PHEROMONES

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Pheromones are wide spread throughout the animal kingdom. In the ten years that have elapsed since the word was coined to describe the sex attractants of insects (Karlson & Butenandt, 1959) our knowledge has greatly increased, not only with reference to chemical communication in the world of insects, but also among mammals, birds and fishes. The meaning of the word "pheromone" has been extended to include chemical communication in a broader sense and in all species. The term now connotes secretions which convey information of many kinds from one individual to others and evoke specific behavioural and physiological reactions in the recipients. Pheromones thus differ fundamentally from hormones, of which the physiological activity finds expression only in the individual within which they originate. Pheromones, like hormones, affect the development, reproduction or behaviour of other individuals. Unlike hormones, which may be active and chemically similar in several species, pheromones appear to be mainly species-specific, but some overlap of activity between closely related insect species occurs (see review by Shorey & Gaston, 1967) and the same is probably true for mammalian pheromones (Bronson, Eleftheriou & Garick, 1964).

1. Mode of Action

Pheromones may act on the recipient by olfaction, by ingestion or by absorption.

The majority of pheromones so far recognized are olfactory; of these, probably the most familiar, and those which have received the greatest study because of their potential value in pest control, are the sex attractants. These have been demonstrated in a great number of insect species. The chemistry of several naturally occurring sex pheromones is known, and a few (five) have been synthesized.

At least one orally active pheromone has been described, the queen substance of honey-bees. Queen substance, produced by the mandibular glands of the queen, prevents the proper development of the ovaries of worker bees, which remain in the juvenile state for as long as the ingestion of queen substance during grooming is maintained by the workers. The chemical constitution has been characterized and the pheromone itself synthesized (Butler, Callow & Johnston, 1959, 1961).

Contact pheromones are also known. The maturation of young locusts is accelerated by a substance produced by the adult male which is probably of importance in the formation of migratory swarms (Loher, 1960). A sex pheromone having aphrodisiac properties is secreted by the female in some lycosid spiders. It is received by the male through contact chemoreception (Hegdekar & Dondale, 1969). A similar situation has been described in cockroaches (Jacobson, 1965).

These two latter types have not yet been reported among mammals. All the mammalian pheromones so far recognized are olfactory.

2. Classification

Whatever the nature of the environmental change, the reactions fall broadly into three types:

i. An immediate and reversible response operated directly through the central nervous system, e.g. recognition, or through rapidly acting neurohumoral channels, as exemplified by the milk-ejection reflex (Cross & Harris, 1952)[1]. Such reactions are described as "releaser", and pheromones which elicit them are known as "releaser pheromones".
ii. The exteroceptive response implicating the anterior pituitary gland (Marshall, 1942). This type is slow to develop, demanding prolonged stimulation which initiates a chain of physiological effects in the recipient. Pheromones producing this type of reaction are called "primer pheromones".
iii. The phenomenon of imprinting. Stimulation at a critical period during development (or the absence of appropriate stimulation) may result in a permanent modification of behaviour in the adult.

Mammalian olfactory pheromones belonging to all three types are recognized.

3. Olfactory Responses

In the present state of knowledge, odour can neither be defined nor adequately measured (see Ottoson, 1963; Amerine, Pangborn & Roessler, 1965). The study of olfactory effects must therefore rely mainly upon behavioural responses. The investigation of olfaction in mammals by default, i.e., by studying responses in the anosmic animal itself, presents problems. Apart from the technical difficulties of surgery, interpretation of the results may be obscured by much individual variation (Whitten, 1956; Lamond, 1958). About nine different categories of behavioural response under control by pheromones are recognized among insects (Wilson, 1965). Some at least have their counterparts in the behaviour of mammals.

4. Mammalian Pheromones

With the exception of man himself, for whom, in appraisal of the environment, the special senses of sight and hearing have greater significance than the sense of smell, the daily life of most mammals is largely determined by olfaction.

[1] See also Cross, *Br. med. Bull.* 1955, 11, 151.—ED.

a. Releaser Pheromones

Sexual behaviour. The first critical report implicating chemical communication in sexual recognition and sexual behaviour is that of Le Magnen (1952). He showed that adult rats discriminate between the sexes, and also between receptive females and those which are sexually quiescent. Later, similar reactions were described in mice (Chanel & Vernet-Maury, 1963). The domestic cat will manifest oestrous behaviour if introduced into a cage recently occupied by a male, unless the cage has been washed (Michael & Keverne, 1968).

Female mice (Whitten, 1956) and female guinea-pigs (Donovan & Kopriva, 1965) from which the olfactory lobes have been removed develop disturbances of receptivity in spite of apparently normal oestrous cycles, but pregnancy follows a normal course once coitus has been effected. Furthermore, sexual behaviour is absent in the adult female rabbit rendered anosmic before puberty (Franck, 1966).

The presence of an olfactory component is apparent in the sexual behaviour of some farm animals. In the oestrous sow the smell of the boar is necessary for the culmination of the immobilization reflex essential to the prolonged act of successful coitus, although both sight and sound also contribute (Signoret & Du Mesnil du Buisson, 1961). The ram discriminates by odour between the oestrous ewes and those which are not receptive. With the olfactory lobes destroyed, the anosmic ram is unable to recognize the oestrous ewe and must find her by a system of trial and error. Copulatory activity and fertility are unimpaired when contact is finally established (Lindsay, 1965). Even the normally unattractive pregnant ewe can be made attractive to the ram by smearing her with a swab taken from an oestrous ewe (Kelley, 1937). Odour is important in the copulatory activity of young stallions, but in experienced stallions sight takes priority over smell (Wierzbowski & Hafez, 1961).

Thus the mammalian sex pheromones, which may be produced either by males, by females or by both sexes, play an important role in the initial recognition of the sexual partner and in the integration of behaviour between the sexes. The precise stage in the sequence of events, collectively described as "sexual behaviour", at which pheromonal intervention occurs may be different for different species. Among the mammalian pheromones so far recognized, the male sex pheromones seem mainly to function as aphrodisiacs for the female, while the female sex pheromones are for identification of sexual state.

Aggression. The causes of aggressive behaviour form the subject of much current debate, but the problem is still largely unresolved. There is, however, convincing evidence that the notorious aggression between adult male mice is released by olfactory signals alone. The aggressive information is contained in urine. Aggression can be induced between familiar pairs of male mice normally living in harmony, by anointing one member with urine from a strange male. Similar treatment with water is without effect (Mackintosh & Grant, 1966). Masking the natural odours of a pair of male mice that are strangers to one another (with Dior scent) has the reciprocal effect. The release of aggression is delayed, presumably until perception of the normal mouse odour, in addition to the unfamiliar scent, permits mutual recognition as strangers. Moreover, while removal of the olfactory lobes eliminates all aggressive behaviour in the anosmic individuals, it does not prevent the anosmic mice from being viciously attacked by intact males (Ropartz, 1968). This behaviour has interesting implications, for it indicates that the production of male mouse aggressive pheromones is independent of olfactory information received from the environment.

Two olfactory pheromones are related to aggressive behaviour in mice: a "urinary factor" corresponding to group odour, which is produced from the coagulating glands when the mice are in close contact; and a "plantar factor", elaborated by the pads of the feet, which characterizes each individual within the group (Ropartz, 1967). These conclusions call to mind the theory adumbrated by Parkes (1960) that "Almost certainly a spectrum of smells is involved, varying slightly in different individuals of the same strain, and substantially between strains".

Other types of activity. Many other examples of activity under the control of releaser pheromones could be quoted, such as territory marking, homing, recognition of status; and there may still be other kinds not yet identified.

b. Primer Pheromones

Control of oestrus. In a number of mammalian species, perhaps in most, the male exerts a controlling influence over oestrus. His exteroceptive effect was first noted among domesticated seasonal breeders—sheep and goats. In both these species the introduction of the male shortly before the start of the breeding season stimulates ovulation and heat in the female, thereby abruptly terminating anoestrus. The olfactory influence of the male reinforces the stimuli supplied by other environmental factors, as a result of which the female will eventually become cyclic. And it is interesting to note that in order to exercise his exteroceptive effect the male must present a novel stimulus. If he remains continuously with the flock there is no acceleration of breeding at the end of anoestrus. Nor is his influence felt when he is introduced after the ewes have become cyclic (for references to the original papers see review by Bruce, 1967).

Most of our information concerned with the exteroceptive effects derives from the mouse, *Mus musculus*, in which these reactions have been extensively studied. The oestrous cycle in the mouse is susceptible to environmental conditions and is readily modified accordingly, both by olfactory stimuli from males and those from females.

When the female mouse is isolated, her cycle is 5–6 days in duration, irregularities are not uncommon and spontaneous pseudopregnancies sometimes occur. Under conditions of community living in all-female groups there is mutual suppression of oestrus; the regular cycle is promptly replaced by a high proportion of pseudopregnancies if the groups are small or by periods of anoestrus if the groups are large. Whatever the previous history of the female the introduction of a male has an immediate effect. A new cycle is initiated among pseudopregnant or anoestrous females so that oestrus becomes synchronized; among females previously isolated the cycle is shortened and irregularities virtually disappear. These reactions induce a striking pattern of mating behaviour, and coitus becomes concentrated over the third night. This synchronization of oestrus is known as the Whitten effect. The influence of the male on the oestrous cycle is transitory, affecting only a single cycle unless the stimulus is renewed in every cycle. Wild *Mus musculus* (Chipman & Fox, 1966) and the deer-mouse *Peromyscus* (Bronson & Marsden, 1964) exhibit the same behaviour towards olfactory male pheromones as do laboratory mice. The ease with which the oestrous cycle of mice can be manipulated by social conditions proves a valuable tool in research.

The Whitten effect is also seen in sheep and in goats but only at the start of the breeding season. In the laboratory rat, oestrus is more stable than in the mouse. The exteroceptive effect of the male is limited to a small proportion of females (Hughes, 1964) unless reinforced by other factors (Cooper & Haynes, 1967). The oestrous cycle of the golden hamster does not seem to be affected by social conditions (Orsini, 1961). Even females belonging to species in which ovulation is induced may not be protected from remote control by strange males. In the prairie vole, *Microtus ochrogaster*, " . . . the female responds to any unusual change in her environment . . . by coming into oestrus . . . Direct exposure to adult males was the most effective treatment . . . " (Richmond & Conaway, 1969).

Control of oestrus by male sex pheromones is clearly a general phenomenon and can be manifest by species having very different patterns of reproduction.

Effects on pregnancy. Even more intriguing than the control of oestrus is the olfactory influence of the male over early pregnancy. For the first four days after coitus the mated female remains vulnerable to olfactory control by males, and pregnancy is protected only after implantation. During the early critical period the intervention of a stranger (but not the stud male) may cause pregnancy to fail, the female to return to oestrus, and to accept the second male within a few days of stud coitus; the subsequent litter is sired by the second male and the original pregnancy blocked (Bruce, 1959, 1960). Pseudopregnancy after an infertile mating may likewise be interrupted.

Anosmic females fail to react to the postcoital intervention of strange males (Bruce & Parrott, 1960); but housing the recently mated intact female in a box soiled by males, or exposing her to male urine, or to air which has passed over male mice (Whitten, Bronson & Greenstein, 1968), can induce the block to pregnancy. The failure of pregnancy is therefore an olfactory effect following exposure of the female to male pheromones. The olfactory block to pregnancy is known as the Bruce effect. Although the exteroceptive control of oestrus has been found in sheep and goats, the block to pregnancy has not so far been reported in farm animals. The Bruce effect has, to date, been demonstrated only in *Peromyscus* (Bronson & Eleftheriou, 1963) and in *Microtus* (Clulow & Clarke, 1968), in addition to laboratory mice. Attempts to induce it in the laboratory rat have failed.

The many experimental results upon which these observations are based have been admirably reviewed recently by Whitten (1966) and by Bronson (1968).

Endocrine background. The endocrine background to pregnancy block has been studied in some detail (see review by Bruce, 1967). The block reflects a failure of implantation, which in turn stems from the failure of the luteotrophic activity that would normally be expected after coitus. The administration of prolactin during the period of exposure to the male, post-partum conception in the presence of a suckling litter and the presence of ectopic pituitary grafts all give protection to the pregnancy by supplying supplementary prolactin, whether exogenous or endogenous in origin. There is, therefore, interference with hypothalamic function. Protection can be given at this level by the administration of reserpine to the female to depress hypothalamic activity (Dominic, 1966).

Male mouse pheromones therefore act by stimulating the hypothalamus, leading to release of follicle-stimulating hormone and luteinizing hormone so that oestrus and ovulation occur; concurrently the release of prolactin is inhibited and implantation fails. This much has been established, but some problems remain unsolved, particularly the possible intervention of the adrenals. There is no relationship between the incidence of pregnancy block and the number of males to which the female is exposed, either in mice (Bruce, 1963) or in *Peromyscus*. The pituitary–adrenal–gonadal response (Christian's "social stress" (Christian, 1957)) is therefore probably not implicated in the olfactory block to pregnancy. But adrenalectomy gives complete protection in one strain of laboratory mice and partial protection in wild mice (Snyder & Taggart, 1967). Different strains of laboratory mice differ in their susceptibility to male mouse pheromones. Some inbred strains fail to manifest the reaction (Marsden & Bronson, 1965) while retaining the oestrous response to the male. The reason why these strains are refractory to pregnancy block remains obscure, but here again the adrenal glands may be involved (Bruce, Land & Falconer, 1968).

Source and control. The site of production of the male sex pheromone is still undetermined, but the pheromone is present in bladder urine, free from any accessory gland secretion (Bronson & Whitten, 1968). The sex pheromone(s) therefore has both a different origin and a different identity from the aggressive pheromones.

Whatever the source, sexpheromone production is under control by the gonads. Control of oestrus by the male is lost after castration; pregnancy-blocking capacity is also lost in the adult and fails to develop if castration is carried out before puberty (Bruce, 1965). Moreover, the capacity to block pregnancy is acquired to a powerful degree by gonadectomized females treated with androgen (Dominic, 1965).

Whether or not two sex pheromones are concerned in these exteroceptive reactions is still an open question. That resistance to pregnancy block can occur without loss of the oestrous response implies a separate pheromone for each activity, but this effect could also result from a change in sensitivity in the pregnant female. This latter suggestion finds support from the greater reliability of the oestrous response. Virtually all females react regularly to the male as regards oestrus, but no test situation has yet been devised in which the block to pregnancy can be expected to occur in all females.

c. *Imprinting Pheromones*

It is widely recognized that disturbances of adult behaviour may result from environmental inadequacy during development. In particular the effect of isolation on young primates has been extensively studied. Less well documented is the importance of the olfactory environment at and shortly after birth in the young mammal. Two reports deserve mention. In mice (Mainardi, Marsan & Pasquali, 1965) and in rats (Marr & Gardner, 1965), social behaviour in the adult may be modified by olfactory experience during the period of suckling. Female mice reared in the absence of the father show a loss of discrimination in sexual selection when adult, and the same deficiency develops if the olfactory atmosphere of the nest is artificially altered by spraying the parents every day with perfume (Parma violet). Young rats reared from birth to about four weeks of age in an olfactorily artificial atmosphere, scented either with Yardley's Red Roses cologne or with oil of wintergreen, also showed modifications of social behaviour when adult. This aspect of primer pheromone activity merits further attention.

5. Primate Pheromones

Our knowledge of non-human primate behaviour rests mainly on observations made under natural conditions, which preclude the experimental approach and are perforce uncontrolled. It is generally conceded that, in these species, olfaction plays only a small part in daily life. Recent reports, however, have produced convincing evidence that even among the higher non-human primates olfactory pheromones may control some aspects of reproductive activity. Michael & Keverne (1968) have shown that sex arousal in the male rhesus monkey, under laboratory conditions, depends upon the reception of pheromones produced by the oestrous female. This could also be so for the chimpanzee. Van Lawick-Goodall (1969) calls attention to the increasing frequency of olfactory inspections, among free-living chimpanzees in Tanzania, of the female's vagina, which are performed by the male at the approach of her oestrous condition.

In the lower primates the situation may be quite different. In a fascinating study of the behaviour of captive ring-tailed lemurs (*Lemur catta*), Evans & Goy (1968) found that social integration is dependent upon a whole series of olfactory responses and they conclude: "Ring-tails may prove to possess an olfactory repertoire whose complexity rivals the more sophisticated visual and acoustic systems of larger brained primates."

Further studies of primate pheromones will be awaited with interest.

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