contained in the food fed by the workers to the larvae in queen cells controls their differentiation into queens, and that at least some essential part of this substance is either highly labile or else is no longer available to larvae after the food has been exposed to the air for some time even in a refrigerator.

It seems probable, therefore, that in order that a given female larva may give rise to a queen she must continually receive liberal supplies of fresh brood food in which the differentiating substance is still active. Furthermore, in order that a normal queen may be produced, the larva must be fed on this diet from at least the 3rd day of life onwards [Weaver, 1957].

Now, in a normal colony of honeybees, the ovaries of the workers remain undeveloped and no eggs are laid by them, and, except under special conditions, the workers do not attempt to rear any new queens. But if a colony loses its queen, perhaps through accident or disease, two things normally occur. First, within a few hours, the workers will have modified one or more worker cells containing young female larvae to form emergency queen cells and, by special feeding of these larvae, will cause them to develop into queens. All being well, one of them will replace the queen that was lost, the others will be destroyed. Secondly, the ovaries of the adult workers, particularly those of the younger ones, will develop slightly [Hess, 1942]. Normally, when a queen has been successfully produced, this process is reversed, but, should there be no larvae present from which the workers can rear a new queen, or should they fail to do so for some other reason, then the workers' ovaries continue to develop until useless, drone-producing eggs are laid. It is clear, therefore, that normally the presence of a queen is sufficient to inhibit both development of the workers' ovaries and the production by them of further queens. The presence of open, occupied queen cells has a similar inhibiting influence [Butler, 1957a].

It has recently been demonstrated that development of the worker ovary is normally inhibited by a remarkably stable substance that the workers of a colony obtain by licking the body of their queen [de Groot & Voogd, 1954; Pain, 1954], and Butler [1954, 1956, 1957b] has shown that this substance is passed from bee to bee in regurgitated food. Furthermore, either the same or some very similar substance, called 'queen substance' by Butler [1954], that is collected in the same way by the workers from their queen, and also distributed in regurgitated food, is responsible, when in sufficient supply, for inhibiting workers from rearing further queens [Butler, 1954, 1957c; Butler & Gibbons, 1958; Simpson, 1958]. It seems probable that a single substance is involved, and biologically effective extracts have been obtained both in acetone and in alcohol [de Groot & Voogd, 1954; Butler, 1957b; Butler & Gibbons, 1958].

The existence of similar ovary-inhibiting substances has recently been demonstrated by Bier [1954] in several species of ants; and Lüscher [1953, 1956] and others have shown that certain queen termites produce a substance that inhibits the development of supplementary reproductives in their colonies.

Again, Carlisle & Butler [1956] have obtained evidence suggesting that honeybee queen substance is interchangeable with a substance found in the sinus glands in the

eyestalks of prawns (*Leander serratus*) which inhibits development of their ovaries; and Butler [unpublished] has obtained evidence indicating that the inhibitory substance produced by at least some species of queen ants is interchangeable with honeybee queen substance. It looks, indeed, as if certain inhibitory substances produced by the queens of at least some social insects, and which play important roles in the organization of their colonies, are to some extent interchangeable. Perhaps they are chemically similar; this we do not know as none of them has yet been isolated and identified.

Dr Callow and Miss Johnston, of the National Institute for Medical Research, and the author are trying to identify the active principle in queen substance. We have also tested a number of steroids to see whether they produce similar effects on bees. So far, we have been able to mimic the action of queen substance in inhibiting ovary development in worker bees with androsterone, but have not been able to demonstrate that this substance prevents them from rearing queens.

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COMMENT

Peacock: I should like to draw your attention to certain long-standing problems of sex in invertebrates. For example, how far the theories of sex determination in the two hymenopterans, the honeybee and *Habrobracon*, discussed by Dr Butler, can be applied to other arrhenotokously parthenogenetic organisms, e.g. saw-flies, ants and other Hymenoptera, rotifers, thrips, remains unknown.

Regarding sex determination in other insects, two particular cases in the Lepidoptera are of interest. Goldschmidt [*Bibl. genet., Lpz.,* 1934, **11**, 1] obtained intersexes of the gypsy moth by crossing different geographical races which differed in the strength of their female- and male-determining factors. More recently, Seiler [*Experientia,* 1949, **5**, 425] obtained different results with *Solenobia* moths. For example, a tetraploid female of a parthenogenetic race, crossed with a male of a diploid race, gave triploid intersexes. Goldschmidt held that all members of a given hybrid brood showed the same degree of intersexuality and that the degree of intersexuality was finally attained only after the competing female- and male-producing determining factors had reacted over a certain period of time. In contrast, Seiler found all grades of intersexes in the same brood, and no switch-over of sex during the larval and later stages, each individual intersex beginning and remaining at the same fixed grade. Seiler therefore suggests that there operate phenotypic factors at present unknown.

In certain Crustacea, other than the decapods to be dealt with by Dr Carlisle, as well as in other invertebrates, we find alternation between the parthenogenetic and bisexual modes of reproduction (heterogony). Environmental factors certainly determine this alternation. By refined culture techniques, in which the effects of food, light, temperature, excretory products, etc., were tested, von Dehn [*Zool. Jb.* 1937, **58**, 241; *Naturwissenschaften,* 1950, **37**, 429] and Buchner [*Z. indukt. Abstamm. VererbLehre,* 1936, **72**, 141], respectively for the crustaceans *Daphnia* and *Moina* and certain rotifers, found that the females of a long-continued parthenogenetic culture can be switched to male production, the main factor involved being the quality of food, the fatstuff content of the latter being important in *Moina*. Also effective to a certain extent in *Moina* were light deficiency associated with food deficiency, and darkness. In the gall-midge *Oligarces paradoxus,* the larva of which is parthenogenetic (paedogenesis), the switch-over to adulthood and bisexuality is effected by the quantity of food, or more strictly, by the constellation of environmental factors (age of parent, population density, etc.) that together influence the food available [Ulrich, *Naturwissenschaften,* 1940, **36**, 569; **37**, 586]. In the bean aphis, where a succession of parthenogenetic generations occurs on alternate host-plants, Davidson [*Ann. appl. Biol.* 1929, **16**, 104] showed that, after prolonged parthenogenetic reproduction, sexual females and males could be produced by reducing the amount of light or by lowering the temperature.

No hereditary factors determining sex have so far been observed by von Dehn, Buchner nor Ulrich in their experimental material. How the environmental factors mentioned produce their effects of sex change or change in the method of reproduction, by influencing chromosomal and/or endocrine processes, is unknown.

SEXUAL DIFFERENTIATION IN CRUSTACEA MALACOSTRACA

By D. B. CARLISLE

The field of study about which I am going to talk today must be so unfamiliar to most of you, engaged in vertebrate endocrinological research, that I feel it may be worth while to present briefly the whole picture of the hormonal control of sexual differentiation in Crustacea of the Order Malacostraca so far as it is known, rather than describe in detail my own work on the subject. The Malacostraca are the higher Crustacea and include such forms as crabs, lobsters, shrimps and sand-hoppers. Apart from my own investigations, most of the research involved has been performed by French workers, and I would refer particularly to the recently published thesis of Mme H. Charniaux-Cotton [1957], who has brilliantly elucidated much of the sexual endocrinology of the sand-hopper, *Orchestia*.

Four organs have been shown to produce hormones influencing the sexual development in Crustacea. The first to be so implicated was the ovary. As early as 1926, Haemmerli-Boveri castrated Crustacea by irradiation, as others also did in the years just before the war [Roux, 1933; Callan, 1940; Knowles & Callan, 1940]. Takewaki & Nakamura [1944] and Charniaux-Cotton [Charniaux, 1952, 1953a, b; Charniaux-Cotton, 1954a, b, 1956a, b, 1957] castrated Crustacea by surgical means, a most difficult technical feat. All are agreed that the only effect is the non-development of the secondary brooding characters—the pregnancy characters as it were—of the gonadectomized females. Castrated males are not affected in any way, not even in behaviour. Both males and females, after castration, develop all the appropriate secondary and accessory sexual characteristics. Hence the control of the differentiation of sexual characters in Crustacea does not lie in any hormonal secretion of the gonads. We must look elsewhere, but first it should be mentioned that it is the ovary undergoing vitellogenesis that secretes the hormone responsible for the development of the brooding characters.

The organ most recently implicated in the sexual differentiation of Crustacea is the gland first described without a name by Charniaux-Cotton [1954b], adjacent to the vas deferens in *Orchestia*. I have called it the vas deferens gland [Knowles & Carlisle, 1956], while Charniaux-Cotton [1956c] has referred to it as the 'glande androgène'. The former name seems preferable, for it is customary to name an organ after its position and anatomical relationships, rather than its presumed function. Charniaux-Cotton, like myself, has also observed the gland in decapod Crustacea. In all, it lies in or alongside the wall of the vas deferens. It betrays histological signs of secretion which vary with the sexual state of the animal. We owe most of our knowledge of its functioning to Charniaux-Cotton, who has experimented exclusively upon *Orchestia*. Briefly, she finds the gland responsible for the entire development of the male characters, both primary and secondary. Removal of both glands from males, whether castrated or not,

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