# Ecdysteroid titre and caste determination in the ant, *Pheidole pallidula* (Nyl.) (Hymenoptera: Formicidae)

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*Summary.* In the ant *Pheidole pallidula,* ecdysteroid level is higher in worker-biased eggs than queen-biased eggs. Moreover queens laying worker-biased eggs exhibit a higher ecdysteroid level than queens laying queen-biased eggs.

In *Pheidole pallidula,* there are 3 female castes: queens, workers and soldiers; only the queens are able to lay eggs. The differentiation of the soldiers occurs in the 3rd and last instar and is trophogenic[1]. On the contrary, the differentiation between queens and workers occurs early, because the eggs are biased. Regardless of culture conditions, the first eggs laid by a queen hatch into queens and/or workers[2]. But by the end of the 1st month after hibernation only worker eggs are laid. The mixed-laying period can however be experimentally lengthened by topical applications of JH to the queen[3]. So caste determination appears to be controlled by the JH level. Within the framework of the hormonal control, it seems to us interesting to study the variations of ecdysteroid titre in queens laying biased eggs and in biased eggs.

*Materials and methods.* Colonies were collected in early spring before egg laying and cultured at 27 °C. Using the radio-immunoassay developed by De Reggi et al.[4], ecdysteroid concentration was measured in individual queens and in batches of 20–50 eggs.

With the queens, 2 series were performed: one involved queens which laid 100% worker-biased eggs; another set concerned those which laid 69–100% queen-biased eggs.

With the eggs, 3 series were performed: one set of assays concerned worker-biased eggs (100%) laid at the end of hibernation; another set involved queen-biased eggs (83–100%) laid at the same period; lastly, for comparison's sake, assays were also run on worker-biased eggs laid just over 2 months after hibernation. The rate of embryonic development is nearly the same for the 2 types of eggs: 7–8 days at 27 °C.

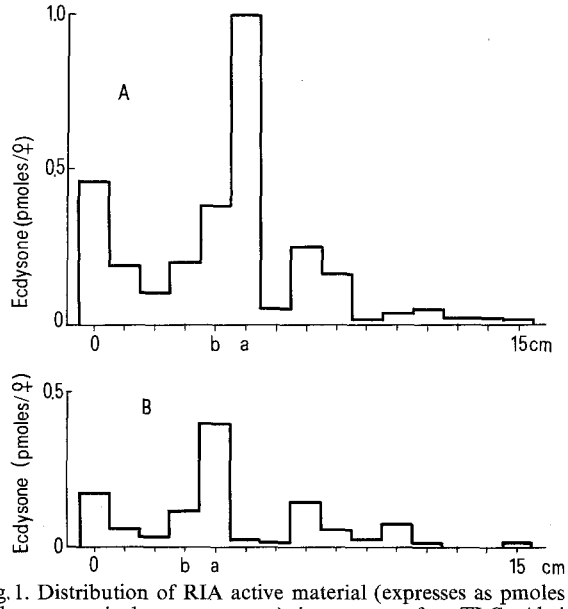


Fig. 1. Distribution of RIA active material (expresses as pmoles of ecdysone equivalent per queen) in queens after TLC. Abcissa represents 1 cm zones scraped and eluted separately (0 cm, origin; 15 cm, front). *A* Queens laying worker-biased eggs (mean of 7 samples). *B* Queens laying queen-biased eggs (mean of 6 samples). a: compounds comigrating with ecdysone; b: compounds comigrating with ecdysterone.

Whole extracts in methanol/water (90:10, v/v) were chromatographed on precoated silica gel plates in a solvent system of chloroform/methanol (80:20, v/v). Samples of ecdysone and 20 hydroxyecdysone were also run as standards. The gel was scraped off according to the position of references.

*Results and discussion.* Queens. Significant amounts of ecdysteroids were present in all queen ants (figure 1). Although we assayed total extracts, it seemed likely that the ovaries were the main origin of these ecdysteroids as has been pointed out for other female insects such as *Aedes*[5], *Locusta*[6], *Galleria*[7], *Blaberus*[8], etc. and for social insects like *Macrotermes*[9] and *Polistes*[10].

When the queens laying worker-biased eggs and those laying sexually-biased eggs were compared, great differences in the quantities of ecdysteroids and compounds comigrating with ecdysone and ecdysterone were revealed (figure 1). However when compared on a percentage basis of RIA active material ecdysone and ecdysterone were roughly equal in both, i.e. 28% ecdysone and 14% ecdysterone in queens laying worker-biased eggs and 27% and 11% for those laying sexually-biased eggs.

Eggs. In eggs the same ecdysteroids were observed as in the queens but in different proportions (ecdysone 41%; ecdysterone 8.5%). By plotting variations in ecdysteroid titre as a function of the time when the different eggs were laid (figure 2), we observed: 1. That the rate was low during the first 3 days but that in the 2nd period of embryonic development it rose considerably. We cannot say whether this increase was due to synthesis or to unmasking of hormones in the embryo. *Pheidole* did not exhibit peaks as in *Blaberus*[8] or *Schistocerca*[11] but embryonic moulting has not been reported in ants. However, daily readings taken from an embryonic development lasting 7 days were perhaps too far apart. 2. That like queens, worker-biased eggs contained more ecdysteroids. Furthermore, we noted that the longer the time after hibernation the higher the ecdysteroid titre and the more the egg was likely to be worker-biased.

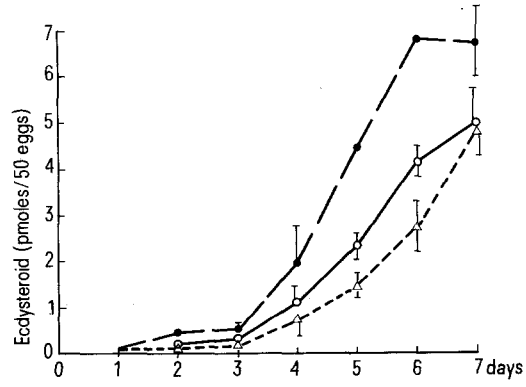


Fig. 2. Evolution of the ecdysteroid rates during the embryonic development. Ordinate: ecdysteroids in pmoles equivalent-ecdysterone per 50 eggs. Abscissa: age in days. – – –, queen-biased eggs laid at the end of hibernation; ——, worker-biased eggs laid at the end of hibernation; — —, worker-biased eggs laid 1 month later.

Our results corroborate the existence of biased eggs in this species. Moreover, they lead us to believe that caste determination is tied to ecdysteroid titre. There appears to be a level below which eggs are sexually-biased, as is the case just after hibernation and above which they are worker-biased. Variations in ecdysteroid titre have also been recorded in *Macrotermes* termites eggs[9] but their destiny is as yet unknown. Also, after caste determination has taken place, the sexually- and worker-biased larvae of honeybees[12] exhibit significantly different ecdysteroid levels.
The roles of JH and ecdysteroids in caste determination can be compared. JH is already known to spur brood sexualization in *Apis*[13] and, in the present case, in *Pheidole*[3]. In *Apis*[14], a great JH increase coincides with caste determination of queen larvae. In *Polistes*[10], JH stimulates oocyte development whereas ecdysterone seems to inhibit it.
A hypothesis based on relative variations of JH and ecdysteroids has already been proposed for termites[15,16]. So it would not be unprecedented if, during caste determination in social insects, a high JH titre accompanies queen biasing while a high ecdysteroid titre goes with worker biasing.


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## Artificial spawning effected in the fresh water teleost, *Cyprinus carpio* by clomiphene citrate


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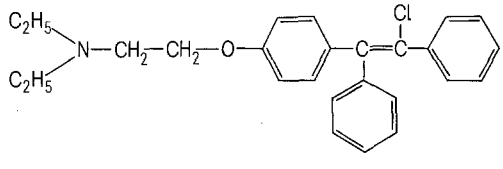
*Summary.* Triweekly i.m. injections of clomiphene citrate (group 1, 25 µg/0.5 ml and group II, 50 µg/1.0 ml) were administered for a period of 3 months during the preparatory period to female fresh water teleosts exhibiting ovarian recrudescence, while a control group received 0.5 ml of physiological saline throughout the period of experimentation i.e., from February through April. 50 µg clomiphene citrate treatment brought about a steady increase in ovarian size, and oocytes began to enlarge and mature and finally ovulation took place in April. This is 4 months ahead of their normal occurrence.


*Chemistry of clomiphene citrate.* Clomiphene citrate (I-p-diethylaminoethoxy) phenyl-I, diphenyl-2 chloroethylene citrate) which is marketed as Clomid® is an established drug for ovulation in human beings. Its structural formula is




For the past decade or so, much work has been done to establish the role of clomiphene citrate in the induction of ovulation in human beings and laboratory mammals. Its mode of action has been explained by a number of workers; to cite a few instances[2-4] in rats and[5-11] in human females. The findings of these authors and many others have proved beyond doubt that this drug is a powerful inducer of mammalian ovulation. But surprizingly only an isolated reference[12] exists as far as its action on fishes is concerned. This coupled with the fact that artificial induction of ovulation using chemical inducers is an important aspect of fisheries development, prompted the authors to undertake the present investigation.

About 200 fishes, more or less of the same weight and length, were collected from local ponds in Varanasi (India) and were acclimatized to laboratory conditions. During this period they were fed on liver slices. The photoperiod was maintained 10 light h/10 dark h throughout the tenure of the experimentation. The fishes were then divided into 3 groups. Group I received fish saline 0.5 ml and formed the control group. Group II and III received 25 µg/0.5 ml and 50 µg/0.5 ml of clomiphene citrate respectively and formed the experimental groups. Clomid was administered i.m. thrice a week on alternate days for a period of 3 months from February through April (the normal preparatory phase of the fish[13]). Fortnightly sacrifises of 5 fishes from each group were made to obtain material for histochemical studies. However, prior to sacrificing, the fishes were examined to see whether they spawned by stroking their abdomen backwards. It was observed that the control fishes did not spawn on day 90 while the 50 µg-treated fishes started spawning from day 60 onwards and the 25 µg-treated fishes started spawning from day 75 onwards.
Histological examination of the ovaries revealed that in the control fishes the ovaries contained mostly stage I and II oocytes while in the experimental groups the ovaries had already become filled with ripe ova. The 50 µg dose of the drug proved more effective in that it brought about earlier spawning. The existing data[12] brings out its action on