

ENDOCRINE REGULATION OF CASTE

DETERMINATION IN ANTS

L. PASSERA

*Laboratoire Associé au C.N.R.S. n° 333
Recherche Coopérative sur Programme du C.N.R.S. n° 645
Laboratoire de Biologie des Insectes, Université Paul-Sabatier
118, route de Narbonne, 31062 Toulouse Cedex France*

SUMMARY

Research work carried out on *Solenopsis invicta*, *Myrmica rubra*, *Pheidole pallidula*, *Plagiolepis pygmaea* and *Aphaenogaster senilis* has pinpointed the role played by hormones at the moment of caste determination in ants.

Role of the JHA's : in strong concentrations they delay metamorphosis and cause morphogenetic problems which can eliminate the brood. At lower levels they play a role in sexualization of the larvae. The JHA's administered either by mixing with the food, or given as a topical application, or by means of injections bring about the production of sexual larvae, mostly females, in the colony. The stages which are sensitive to JH vary according to the species. *Pheidole pallidula* is sensitive the earliest, determination occurring during oogenesis ; in *Solenopsis invicta* larvae in the first stage react to the introduction of JH, whereas in *Myrmica rubra* this occurs in the last larval stage. Ligation and removal of the *corpus allata* (CA) (*Myrmica*), use of Precocen (*Pheidole*) eliminate the CA's or prevent their action thereby favouring the appearance of workers.

Role of ecdysteroids : the use of ecdysone (*Myrmica*) or of ecdysterone (*Pheidole*) dramatically lowers the percentage of sexual larvae. It can also be observed that in *Pheidole* the level of ecdysteroids is higher in the queens laying eggs with worker orientation than in those laying eggs with sexual orientation. The same is true of the eggs themselves.

The juvenile hormones and the ecdysteroids thus have antagonistic roles. Their relative variations which are controlled by external factors, such as the composition of the colony or climatic conditions determine the orientation of the brood.

RÉSUMÉ

Régulation endocrine de la détermination des castes chez les fourmis

Les travaux effectués chez *Solenopsis invicta*, *Myrmica rubra*, *Pheidole pallidula*, *Plagiolepis pygmaea* et *Aphaenogaster senilis* conduisent à préciser le rôle joué par les hormones lors de la détermination des castes chez les fourmis.

Rôle des JH : à forte concentration, elles retardent la métamorphose et déterminent des troubles morphogénétiques pouvant faire disparaître le couvain. A doses plus faibles elles ont un rôle dans la sexualisation des larves. Les JHA distribués soit mélangés à la nourriture, soit par applications locales ou encore par injections entraînent la formation de larves sexuées, surtout femelles, dans la société. Les stades sensibles à la JH sont variables selon les espèces. *Pheidole pallidula* est la plus précoce, la détermination se produisant pendant l'ovogenèse ; chez *Solenopsis invicta* c'est la larve du premier stade qui réagit à l'apport de JH, alors que chez *Myrmica rubra* c'est celle du dernier stade. Les ligatures et allatectomies (*Myrmica*), l'utilisation du Précocène (*Pheidole*) en éliminant les CA ou en bloquant leur fonctionnement favorisent au contraire l'apparition des ouvrières.

Rôle des ecdystéroïdes : un apport d'ecdysone (*Myrmica*) ou d'ecdystérone (*Pheidole*) diminue fortement le pourcentage des larves sexuées. Chez *Pheidole* on note d'ailleurs que le taux des ecdystéroïdes est plus élevé chez les reines pondeuses d'œufs à orientation ouvrière que chez celles pondeuses d'œufs à orientation sexuée. Il en est de même pour les œufs eux-mêmes.

Les hormones juvéniles et les ecdystéroïdes ont ainsi un rôle antagoniste. Leurs variations relatives réglées par les facteurs externes comme la composition des sociétés ou les conditions climatiques déterminent l'orientation du couvain.

INTRODUCTION

The study of caste determination in ants has been mainly centred, over the last few years, around the importance of social factors (composition of societies) and environmental restrictions (climatic factors). The best known species in this respect are the *Formica* of the group *rufa* (Gösswald et Bier, 1953, 1954a, 1954b ; Bier, 1954, 1958 ; Schmidt, 1974), *Myrmica rubra* (Brian, 1974a, 1979), *Leptothorax nylanderi* (Plateaux, 1971) et *Plagiolepis pygmaea* (Passera, 1969, 1974). From these investigations and those concerning 20 or so other species, the following general rules can be drawn :

- Orientation usually occurs at the last instar suggesting trophogenic determinism (with one exception) ;
- The queen or queens have an inhibiting action, of varying degree, on the development of potential queen larvae.

– The winter or seasonal dormancy period of the nursing workers and of the larvae (societies with a dormant brood) is indispensable for the appearance of sexual brood.

– The larvae are orientated by the workers which give a diet adapted to the chosen caste.

These investigations tell us WHEN and HOW a larva becomes orientated towards one caste or another but studies giving an indication of WHY are much more rare. Attention is required not in the direction of the whole colony but rather towards the individual and in particular its physiology.

CYTO-ANATOMICAL DATA

In adults

In various species the size of *corpora allata* was studied and authors found that the volume of the glands was proportional to the size of the ants : they are larger in queens than in worker (Hultin, 1947 ; Gawande, 1968 ; Bressac and Bitsch, 1969).

In larvae

In *Myrmica rubra*, Brian (1959) noted that the CA of the queen larvae – although larger than those of the workers in absolute terms – are no longer so when considered in relative terms. A similar modification was noted by Lappano (1958) in the larvae *Eciton burchelli* a species with widespread polymorphism among the workers : the size of the CA is directly proportional to that of workers themselves.

In *Plagiolepis pygmaea* (Suzzoni and Grimal, 1981), the results are as follows :

Worker biased larvae : the absolute volume remains almost stable (about 4 000 μm^3) throughout the determination phase (fig. 1). The weight increase of the larvae during this period being moderate, the weighted volume of the CA decreases fairly slowly (fig. 1). The number of cells seems to be constant varying between 7 and 8. The cell volume (fig. 2) is fixed between the end of hibernation and the pharate pupal stage at 500 μm^3 .

Queen biased larvae : the absolute volume (fig. 3) increases three fold going from 3 000 μm^3 , when activity starts again, to 9 500 μm^3 , at the pharate pupal stage. The ratio is therefore 1.7 in favour of queen larvae. The growth of the sexed larvae being particularly rapid, the weighted volume (fig. 3) decreases faster than in worker larvae. The number of cells increases slightly

from 7 or 8 to 9 or 10 but the most remarkable fact is that the cell volume passes from 500 to 1 240 μm^3 (fig. 2). Overall, it can be noted that the characteristics of the CA in both series show differences which could be explained by differing physiological activities.

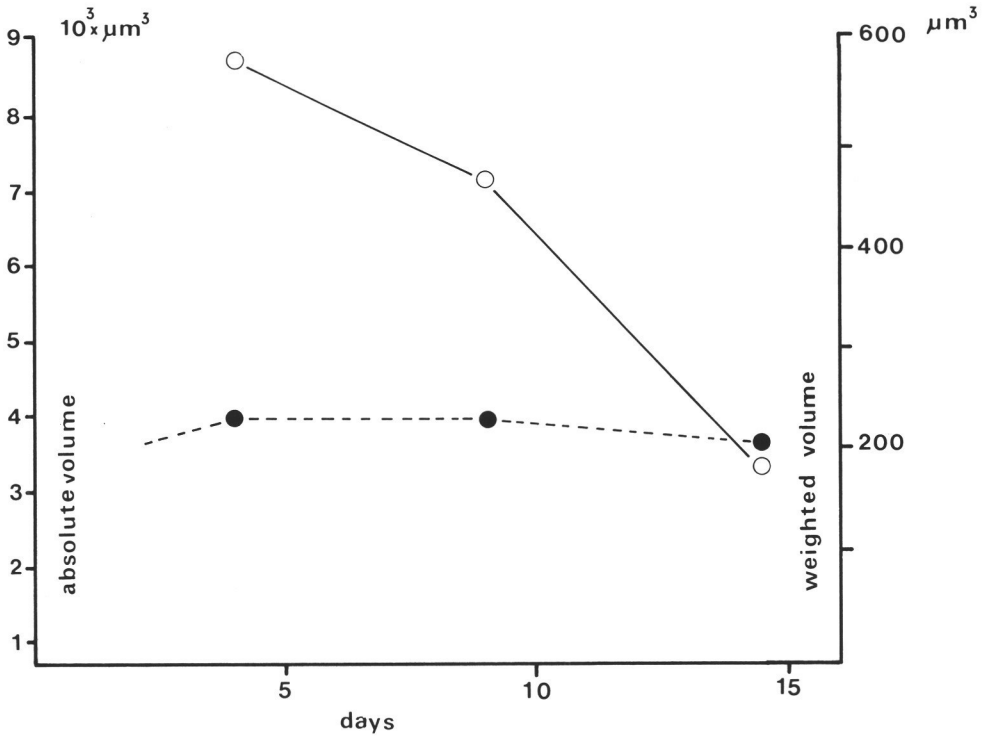


Fig. 1 — Corpora allata volume of the worker-biased larvae according to the age from the end of the hibernation to the naked prenymphe stage (*Plagiotelepis pygmaea*). Broken line : absolute volume in $10^3 \mu\text{m}^3$. Solide line : weighted volume in μm^3 . From Suzzoni and Grimal (1981).

Fig. 1 — Volume des corps allates des larves-ouvrières en fonction de l'âge depuis la sortie d'hibernation jusqu'au stade prénymphe chez *Plagiotelepis pygmaea*. Trait pointillé : volume absolu en $10^3 \mu\text{m}^3$; trait plein : volume pondéré en μm^3 . D'après Suzzoni et Grimal (1981).

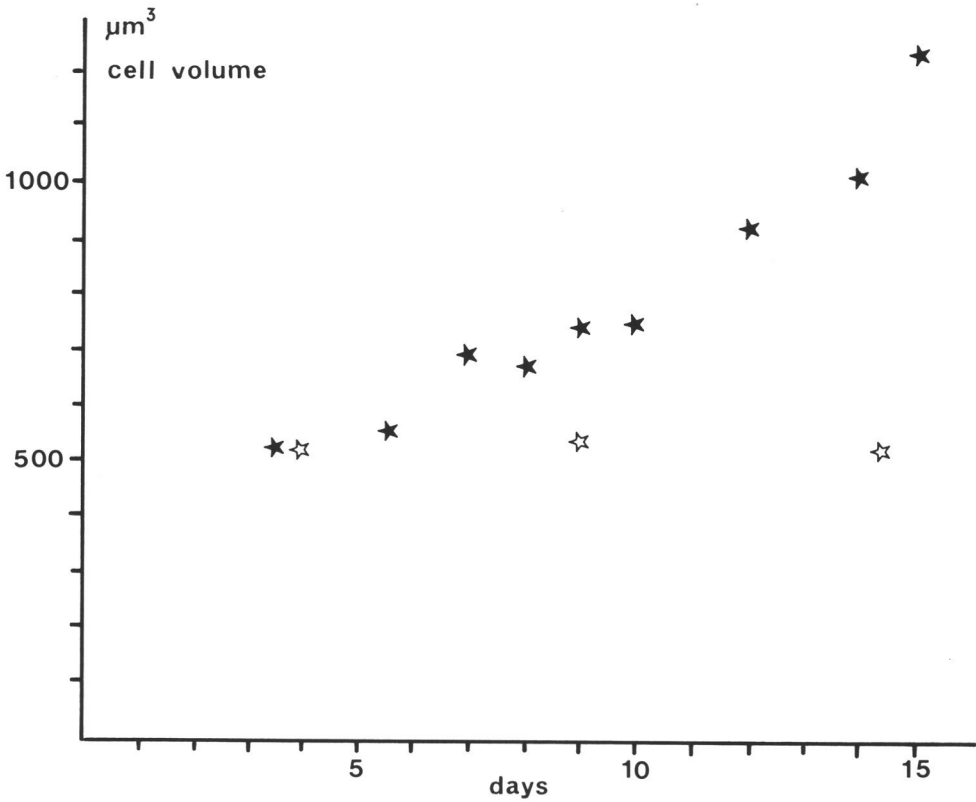


Fig. 2 - Evolution of the mean cellular volume (absolute volume /cell number per corpus allatum) in μm^3 during the development between the end of hibernation and the naked prenymp stage (*Plagiolepis pygmaea*). Black stars : queen-biased larvae ; white stars : worker-biased larvae. From Suzzoni and Grimal (1981).

Fig. 2 - Evolution du volume cellulaire moyen (volume absolu/nombre de cellules par corps allate) en μm^3 au cours du développement depuis la sortie de l'hibernation jusqu'au stade prénymphe chez *Plagiolepis pygmaea*. Etoiles noires : larves à orientation reine ; étoiles blanches : larves à orientation ouvrière. D'après Suzzoni et Grimal (1981).

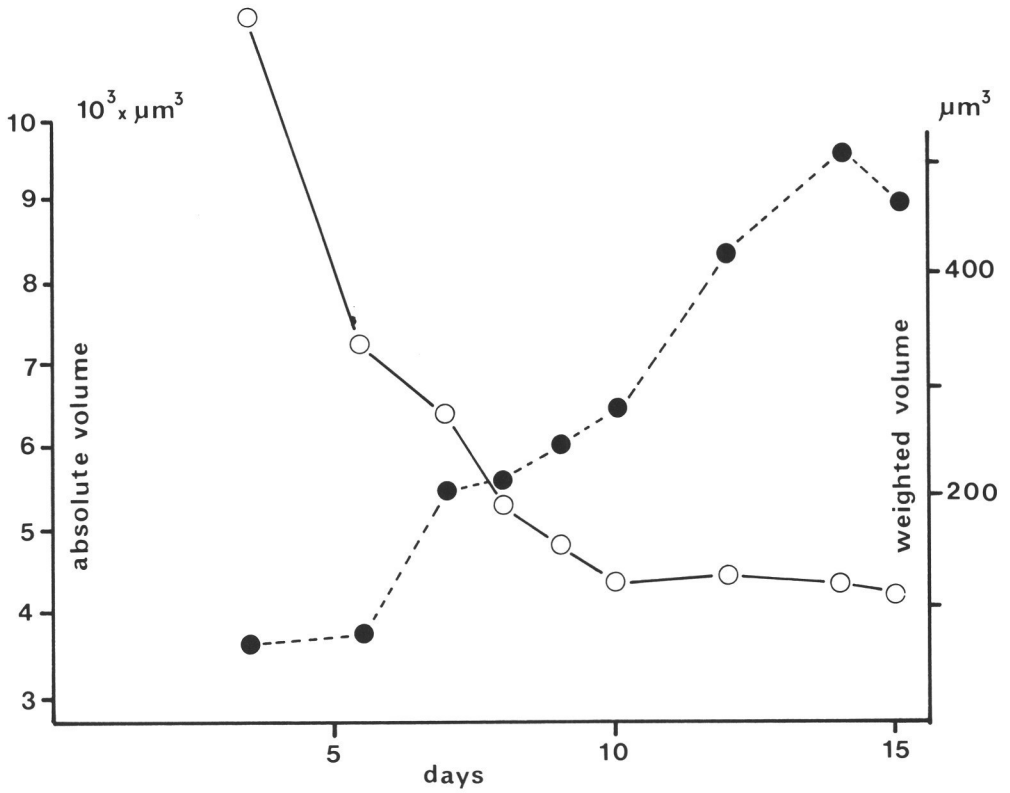


Fig. 3 – Corpora allata volume of the queen-biased larvae according to the age from the end of the hibernation to the naked prenymphe stage (*Plagiotelepis pygmaea*). Broken line : absolute volume in $10^3 \mu\text{m}^3$; solid line : weighted volume in μm^3 . From Suzzoni and Grimal (1981).

Fig. 3 – Volume des corps allates des larves-reines en fonction de l'âge depuis la sortie d'hibernation jusqu'au stade prénymphe chez *Plagiotelepis pygmaea*. Trait pointillé : volume absolu en $10^3 \mu\text{m}^3$; trait plein : volume pondéré en μm^3 . D'après Suzzoni et Grimal (1981).

THE ACTIVITY OF THE JUVENILE HORMONES

*The case of Myrmica rubra**Topical applications and injections*

Brian (1974b, 1976) used post-wintering larvae which are queen-potential. They received JHA's as topical applications or as injections. Metamorphosis was clearly retarded in all treated larvae. All the nymphs obtained from both treated and control larvae were queens but the former weighed more than the latter. In another series of experiments the author chose average-sized post-wintering larvae known to have less chance of developing into queens than their larger counterparts. Here, he obtained 84 % queen nymphs in the treated group and only 60 % in the controls. So JH analogues retard metamorphosis, prolong the growth period – and thus increase the size of the queens – and increase the percentage of larvae which become transformed into queens.

Ligature and corpus allatum extraction

Comparison of the imaginal discs of legs and wings : the large post-wintering larvae used in these tests are queen-potential and so possess imaginal wing discs. The degree of sexualization of the larvae can be judged by measuring the surface areas of the wing buds and comparing them with those of the leg buds 6 days after treatment : as sexualization increases the rate of growth of the imaginal wing discs increases whereas that of the legs is slowed down.

The treated larvae either undergo cephalic ligature short-circuiting the CA or removal of the CA. Under these conditions, the surface area of wing buds in the control animals was either equal to that of the leg buds (60 % of the cases), or bigger (in 40 % of cases), and was never smaller ; the wings develop therefore more quickly than the legs. On the other hand in the group of treated animals the surface area of the wing buds was either equal to that of the leg buds (in 33 % of cases) or smaller (in 67 % of cases) and was never larger ; therefore the absence of the CA or their short-circuiting causes an stoppage in the inhibiting action controlling growth and differentiation of the legs.

Leg segmentation : in this experiment the development of the imaginal leg discs was estimated by counting the number of segments ; rapid segmentation taking place at the expense of imaginal wing disc growth indicating a difficult or at least retarded sexualization. The results show that for cephalic ligature isolating the CA, a much higher degree of segmentation is noticeable

than in the control group. Furthermore it is possible to retard the differentiation of the legs by immersing the ligatured animals in solution of JH analogues. These experiments confirm the previous ones as to the role of JH ; it retards growth of the leg buds in favour of growth of the wing buds. JH has therefore a positive action on sexualization.

The case of *Aphaenogaster senilis*

Topical applications of a JHA are performed on larvae of varying ages in the presence of their queen (Ledoux, 1976). In the control group the queen caused total inhibition, only workers resulted. In the treated group the appearance of gynaecoid workers and even a few nymphs was observed.

The case of *Solenopsis invicta*

Action on mortality and morphogenesis

Cupp and O'Neal (1973), Vinson et al. (1974), Troisi and Riddiford (1974), Vinson and Robeau (1974) treated a brood of *Solenopsis* with two JHA's, ZR 512 and ZR 515 (methoprene). The results concern the action on larval mortality and disturbances noticed during metamorphosis. Larvae develop into nymphs without legs or antennae, sometimes having large mandibles without teeth. Some of these nymphs develop into imagos which are carriers of abnormalities, in particular with relation to the legs.

Action on caste

By submitting the colony to the action of JHA, Troisi and Riddiford (1974) obtained noteworthy results concerning the equilibrium of the societies. ZR 512 injected at the rate of 100 μg /cockroach fed per week results in fewer larvae but, more important, it was noticed that 10 days after the beginning of this treatment 75 % of the larvae were of the sexual type, most of them developing into males ; the workers only forming 25 % of the total. With an even lower dose (10 μg /cockroach per week) the number of larvae produced becomes normal again and the brood is composed of 25 % males against 75 % workers. In all these cases the colonies both before the beginning and after the end of treatment only produced workers. As to ZR 515, it appears that although it too lowers the larval population it seems less effective in producing winged animals. If the drop in larval production can be explained by the action of JHA on the larvae, the appearance of numerous males at the

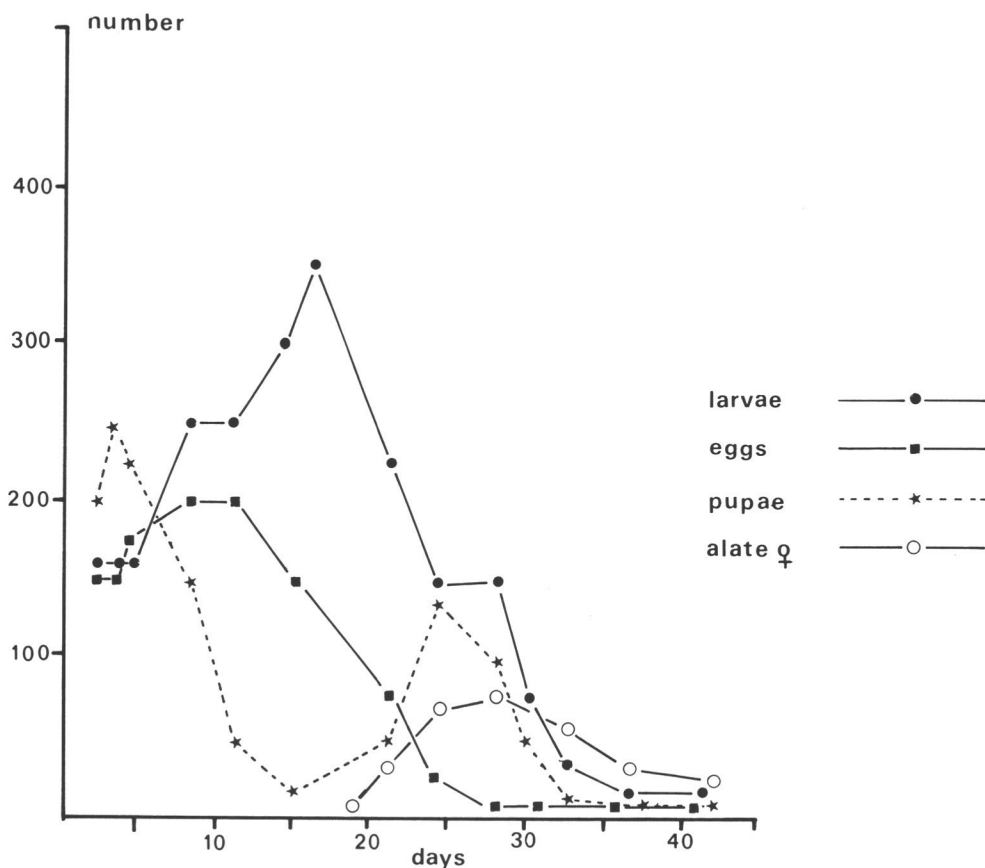


Fig. 4 – Number of imported fire ants in each stage after feeding the colony doses of JHA in soybean oil (50 mg/colony). From Vinson and Robeau (1974).

Fig. 4 – Evolution numérique du couvain de *Solenopsis invicta* dans une société nourrie avec de l'huile de soja contenant des analogues de la JH (50 mg/colonie). D'après Vinson et Robeau (1974).

expense of workers tends to indicate an action on the queen which would then lay unfertilized male eggs.

The use by Vinson and Robeau (1974) of 6 JHA's mixed with food in doses varying from 0.05 to 50 mg/ml provides interesting results. Whatever JHA used, at whatever dose there is always production of sexual larvae 20-30 days after the beginning of the treatment (fig. 4). In the controls sexual larvae were never produced, these colonies always being quite young. Like the rest of the brood large numbers of these larvae died before the nymph

stage ; those that survived and reached the nymph stage were all queens and nymphosed into winged queens. This result is obviously different to that of Troisi and Riddiford who obtained males. Thus incorporation of JHA into the food has the effect of sexualizing a part of the brood of *Solenopsis*.

In a subsequent phase the same authors (1976) attempted to determine whether the sexualization of the brood is due to an effect of the JH on the queen and the nature of the eggs laid or if the effect takes place later, during the larval stages. To this end they treated the queen of a colony by topical application. After a 2 days pause in laying, the eggs produced developed normally and the larvae all become workers. In another series of tests, the colonies possessing a brood in all stages but lacking their queen were treated by JHA. In the days that followed a drop in the number of larvae and nymphs of «minor» workers was noticed, followed by their disappearance ; during this time however larvae and nymphs of major workers and queens were produced. In the control colonies there was never production of either «major» workers or queens. The absence of sexualization when only the queen was treated lead one to think that the JH does not act during ovogenesis, but later during larval development.

Banks et al. (1978) looked for the stage which is sensitive to the action of JH. The eggs laid in a colony fed with JH oil solution were separated into 2 groups ; the first was reared by workers which were fed normally ; they all gave rise to worker nymphs ; the second group was reared by workers which were fed JH and it resulted in sexual larvae. Therefore JH has no action at all on queens or on eggs. Furthermore larvae in the third or fourth stages present in the nest at the time of treatment developed into worker larvae. It appears therefore that the first larval stage and perhaps the second are sensitive to action by JHAs.

Distribution and circulation of JH

The distribution and circulation of the JHA's in the colonies was followed using radioactive analogues (Wendel and Vinson, 1978 ; Bigley and Vinson, 1979). In the case of topical applications the rates of absorption, then of degradation and lastly of excretion are found in the following decreasing order : workers, larvae, nymphs and prenympths, each category having a means of degradation of its own. When the JHA is mixed with the food the workers are immediately contaminated and then lose the compound at a steady rate of which only the metabolites are found 34 days later (fig. 5). The larvae presented maximum contamination 1.5 days after the introduction of the radioactive JHA ; they had at that point 7 % of the total radioactivity which they then lost progressively. Although it appears normal that the nymphs

which are not fed are practically free from contamination, it is more surprising in the case of the queens. In any case it confirms that JH acts on the larvae and not on the queen since she is not involved in the circulation of the labelled product.

Briefly, JHA's introduced into a colony have a double action in *Solenopsis invicta* :

- Actions on metamorphosis : they bring about the disappearance of developing «minor» worker larvae and nymphs and determine morphogenetic abnormalities of rescued nymphs ; in addition at high doses they can slow down or stop the queen laying ;

- Action on the determination of castes : if the dose administered is moderate enough to allow the queen to lay, the larvae which develop will give rise to «major» workers but above all sexual males and/or females.

The appearance of winged males implies an action on the queen which then lays unfertilized eggs ; the appearance of winged females results from action by the JHAs on the young brood in the first or second stages, the queen no longer being concerned.

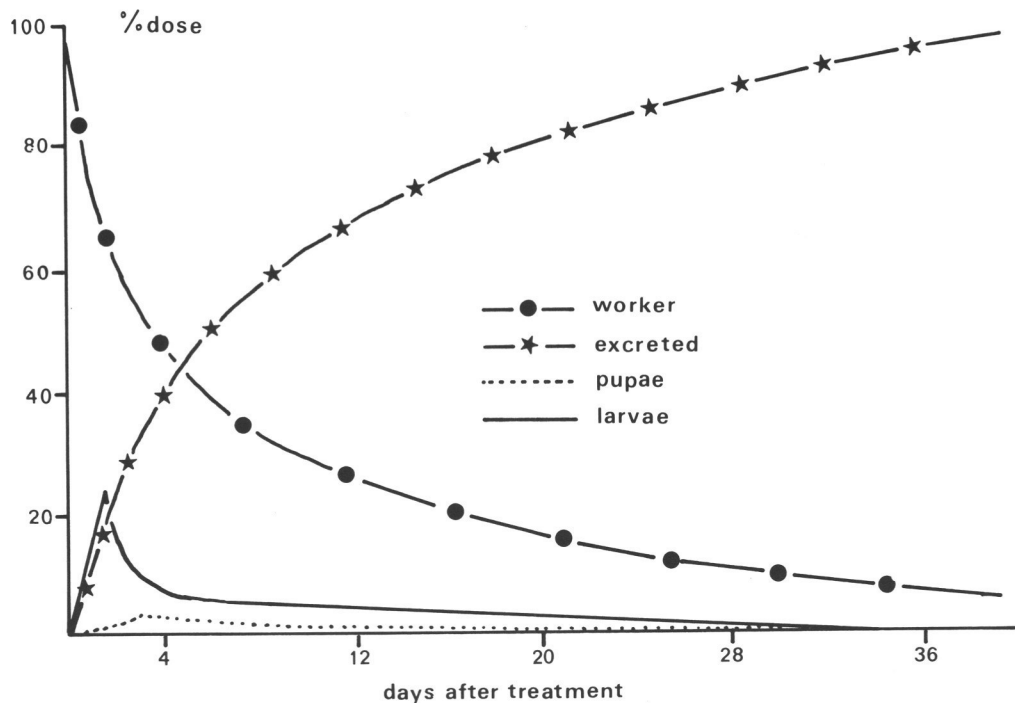


Fig. 5 - Distribution of radioactivity after feeding of the juvenile hormone analogue R-20458 formulated in soybean oil to adults within colonies of the imported fire ant. From Wendel and Vinson (1978).

Fig. 5 - Evolution de la radioactivité dans des colonies de *Solenopsis invicta* nourries avec de l'huile de soja contenant un analogue de la JH (R-20458). D'après Wendel et Vinson (1978).

The case of *Pheidole pallidula*

Sexual individuals result here from the first brood of spring : at the end of hibernation most of the queens lay biased eggs which all develop into sexed larvae, even in bad breeding conditions. Later the eggs are all worker biased (Passera, 1980). This characteristic makes it possible to use eggs with a known future : sexual or workers.

Treatment of whole societies

Whole societies were experimented on more than one month after the end of hibernation, that is to say when they were only producing worker or soldier broods. They were fed with *Tenebrio* larvae which had received an injection of 10 μg of a JHA (Ayerst's AY 22, 342, a mixture with 8 isomers of JHI). The eight societies treated produced a sexed brood between the second and sixth week following the beginning of the treatment whereas the controls only produced worker and soldier broods (Passera et Suzzoni, 1978a).

Treatment of queens

JHA dissolved in acetone or olive oil was used in topical applications at weekly doses of 1 μg , 0.1 μg or 0.01 μg (Passera and Suzzoni, 1978b, 1979a). The treatments were carried out one month after the end of hibernation, after the period during which the sexual brood appeared. 28 colonies out of 29 once more gave rise to sexual larvae. The result was especially remarkable for the 11 colonies which had not given rise to sexual larvae at the end of hibernation.

The controls however continued to produce workers. Sexualization appears not to depend on the nature of the solvent but rather on the dose of JHA (fig. 6), 0.01 μg is close to the effect threshold. More often than not sexed larvae appeared during the third week following treatment ; this rather long interval shows that JHA acts on queens during ovogenesis ; indeed if it were a case of action on the larvae the effect would be immediate ; there being brood at all stages when the first application was given. Besides, eggs collected during the few hours quarantine undergone by the queens at the time of each treatment, develop in part into sexual larvae when they are placed in untreated queenless colonies. Therefore the ovary in the queens appears to be sensitive to the hormone environment.

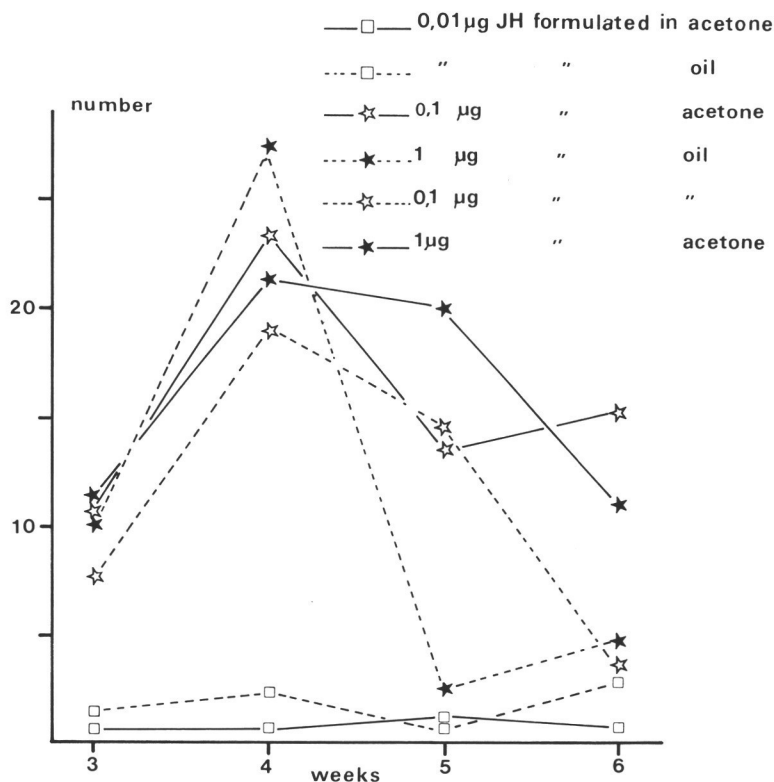


Fig. 6 — Effets of topical applications on queens (*Pheidole pallidula*) according to mode of JH administration. Along the abscissae : number of weeks after the beginning of the treatment. Along the ordinates : average number of sexual larvae produced in each treatment.

Fig. 6 — Effets des applications topiques des reines de *Pheidole pallidula* en fonction des modes d'administration de la JH. En abscisses : nombre de semaines après le début du traitement ; en ordonnées : nombre moyen de larves sexuées produites pour chaque traitement.

Treatment of the brood

Brood taken from colonies producing workers and soldiers is distributed to colonies of queenless workers. The eggs and the larvae of the three stages were immersed in a 0.5 % solution of JHA (Passera and Suzzoni, 1979a) for a few seconds. When eggs were the subject of the treatment, sexual larvae resulted six times out of seven. On the other hand treated larvae of the first, second and third stages only gave rise to workers. It appears then that JH acts only on eggs, either during ovogenesis, before laying, or during embryogenesis.

However the larvae of the three stages, whether they were treated directly or fed by treated workers showed no tendency to sexualization. The caste is therefore finally fixed in the egg in *Pheidole pallidula*.

A comparison of the activity of the 3 JHs and the anti-JHs

Weekly applications of 0.1 μg of JHI, JHII and JHIII were carried out on queens which belonged to societies producing worker biased larvae. Over a period of 5 weeks larvae were collected as soon as they became identifiable. As before, sexualization of the brood was obtained (Passera and Suzzoni, 1979b). It can clearly be seen (fig. 7) that when the average number of sexual larvae collected every week in the different colonies is

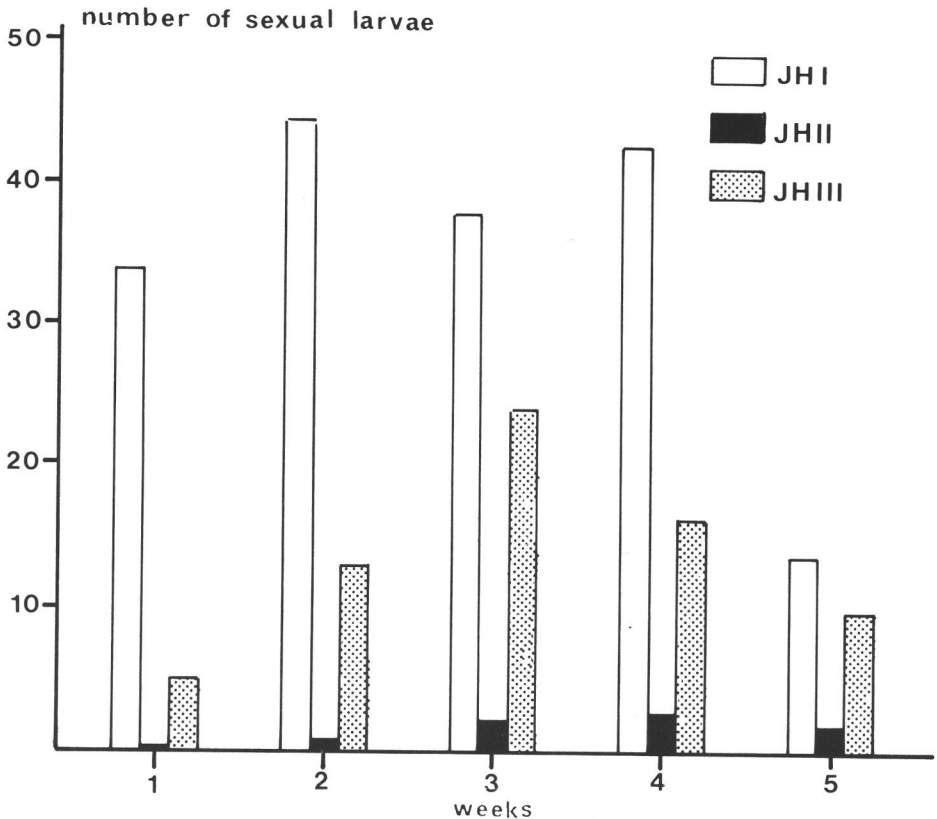


Fig. 7 — Compared effects of JHI, JHII and JHIII on appearance of sexual larvae after topical treatments on the queens (*Pheidole pallidula*). Along the abscissae, the weekly date. Along the ordinates, the number of sexual larvae produced (average by colony).

Fig. 7 — Action comparée des JHI, JHII et JHIII sur l'obtention de larves sexuées après applications topiques des reines de *Pheidole pallidula*. En abscisses, les relevés hebdomadaires ; en ordonnées, le nombre de larves sexuées obtenues (moyenne par élevage).

compared, JHI is the most effective. Each culture treated with JHI produced on average 172 sexual larvae whereas, under the same conditions, only 68 were obtained with JHII. If the results are expressed as percentages of sexual larvae with respect to the whole brood 53 % of the brood were sexual with JHI, 1,6 % with JHII and 24.7 % with JHIII.

The role of the *corpora allata* appears to be fundamental in the formation of castes in *Pheidole pallidula*. The very small size of the queens made excision of the CA impracticable and this difficulty was overcome by the use of topical applications of Precocene II, a substance which brings about degeneration or atrophy of the CA. The queens were treated twice during hibernation, once at the time of the break in hibernation and once two days later (1.5 μg Precocene : 1 μl Acetone). After 3 days of laying the queen was eliminated and the composition of the brood noted. Whereas 79 % of the untreated nests yield sexual larvae after hibernation this percentage fell to 33 % in the case of cultures treated with Precocene. If one considers the number of sexual larvae collected with respect to the total brood produced, 54 % sexual larvae were present in the untreated cultures as against 7 % in treated cultures. These figures suggest an inhibitory action by Precocene but they must be interpreted with caution because acetone which was used as the vehicle also lowers the sexualization of the brood. *Pheidole pallidula* was shown to be fairly different to *Myrmica* or to *Solenopsis*.

– at the doses used no action, either on metamorphosis or on morphogenesis, was noticed : in particular intercastes never appeared ;

– the sensitive stage is very early because sexualization results from an intervention during oogenesis. The larval stages are not involved, as opposed to observations made on *Myrmica* or *Solenopsis*.

ACTIVITY OF ECDYSTEROIDS

The case of *Myrmica rubra*

Ecdysone in solution

Large post wintering larvae with queen potential were injected with a solution of ecdysone in a water-ethanol mixture. In 9 groups out of 14 metamorphosis occurs earlier and in 3 groups Brian (1974b) obtained a higher percentage of workers (44 %) than in the control groups (9 %).

Crystallized ecdysone

In this experiment 1 to 2 μg of ecdysone in its crystalline form was implanted per post-wintering larva. The early treatment of larvae during the pre-segmentation period appears to be ineffective. On the other hand a later

implantation during the segmentation period gave a double result : metamorphosis is accelerated and a higher proportion of workers and a lower number of queens was obtained. Therefore in the treated groups 29 % worker nymphs, 47 % intercaste nymphs and 24 % queen nymphs were obtained as opposed to 11 %, 33 % and 56 % respectively, in the control groups. Furthermore queens obtained after treatment were very small and can be considered as microgynes. The sum up, ecdysone treatment leads to early metamorphosis and favours the appearance of workers whereas the few queens produced were very small.

The case of *Pheidole pallidula*

Treatment of queens by topical application at the end of hibernation, when they are potential layers of sexual eggs, was carried out using ecdysterone dissolved in a mixture of acetone and ethanol. The fertility of the queens does not appear altered because their average production during the first 5 days following hibernation was the same in the treated cultures and in the control cultures. On the other hand the differences concerning the construction of the castes are significant because an important drop was noticed in the number of cultures with sexed progeny : 69 % for untreated cultures as against 31 % for cultures treated with ecdysterone. The average number of sexed larvae per culture fell from 37 in the controls to 11 in the treated groups. Ecdysteroids appear to slow down the sexualization of the brood thus opposing the action observed with JH.

This hypothesis received some support from a study aimed at assaying ecdysteroids using radio-immunoassay techniques with microdialysis (Suzzoni, Passera, Strambi, 1980). The queens which laid worker biased eggs showed higher levels of ecdysteroids and other substances co-migrating with ecdysone and ecdysterone than the queens which laid queen biased eggs (fig. 8). The same assays carried out on eggs with different potentials and laid at different points in the cycle also support this hypothesis (fig. 9).

– like the queens, it was the worker biased eggs that were richest in ecdysteroids ;

– the comparison of worker biased eggs laid at the beginning of the season, with other laid in the middle of the season show that the latter possess more ecdysteroids than the former. Furthermore it is in the middle of the activity period that the ant nest produces most workers.

Therefore in *Pheidole pallidula* the appearance of sexual larvae seems to be assisted by a high level of JH whereas the appearance of workers coincides with a high level of ecdysteroids.

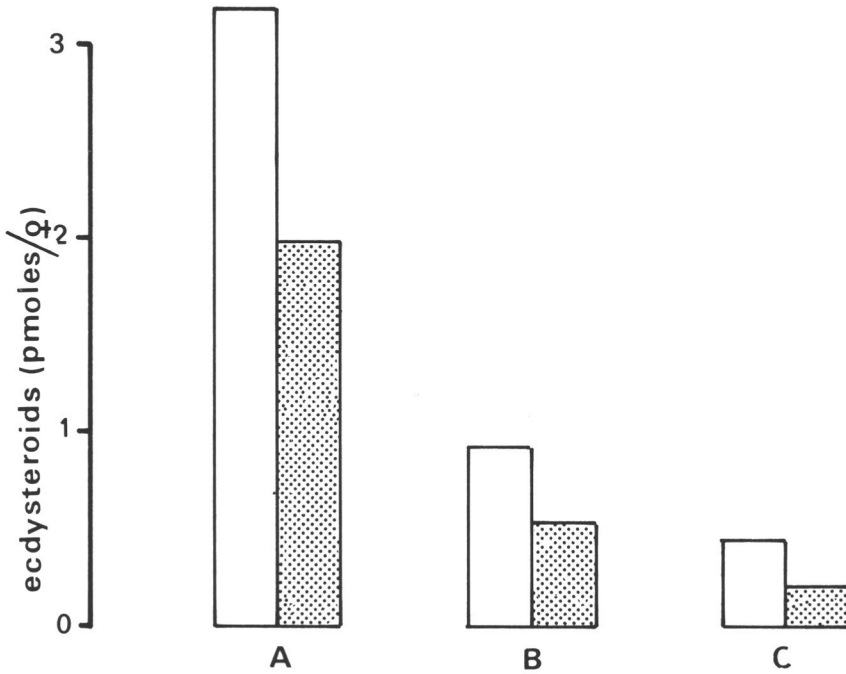


Fig. 8 – Ecdysteroid titred in queens (*Pheidole pallidula*) expressed as pmoles of ecdysterone equivalent per 1 queen. A : total ecdysteroids – B : ecdysone – C : ecdysterone. Open bars : queens laying worker-biased eggs. Spotted bars : queens laying queen-biased eggs.

Fig. 8 – Dosages des ecdystéroïdes chez les reines de *Pheidole pallidula* exprimés en pmoles d'équivalent ecdystérone pour une reine. A : ecdystéroïdes totaux – B : ecdysone – C : ecdystérone. Colonnes blanches : reines pondéuses d'œufs à orientation ouvrière. Colonnes pointillées : reines pondéuses d'œufs à orientation sexuée.

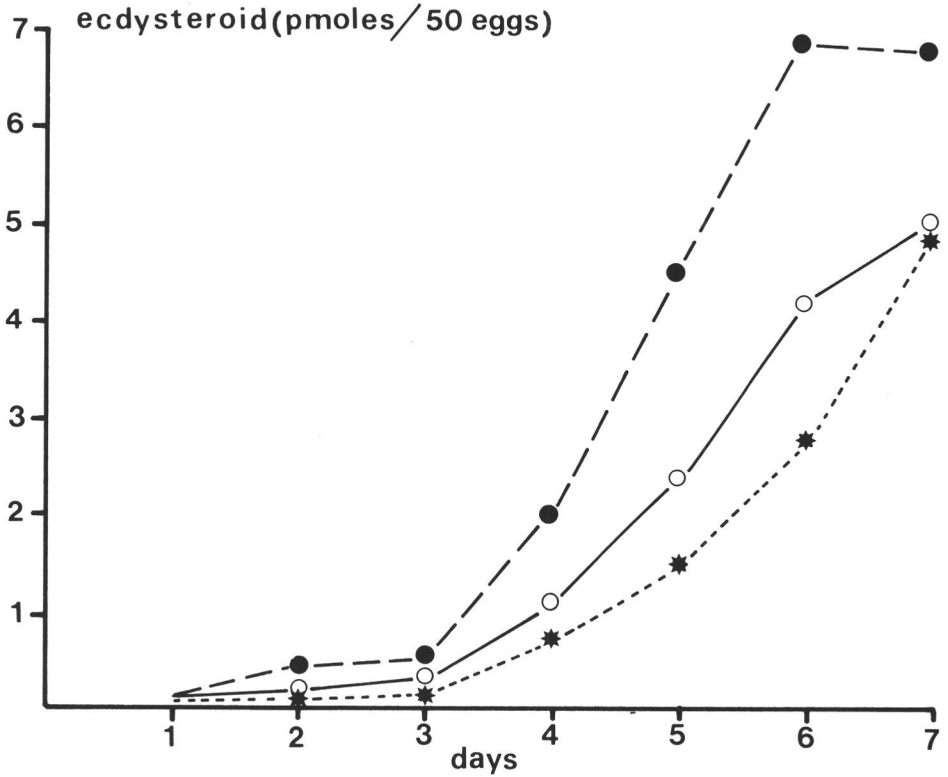


Fig. 9 — Evolution of the ecdysteroid rates during the embryonic development (*Pheidole pallidula*). Ordinate : ecdysteroids in pmoles equivalent-ecdysterone per 50 eggs. Abscissa : age in days. Spotted line : queen-biased eggs laid at the end of hibernation ; solid line : worker-biased eggs laid at the end of hibernation ; broken line : worker-biased eggs laid 1 month later.

Fig 9 — Evolution du taux des ecdystéroïdes pendant le développement embryonnaire (*Pheidole pallidula*). Ordonnées : ecdystéroïdes en pmoles d'équivalent ecdystérone pour 50 œufs ; Abscisses : l'âge des œufs en jours. Traits pointillés : œufs à orientation sexuée pondus à la fin de l'hibernation ; traits continus : œufs à orientation ouvrière pondus à la fin de l'hibernation ; traits interrompus : œufs à orientation ouvrière pondus 1 mois plus tard.

DISCUSSION

The sex determination role played by JH's appears well-established in experimental conditions in several ants. It is generally consistent with that found in other social Hymenoptera : *Apis* (Wirtz and Beetsma, 1972 ; de Wilde, 1976 ; Goewie, 1978 ; Beetsma, 1979), *Bumblebees* (Röseler, 1977), *Melipons* (Velthuis and Velthuis-Kluppel, 1975 ; Campos, 1978), *Trigons* (Campos, 1979). The phenotypic expression of caste could result from the activation of «queen» genes by juvenile hormone.

The point in the cycle where the biological material becomes sensitive to the JH is variable according to the species. In *Pheidole pallidula* it is the earliest because the JH must be supplied during ovogenesis, therefore via the maternal organism. In *Solenopsis invicta* only larvae in the first stage and perhaps in the second are affected. The same is found in *Aphaenogaster senilis*, the earlier stages being more readily affected by JH than the later stages. Lastly, in *Myrmica rubra* however, it is the third and fourth stages which can be affected. The sensitive period must, furthermore, be very short and difficult to determine. Without doubt this is what explains the failures experienced using certain species whose brood orientation it has so far been impossible to modify in spite of massive doses of JHA : e.g. *Plagiolepis pygmaea*, *Temnothorax recedens*, *Solenopsis fugax*, *Iridomyrmex humilis* (Passera and Suzzoni, unpublished).

Until now it has only been possible to study the action of exogenous juvenile hormone in ants. A supplementary proof could be provided by assaying the endogenous JH's circulating at different moments of the cycle as has been done in *Apis* (Lensky et al., 1978) but the difficulty of obtaining a large enough volume of material in *Pheidole* is a serious obstacle. Another argument in favour of the CA having specialized functions was suggested by the results obtained using antiallatotropic substances in *Pheidole pallidula*. Precocene II prevents sexualization of the brood ; these results are similar to those obtained by Goewie et al. (1978) in *Apis* : the queen biased larvae which were treated with Precocene II develop into workerlike intermediates, following atrophy of the CA. Other less detailed results were obtained again in *Apis* by Dietz et al. (1979) : Precocene II in association with JH increased slightly the number of workers obtained (83 % as against 79 %) but the preparation was applied late after the orientation period.

Less is known about ecdysteroids because data is restricted to *Myrmica rubra* and *Pheidole pallidula* ; however the theory that they act in an opposite direction to JHA by favouring the production of workers, appears well-established. This being the case, in *Pheidole*, eggs rich in juvenile hormones

and poor in ecdysteroids give rise to worker larvae. A similar hypothesis based on the relative variations of JH and ecdysteroids has already been proposed for Termites by Lüscher (1976) and by Noirot (1979). Caste determination could therefore result from a succession of two series of factors ; first of all external factors related to the composition of the societies, to the quantity of available food and also the circulation of royal pheromones ; these external factors would have then as target the endocrine organs at the sensitive stage. These organs react by giving priority to either of the two antagonistic sets of hormones.

References

- BANKS W.A., LOFGREN C.S., PLUMLEY J.K., 1978. — Red imported fire ants : effects of insect growth regulators on caste formation and colony growth and survival. *J. Econ. Entomol.*, 71, 75-78.
- BIER K., 1954. — Ueber den Einfluss der Königen auf die Arbeiterinnen-Fertilität im Ameisenstaat. *Ins. soc.*, 1, 7-19. — 1958 — Die regulation der Sexualität in den Insektenstaat. *Ergebn. Biol.*, 20, 97-126.
- BIGLEY W.S., VINSON S.B., 1979. — Degradation of (¹⁴C) methoprene in the imported fire ant, *Solenopsis invicta*. *Pest. Bioch. Physiol.*, 10, 1-13.
- BRIAN M.V., 1959. — The neuro-secretory cells of the brain, the *corpora cardiaca* and the *corpora allata* during caste differentiation in an ant. In *The ontogeny of insects. Acta symposii de evolutione insectorum*, Praha, 167-71. — 1974a — Kastendetermination bei *Myrmica rubra* L. In *Sozialpolymorphisme bei Insekten*, 565-89, Ed. G.H. Schmidt, Wiss. Verlagsges, Stuttgart. — 1974b — Caste differentiation in *Myrmica rubra* : the role of hormones. *J. Insect Physiol.*, 20, 1351-65. — 1976 — Endocrine control over caste determination in a Myrmicine ant. In *Phase and Caste Determination in Insects*, 63-70. Ed. M. Lüscher, Pergamon Press, New York. — 1979 — Caste differentiation and division of labor in Social Insects, vol. I, 122-222, Ed. H.R. Hermann, Academic Press, New York.
- BRESSAC C., BITSCH J., 1969. — Observations sur la structure du système nerveux céphalique (cerveau, masse sous-œsophagienne et complexe rétro-cérébral) de la fourmi *Aphaenogaster senilis* (Mayr, 1853) (Hymenoptera, Myrmicinae). *Ins. soc.*, 16, 135-48.
- CAMPOS L.A.O., 1978. — Sex determination in bees. VI. Effect of a juvenile hormone analogue in males and females of *Melipona quadrifasciata* (Apidae). *J. Kansas Entomol. Soc.*, 51, 228-34. — 1979 — Determinação das castas em *Partamona cupira* (Hymenoptera, Apidae). Papel do hormônio juvenil. *Ciencia e Cultura*, 31, 65-69.
- CUP E.W., O'NEAL J. 1973. — The morphogenetic effects of two juvenile hormone analogues on larvae of imported fire ants. *Environ. Entomol.*, 2, 191-94.
- DIETZ A., HERMANN H.R., BLUM M.S., 1979. — The role of exogenous JH I, JH III and anti-JH (precocene II) on queen induction of 4-5 day-old worker honey bee larvae. *J. Insect. Physiol.*, 25, 503-12.
- GAWANDE R.B., 1968. — A histological study of neurosecretion in ants. *Act. ent. bohemoslov.*, 65, 349-63.

- GOEWIE E.A., 1978. — Regulation of caste differentiation in the honey bee (*Apis mellifera* L.). *Meded. Landbouwhogeschool. Wageningen*, 78-15, 1-75.
- GOEWIE E.A., BEETSMA J., de WILDE J., 1978. — Wirkung von Prececone II auf die Kastendifferenzierung der Honigbiene (*Apis mellifera* L.). *Mitt. dtsh. Ges. allg. angew. Ent.*, 1, 304-05.
- GÖSSWALD K., BIER K., 1953. — Untersuchungen zur Kastendetermination in der Gattung *Formica*. *Naturwissenschaften*, 40, 38-39. — 1958a — Untersuchungen zur Kastendetermination in der Gattung *Formica*. 3. Die Kastendetermination von *Formica rufa rufo pratensis minor* Gössw. *Ins. soc.*, 1, 229-246. — 1954b — Untersuchungen zur Kastendetermination in der Gattung *Formica*. 4. Physiologische Weisellosigkeit als Voraussetzung der Aufzucht von Geschlechtern in Polygynen Volk. *Ins. soc.*, 1, 305-18.
- HULTIN T., 1947. — The *corpora allata* in various castes of ants. *Kungl. Fysiografiska Sällskapets I Lund Förhandlingar*, 17, 107-13.
- LAPPANO E.R., 1958. — A morphological study of larval development in polymorphic all-worker broods of the army ant *Eciton burchelli*. *Ins. soc.*, 5, 31-66.
- LEDOUX A., 1976. — Action d'un dérivé du farnésol sur l'apparition des femelles ailées chez *Aphaenogaster senilis* (Hym. Formicoidea). *C.R. Acad. Sci., Paris, D*, 282, 569-70.
- LENSKY Y., BAEHR, J.C., PORCHERON P., 1978. — Dosages radio-immunologiques des ecdysones et des hormones juvéniles au cours du développement post-embryonnaire chez les ouvrières et les reines d'Abeille (*Apis mellifica* L. var. *ligustica*). *C.R. Acad. Sci., Paris, D*, 287, 821-24.
- LÜSCHER M., 1976. — Evidence for endocrine control of caste determination in higher Termites. In : *Phase and Caste Determination in Insects*, 91-103. Ed. M. Lüscher, Pergamon Press, New York.
- NOIROT C., 1977. — Various aspects of hormone action in social insects. *Proc. 8th Congr. IUSSI*, Wageningen, 12-16.
- PASSERA L., 1969. — Biologie de la reproduction chez *Plagiolepis pygmaea* Latr. et ses deux parasites sociaux *Plagiolepis grassei* Le Masne et Passera et *Plagiolepis xene* Stärcke (Hymenoptera, Formicidae). *Annls. Sci. Nat., Zool. Biol. anim.*, 12ème série, 11, 327-482. — 1974 — Kastendetermination bei der Ameise *Plagiolepis pygmaea* Latr. In *Sozialpolymorphismus bei Insekten*, 513-32, Ed. G.H. Schmidt, Wiss. Verlagsges, Stuttgart. — 1980 — La ponte d'œufs orientés chez la Fourmi *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae) après traitement par l'hormone juvénile. *C.R. Acad. Sci. Paris, D*, 286, 615-18.
- PASSERA L., SUZZONI J.P., 1978a. — Sexualisation du couvain de la fourmi *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae) après traitement par l'hormone juvénile. *C.R. Acad. Sci. Paris, D*, 287, 1231-33. — 1979a — Le rôle de la reine de *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae) dans la sexualisation du couvain après traitement par l'hormone juvénile. *Ins. soc.*, 26, 343-53. — 1979b — Activités comparées des hormones juvéniles (JH I, JH II, JH III) sur le déterminisme des castes chez *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae, Myrmicinae). *C.R. Acad. Sci. Paris, D*, 289, 1315-17.
- PLATEAUX L., 1971. — Sur le polymorphisme social de la fourmi *Leptothorax nylanderi* (Förster). II. Activité des ouvrières et déterminisme des castes. *Annls. Sci. Nat., Zool. Biol. anim.*, 12ème série, 13, 1-90.
- ROBEAU M.R., VINSON S.B., 1976. — Effects of juvenile hormone analogues on caste differentiation in the imported fire ant, *Solenopsis invicta*. *J. Georgia Entomol. Soc.*, 11, 198-203.

- RÖSELER P.F., 1977. — Endocrine control of polymorphism in Bumblebees. *Proc. 8th Congr. IUSSI*, Wageningen, 22-23.
- SCHMIDT G.H., 1974. — Stenerung der Kastenbildung und Geschlechtsregulation im Waldameisenstaat. In *Sozial polymorphismus bei Insekten*, 404-512, Ed. G.H. Schmidt, Wiss. Verlagsges, Stuttgart.
- SUZZONI J.P., GRIMAL A., 1981. — Variations biométriques des corps allates pendant la différenciation des castes reine et ouvrière chez *Plagiolepis pygmaea* Latr. (Hymenoptera : Formicidae). *Ins. soc.*, 27, 399-414.
- SUZZONI J.P., PASSERA L., STRAMBI A., 1981. — Ecysteroid titre and caste determination in the ant *Pheidole pallidula* (Nyl.) (Hymenoptera : Formicidae). *Experientia*, 36, 1228-29.
- TROISI S.J., RIDDIFORD L.M., 1974. — Juvenile hormone effects on metamorphosis and reproduction of the fire ant, *Solenopsis invicta*. *Environ. Entomol.*, 3, 112-16.
- VELTHUIS H.H.W., VELTHUIS-KLUPPEL F.M., 1975. — Caste differentiation in a stingless bee, *Melipona quadrifasciata* Lep. influenced by juvenile hormone application. *Proc. Kon. Ned. Akad. Wetenschappen*, Ser. C, 78, 81-94.
- VINSON S.B., ROBEAU R.M., 1974. — Insect growth regulator : effects on colonies of the imported fire ant. *J. Econ. Entomol.*, 67, 584-87.
- VINSON S.B., ROBEAU R., DZUIK L., 1974. — Bioassay and activity of several insect growth regulators on the imported fire ant. *J. Econ. Entomol.*, 67, 325-28.
- WENDEL L.E., VINSON S.B., 1978. — Distribution and metabolism of a juvenile hormone analog within colonies of the imported fire ant, *Solenopsis invicta*. *J. Econ. Entomol.*, 71, 561-65.
- WILDE J. (de), 1976. — Juvenile hormone and caste differentiation in the honey bee (*Apis mellifera* L.). In *Phase and Caste Determination in Insects*, 5-20. Ed. M. Lüscher, Pergamon Press, New York.
- WIRTZ P., BEETSMA J., 1972. — Induction of caste differentiation in the honeybee (*Apis mellifera* L.) by juvenile hormone. *Ent. exp. appl.*, 15, 517-20.