Table 2. Variation of the average fecundity of treated and control couples in the period following metamorphosis

Days	Treated insects		Controls		t
	No. females	No. eggs	No. females	No. eggs	
1–5	74	12.82 ± 1.21	65	16.95 ± 1.59	2.09*
6–9	74	34.03 ± 1.74	65	40.54 ± 1.99	2.47*
10–13	74	28.42 ± 1.31	65	30.00 ± 1.29	0.85
14–17	74	20.07 ± 1.33	65	25.78 ± 1.55	2.81*

* $p < 0.05$.

treated and control females. No females died during the study and all laid eggs.

The pairs treated with the 1:100 dose laid the same number of eggs on the average as the controls. Those treated with the 2 larger doses, instead, laid fewer. The variance, according to Bartlett's test, was found to be homogeneous and therefore not altered by the treatment.

Table 2 shows the average number of eggs laid in the 4 consecutive time intervals by all treated and control pairs. The treated pairs always laid fewer eggs than the controls. The effect of the extract on their fecundity was almost immediate; their 1st lot of eggs was already smaller on the average than those of the controls. The reduction of fecundity remained nearly constant throughout the period of extract administration and there was no accentuation of the effect. 3 treated females (out of 74) and 7 controls (out of 65) only began laying eggs after the 5th day; the treatment thus did not delay the onset of oviposition but only reduced the number of eggs laid.

Some eggs laid either by the control pairs (11.8%) or by the treated ones (12,6%) dried up, remaining whitish; no significant differences were found.

On the whole the queen bee extract given by mouth to pairs of *T. molitor* acted very quickly in reducing the average fecundity of females, but did not induce complete sterility nor produce cumulative effects on fecundity.

Some researches have shown that the 'royal substance' of *Apis mellifera* hinders the enlargment of the corpora allata in orphan worker bees[14] and in orphan pseudergates of *K. flavicollis*[15]. It may act in the same way in other insects as well: reducing, that is, the fecundity of females by making the corpora allata wholly or partly inactive.

It is even more interesting, however, that the 'royal substance' of *Apis mellifera* has been shown to be active in an ever wider number of species, and thus does not have a strictly specific action.

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Measuring nanogram amounts of DNA by photographing fluorescence in thin layers of agarose

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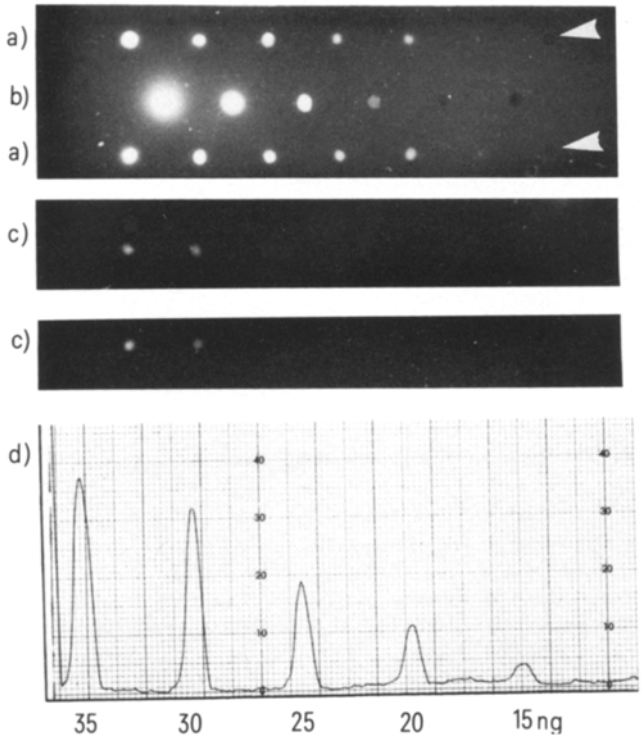
Summary. A method is described which permits the measurement of DNA amounts of as low as 0.5 ng by photographing the fluorescence of ethidium bromide after complete removal of unbound dye. The DNA is immobilized in thin layers of agarose.

A reliable and easily-reproducible assay for the measurement of minute quantities of DNA is desirable when the results depend very much on the precise determination of these values. Normally the evaluation has to be made with a sample that is taken from a small aliquot with limited amounts of DNA. Typical applications of such assays are during DNA-protein binding studies and for the determination of specific radioactivities where only the removal of amounts of DNA in the ng range is acceptable. Previous attempts to solve this problem have been published[3–6] and the assay which is described relies on information conveyed in all of them. The given method may be used to estimate DNA amounts of as low as 0.5 ng.

The following DNAs were used: calf thymus and plasmid col. E1 (Boehringer, Mannheim); phage lambda (a gift of H. Bujard, Heidelberg); lambda rif^{d18} (a gift of P. Venetianer, Szeged); pBR 322 and several DNA inserts in this plasmid; *E. coli* chromosomal, and several chloroplast DNAs[7]. The latter DNAs were prepared as described before[7] and all were stored sterile at 4 °C in 0.5 mM EDTA in concentrations of approximately 0.5 mg/ml. DNA concentrations were determined photometrically on the basis of a molar extinction coefficient of 6800 M^{-1} cm^{-1} at 260 nm in 1 × SSC, pH 6.8.

For the assay, 4 ml of 1% high-temperature gelling agarose (MCI, Rockville) in 10 mM NaCl were allowed to solidify

on a microscope slide. From the resulting 3 mm thick agarose layer, holes with diameters of 0.5 or 1 and 2 mm were punched (LKB gel puncher). Samples of a standard dilution series with ranges from 0.2 to 200 ng of DNA were mixed with equal amounts of 2% low-temperature gelling agarose (MCI, Rockville) containing the dye in 10 mM NaCl at 40 °C and 5 or 10 and 20 µl aliquots (0.1–100 ng of DNA) were transferred into the holes on the slide and allowed to solidify (ethidium bromide 0.5 or 1 µg/ml). During the following 2–30 h floating period of the agarose layers in water diffusion of DNA did not affect the results. When placed on a transilluminator (C-63, UVP, San Gabriel, at 302 nm) the agarose could be photographed with a Polaroid MP-4 camera through a red-orange (factor × 4) filter on type 665 negative film. This transilluminator was used since it gave a more even illumination of the filter area, although the slightly higher resolution at 254 nm was sacrificed by this. Exposure times varied with the age of the UV-filter and were in the range of 1–8 min. Alternatively the agarose pieces could be photographed on 36 mm film. The Agfapan (25 ASA) film and exposure times of 2–8 min were compared with results obtained on Polaroid material. Experiments showed that values from 0.5 to 20 ng of DNA should be photographed using long exposures (e.g. 5 min), while for amounts from 10 to 60 ng of DNA short exposures (e.g. 1 min) should be taken (fig. a–c). Negatives were scanned with a Joyce/Loebl chromoscan or a Beckman Acta 3 photometer equipped for scanning. During the preparations for an assay due care should be taken to avoid spillages since incompletely filled holes would cause reflexions and loss of sensitivity.



Photography and scanning of DNA-ethidium-bromide fluorescence. *a* From left: 50–40–30–20–10–2.5–0 ng of DNA. The arrows point to wells from which, in the absence of DNA, ethidium bromide was removed totally. (1 min exposure, Polaroid 665). *b* 5 dilutions of an unknown DNA sample (chloroplast DNA: 1000–250–50–5–2.5–0 ng of DNA). The DNA amount was determined photometrically. *c* From left: 3–1.5–0.75–0.375–0.2–0.1–0 ng of DNA (6 min exposure, Polaroid 665); the lowest amount of DNA which could be photographed reproducibly was between 1 and 0.5 ng. *d* Scan of a dilution series comparable to a) in a Beckman Acta III photometer with a gel scanner at 550 nm. Scans are quantitated by peak height at a scanner speed of 1 cm/min.

As DNA dilution series and the agarose stock, without ethidium bromide, can be stored frozen and are thus readily available the only time-consuming step of this assay is the removal of unbound ethidium bromide from the sample-containing holes. The use of the given low amount of dye, apart from destaining being rapid, implies that between 4 moles of ethidium bromide per mole base pairs (using 1 ng of DNA) and 0.07 or 0.14 moles/mole base pairs (with 60 ng of DNA) may be available. In spite of these small values the increase in fluorescence was linear for up to more than 200 ng of DNA/hole. Without DNA being present the removal of unbound dye could be demonstrated to be fast and virtually complete (fig. a, arrow) during the immersion in water. The extent to which a linear increase in fluorescence with the DNA amount per hole existed was tested in a series with values from 10 to 70 ng DNA/hole (fig. d). The figure illustrates the upper range of this method and points to its limitations with the photographic equipment used when high DNA concentrations are being scanned.

For measurements of unknown DNA values[8] 3–4 dilutions by a factor of 10 were normally sufficient to find an appropriate dilution (fig. b). For the confirmation of known amounts of DNA only 2 dilutions by a factor of 3 were usually required. In the assays, always the unknown samples were on the same agarose slide as the standard dilution series.

Calibration curves were normally performed with linear DNAs. It should, however, be emphasized that when covalently closed circular DNAs were used fluorescence yields were reduced depending on the amount of ccc DNA in the probe. A plasmid col. E1 DNA preparation consisting of approximately 40% of ccc DNA which was determined by agarose gel electrophoresis of the unrestricted plasmid DNA resulted in a 15% lower determination of DNA amount when compared with the same DNA after restriction endonuclease digestion with *Eco* RI.

In contrast to the fast coloriometric method for the determination of DNA amounts which requires approximately 1 µg of material[9], fluorometric methods are more economical[3–6]. It would be preferable if the sensitivity and reliability could be maintained even when values of only 1–10 ng of DNA are used. From published data the method by Reis[6] is similar to the one presented here but is limited to amounts of above 10 ng as some non-removable ethidium bromide persists as background. The major advantage of the method presented here is that with the removal of unbound dye DNA values of as little as 0.5 ng may be measured in aliquots of 5-µl volume.

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