Juvenile hormone and reproduction in the cricket. II. Effect of rearing temperature on corpus allatum activity (in vitro) in adult females

W. Espig and K. H. Hoffmann*

Allgemeine Zoologie (Biologie I), Universität Ulm, Oberer Eselsberg, D-7900 Ulm/Donau (Federal Republic of Germany), 9 July 1984

Summary. In the Mediterranean field cricket, *Gryllus bimaculatus*, reproduction is controlled by temperature and the corpus allatum (CA) hormone JH III. In CA of females reared at 24°:12°C (16:8 h) (high reproduction rate) a first peak in JH III synthesis is reached about 4 days earlier than in those of 20°C females (low reproduction rate). Furthermore, in 20°C animals CA activity is low during the entire oviposition period, whereas at 24°:12°C high CA activity is found during this period of adult life. The results indicate a stimulation of CA activity and reproduction by thermoperiods around a constant low temperature.
Key words. Crickets; corpus allatum activity; juvenile hormone III; temperature; reproduction.

In the Mediterranean field cricket, *Gryllus bimaculatus* DEG (Insecta, Saltatoria, Gryllidae), daily alternating temperatures enhance ovarian development and stimulate oviposition[1]. Under constant temperature conditions the zero point for reproduction is slightly below 20°C. However, in 'normal' thermoperiods around such a low temperature (e.g. in 24°:12°C, 16:8 h; corresponding to a mean constant value of 20°C) a tremendous increase in egg number per female can be observed. Although there is little direct evidence, it is generally accepted that the effect of temperature on development processes is not only direct, but also indirect[2]. The latter is thought to be transduced via the endocrine system[3]. In *Gryllus bimaculatus* direct support for a temperature sensitive endocrine mechanism comes from findings that ecdysteroid titres are evoked by temperature changes[4,5]. The present experiments were designed to analyze the relationship between thermoperiodic control and corpus allatum (CA) activity during sexual maturation and the oviposition period in female crickets.

Materials and methods. The insects were reared individually either at a constant temperature of 20°C (LD 16:8 h; 04.00–20.00 h light, about 400 lx) or in 16 h at 24°C: 8 h at 12°C coinciding with a LD cycle of 16:8 h as described previously[4]. For mating, females were paired with males, beginning on the 2nd day after ecdysis. The whole body fresh weight and the oviposition rate of females were determined every two days up to the age of CA dissection. Fat body and ovarian fresh weight of the experimental females were measured at the time of CA incubation. The radiochemical in vitro assay for measurement of juvenile hormone (JH) biosynthesis by the CA, as introduced by Tobe and Pratt[6] was performed as recently described[7]. Incubations of corpora cardiaca/corpora allata (CC/CA) complexes were performed at 20°C for 4 h. The mean cumulative JH release into the incubation medium was approximately linear during this period of time. From radioscans of TLC-separated incubation products it became evident that the samples contained JH III only. A minor incubation product, which is only little released into the incubation medium, was found when CA/CC complexes were analyzed together with the incubation medium. Former experiments[7] confirmed the identity of this product as methylfarnesoate (MF), a precursor of JH III. Activity values are means of 10–22 separate determinations (see figs 1 and 2). Vertical bars in the figures represent SEM ($SD/\sqrt{n}$).

Results. CA taken from immature females (days 0–8 after ecdysis at 20°C and 0–4 at 24°:12°C, respectively) released very little JH III into the incubation medium (figs 1C and 2C). During the following 4 days JH synthesis by freshly-dissected CA increased drastically under both temperature regimes. The change in the activity of the CA corresponds with a dramatic increase in the ovarian weight of the females from which the incubated CA were taken (figs 1A and 2A). Maturing ovaries increase in weight because of yolk deposition (see arrows 'vit' in figs 1C and 2C; S. Kempa-Tomm, unpublished). In CA of 24°:12°C females a first peak in JH III synthesis was reached at day 8 after imaginal molt (IM) as against day 12 in 20°C females. Absolute amounts of JH III synthesis, however, were similar in both experimental groups during this time (ca 3.5 pmoles · h^{-1} · pair CA^{-1}). In both temperature regimes oviposition started exactly at the time of the first JH III peak (8 and 12 days after ecdysis, respectively), but in 24°:12°C crickets the increase in egg deposition rate was drastically higher ($p < 0.01$) than in 20°C females (figs 1B and 2B). During this increase in egg laying the rates of JH release declined in both experimental groups, before increasing again to high rates, but only when the glands had been taken from 24°:12°C females. In 24°:12°C females high rates of egg deposition contin-

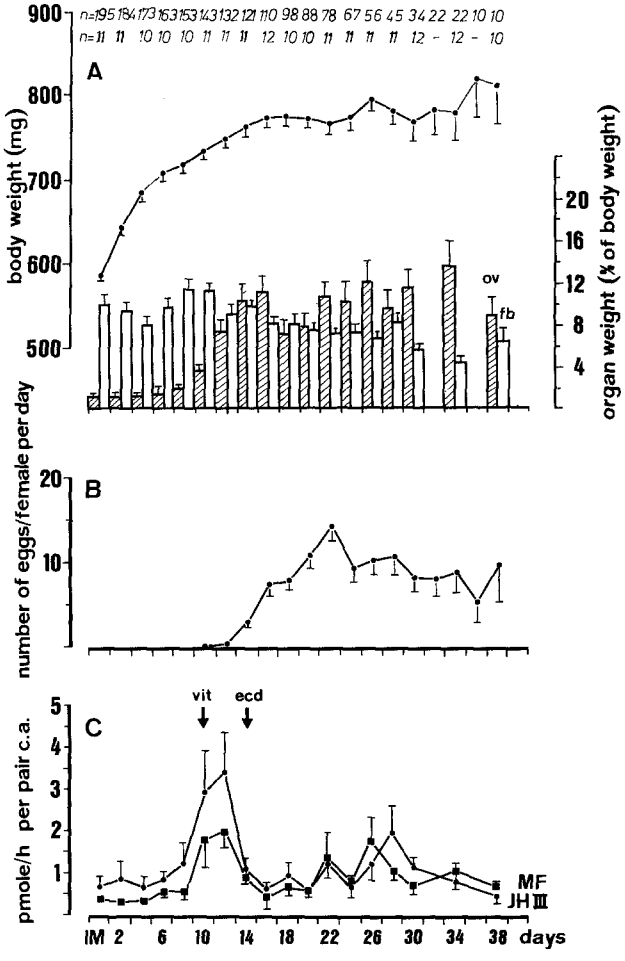

Figure 1. *A* Life time whole b.wt (n = 10–195) and organ weights (n = 10–12) in females reared at 20°C. ov, ovary; fb, fat body; IM, imaginal molt. *B* Life time egg production in females reared at 20°C (n = 10–195). *C* Changing CA activity (in vitro) for females reared at 20°C. Incubation media were extracted together with the incubated CA/CC complexes (see 'materials and methods'). JH III, juvenile hormone III; MF, methylfarnesoate; vit, time of first vitellogenin peak in hemolymph (S. Kempa-Tomm, unpublished); ecd, time of first ecdysone peak in hemolymph (from Hoffmann et al.[4]) (n = 10–12).

ued for 8–10 days. Since at this stage of the oviposition period cycles of oocyte development considerably overlap within the ovaries, it is unlikely that the pattern of JH production by CA will be correlated with vitellogenic oocyte growth.

MF, which was predominantly extracted from the glands and not from the medium, showed a similar time course of synthesis to JH III, but its concentration never exceeded that of JH III (figs 1C and 2C).

Discussion. In adult insects JH functions range from the regulation of yolk deposition in oocytes to pheromone production and the initiation of sexual behavior (see Engelmann[8] for review). Using an in vitro radio-biosynthetic assay to measure the actual JH synthesis of the incubated CA, a clear correlation between CA synthetic activity and rapid oocyte development has been demonstrated in various orthopteran insect species (for summary see McCaffery and McCaffery[9]). Besides hormonal effects, oocyte development and reproduction in insects are strongly temperature dependent[3]. Whilst several papers deal with the effect of photoperiod and starvation on CA activity in insects[10–14], only a little information is available on the effect of temperature on CA activity. As early as 1966, Clarke[15] demonstrated considerable histological differences in the CA of adult *Locusta migratoria* reared at 15°, 30°, 45° and 30 ± 10 °C: In females of another locust species, *Schistocerca gregaria,* the following phenomena were induced as a result of decrease in diurnal rearing temperature from 33° to 28 °C; a delay in the appearance of maximal levels of JH III and ecdysteroids in the hemolymph, a slowing down of oocyte growth, and an accumulation of hemolymph proteins[16]. As far as we know, the effects of rearing temperature on CA activity (in vitro) in adult female insects have been demonstrated for the first time by the work described in this paper.

The chronological sequence of increasing CA activity, ovarian weight, and oviposition in *Gryllus bimaculatus* suggests that high rates of JH biosynthesis during the preoviposition period are correlated with rapid oocyte growth. If there is to be a continuation of high egg deposition rates, such as are seen at a daily alternating temperature (24°:12 °C) or at a constant high temperature (for results on 27 °C females see Koch and Hoffmann[7]), further peaks of JH synthesis during the entire oviposition period are unavoidable. At 20 °C, where reproduction is low, JH synthesis during the oviposition period is also low. If, however, a low endogenous JH synthesis by CA at 20 °C is counteracted by repeated JH III applications, the females reach a total fecundity similar to that in 24°:12 °C crickets[7].

The mechanisms by which CA activity is regulated in a temperature-dependent manner are still largely unknown. Previous experiments have shown that in female crickets free ecdysteroid titres in hemolymph reached maximal values three times higher, and reached them about 4 days earlier, when animals were reared at 24°:12 °C than under constant 20 °C[4]. Repeated injections of ecdysone and 20-hydroxyecdysone into females with a low endogenous molting hormone (MH) titre (20 °C animals) stimulated fecundity, thus mimicking the timing of increase in endogenous level of 24°:12 °C animals[17]. The results of this paper indicate that the primary onset of vitellogenin synthesis cannot be initiated by ecdysteroids, since CA activity and hemolymph vitellogenin titre are high when ecdysteroid levels are still low (figs 1C and 2C). Ecdysteroids, however, may play a role in later ovarian maturation[17,18]. Recent experiments, which have shown that antennal amputations stimulate the fecundity of female crickets at suboptimal rearing temperatures[5], support our hypothesis that CA are affected by a removal of antennal cold receptors via CNS neurosecretory cells.

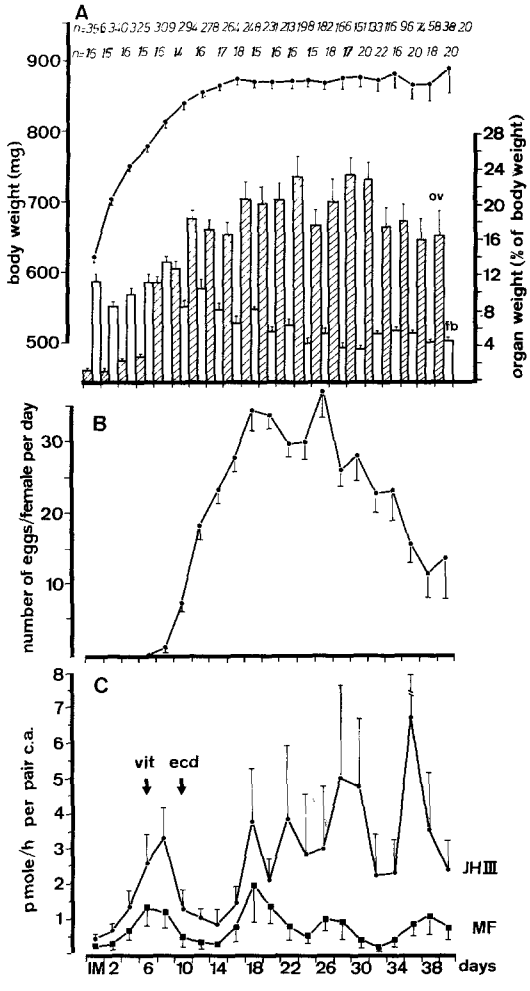

Figure 2. B.wt change (*A*, upper curve) and organ weights (*A*, lower part), egg production rate (*B*), and CA activity (in vitro) (*C*) for females reared at 24°:12 °C (16:8 h). For details see figure 1.

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