ORIGINAL ARTICLE

Suppression of worker fertility in the honey bee (Apis mellifera) by treatment with X-rays

Jakob Wegener [1], Fred Zautke [1], Stefan Höcht [2], Britta Köhler [2], Kaspar Bienefeld [1]*

[1] Institute for Bee Research, F.-Engels-Straße 32, 16540 Hohen Neuendorf, Germany

[2] Poliklinik für Radioonkologie und Strahlentherapie, Charité Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany

Received 24 June 2005, accepted subject to revision 5 December 2005, accepted for publication 28 December 2005

* Corresponding author

Summary

Breeding of the honey bee normally involves measurement of traits at the colony level. Nevertheless, there are traits that can be observed at the level of the individual worker. In this case, workers with interesting features are artificially induced to parthenogenetically produce offspring. In order to promote fertility in the worker bee of interest, it is useful to suppress the fertility of all other workers. In this study, our aim was to produce sterile bees by the application of ultra-hard X-rays. We irradiated capped brood of different ages with a dose of 20 Gy. This treatment suppressed fertility in all treated workers, while controls laid normal amounts of eggs. However, rare cases of fully developed ovaries occurred in workers when pupae were irradiated 0–4 days prior to emergence. In these bees, ovary development was retarded compared to controls. Hypopharyngeal glands degenerated more quickly in irradiated bees than in the controls, and mortality was heightened. Production of wax also was reduced. Radiosensibility of pupae dropped after approximately 110 h of age, egg and larval stages included. Given the rapid degeneration of hypopharyngeal glands in the irradiated bees, the usefulness of irradiated workers for breeding from single workers seems doubtful.

Keywords: Ionising radiation, sterilization, worker reproduction

Introduction

Most breeding traits of the honey bee (*Apis mellifera*), such as honey production or swarming behaviour, are the result of a coordinated action of many workers and a queen, and can only be assessed at colony level. Yet there are many traits that can also be assessed by observing individual workers (Harris and Harbo, 1991). Examples include duration of development, morphological traits, and some behavioural traits such as grooming or the removal of diseased brood. Observation of individual workers could be especially interesting in the case of quantitative traits occurring at measurable levels only in a few workers per colony. In such a situation, selection at the colony level may be difficult, because the existing variability in the population is too low to be detectable.

The number or proportion of workers showing the trait can then be used for selection at the colony level. Additionally, the selected workers themselves can serve to produce drones. The use of such worker-laid drones for breeding purposes was suggested by Böger (1969). Obtaining such worker sons is of high interest, since they are related to the animal showing the trait by r=0.5 (r: relatedness coefficient). The sons of the mother of the worker, which present the second best choice, are only be related to the worker by r=0.25. Using them, one misses out on the alleles of the father drone of the selected worker. There is a chance to recover these alleles on the female side of the breeding program, if super-sisters of the worker are used as queens. Yet, if the mother queen was mated by a hypothetical number of 10 drones, the probability of obtaining a super-sister of the selected worker is only 10% for every daughter queen reared. Therefore, the use of worker's sons may potentially fasten considerably the selection of rare traits with a low variability.

There are however obstacles to the use of worker bees in a selection program. The biggest one is that it is difficult to specifically induce fertility in a selected worker. Queenless workers compete with each other for the development of fertility (Page and Erickson, 1988; van de Blom, 1991), and the result of this race may not necessarily favour the selected bee. To solve this problem, Harris and Harbo (1991) suggested the use of nurse bees with a genetically determined delay of ovary development. When bees selected for fast ovary development were placed in the company of nurses with slow ovary development, only the former reproduced. However, this approach seems to be relatively labour-intensive, because such a line of bees with slow ovary development has to be identified and maintained specifically for this purpose. Also, whenever the breeding material is of a subspecies in which ovary development is slow, as in *A. m. carnica* Pollmann or in *A. m. mellifera* L. (Ruttner and Hesse, 1981), it may be very difficult to find bees with an even slower development.

In the present study, we therefore tried to sterilize worker bees to serve as nurse bees to single workers with developing fertility. We used ultra-hard X-rays to achieve this aim.

The method of sterilizing both male and female insects via irradiation is common in pest control (reviewed by Lindquist, 1963; O'Brien and Wolfe, 1964; Bakri *et al*., 2005). Most irradiated tissues stay functioning until cell division is initiated. In insects, unlike in mammals, very little cell division occurs in the adult stage (Bakri *et al*., 2005). Cells of the germ line generally divide more frequently than cells of other tissues, therefore the sterilizing dose of radiation is often well below the lethal dose (Lindquist, 1963).

As they contain more dividing cells, early developmental stages are generally more sensitive than later ones (O'Brien and Wolfe, 1964). In our study, we irradiated capped brood of different ages in order to determine the stage in which suppression of fertility is accompanied by as little somatic damage as possible. In order to quantify the damage, we measured the mortality of the irradiated animals, the development of their hypopharyngeal glands, comb building, and the date at which defecation first occurred in the cages. The development of the hypopharyngeal gland was of special interest to us, because the capacity to produce jelly is a crucial quality for a nurse bee. Defecation is recorded because in some insects, certain tissues of the digestive tract are especially radiosensitive (Riemann and Flint, 1967). As data on the radiation biology of the *Hymenoptera* is scarce (Bakri *et al*., 2005), we also discuss our results from a physiological point of view.

Materials and Methods

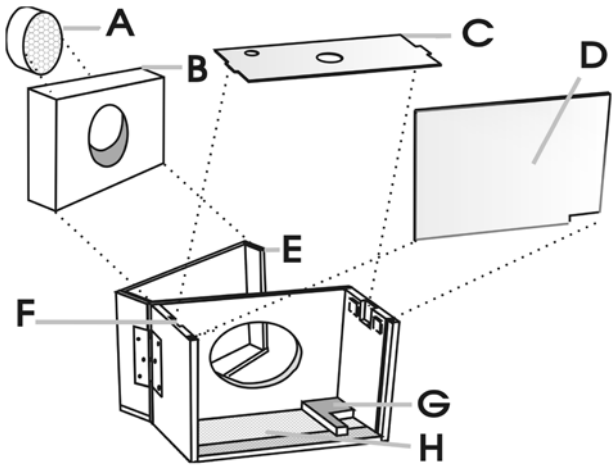
One brood comb was collected from each of 8 *A. m. carnica* colonies from the stock of the Institute for Bee Research, all from one apiary and similar in size and age of the queen. The combs contained capped brood of different stages at comparable ratios. They were transferred to the irradiation facility, where three combs were arbitrarily chosen to serve as controls. The remaining 5 combs were irradiated with a photon energy of 6 MeV and a dose rate of 3.9 Gy/minute. Photons from a standard clinical linear accelerator were used with sufficient bolus material added on the combs to ensure a very homogeneous dose distribution. Dose distribution was checked by thermoluminescence dosimetry and calculation in a human therapy planning system during a dummy run procedure prior to the experiments.

Preliminary experiments had shown that workers emerging from young pupae that had been irradiated with a dose of 15 Gy were still able to produce eggs, while a dose of 50 Gy produced strong mortality shortly after hatching. In the current study, we therefore chose a dose of 20 Gy, which was applied in two portions. First, the combs were irradiated with 10 Gy from one side, then they were turned over and another 10 Gy were applied. Back at the Bee Research Institute, all bees that had emerged during irradiation or transportation were removed. The combs were then fitted into pockets made from wire mesh and placed into foster colonies. The bees emerging inside the pockets were collected after 2, 4, 6, 8, and 10 days. Hereafter, we will refer to these groups of bees by their age at irradiation, as estimated from the time between irradiation and hatching. Thus, for example, group 19–21d corresponds to the bees collected after 2 days in the foster colony. The bees were filled into cages of 50 workers each. Ten days and more after irradiation, only very few bees emerged from the treated combs, and these were discarded. From the bees of age group 19–21d, we formed 16 cages of irradiated and 9 cages of control workers. Age groups 17–19d to 13–15d were each dispatched into 6 irradiated and 3 control cages. Age group 11–13d was used to form 4 cages of irradiated and 3 cages of control bees. In total, 59 cages were filled. They were placed into incubators at 32°C and 50–70% relative humidity. The cages received water, honey, and bee candy consisting of powdered sugar (53.1% weight), bee bread (pollen extracted from pollen combs, 35.4% weight), and water (11.5% weight). Each age group was observed for 26 days.

The cages we used were specially designed to facilitate the counting and removal of any eggs produced. Figure 1 shows their functioning. A piece of drone comb is pressed onto an opening in the side wall of the cage from the exterior. A plastic screen can be inserted between the comb and the cage, separating the bees from the comb, which can then be removed and examined. Each cage also contained a piece of wax foundation to monitor cell building.

Egg laying and worker mortality were recorded every day. Every day, the cages were checked for the occurrence of faeces. After the experiment had ended, the depth of the deepest cell built on the strip of foundation was determined and used as a measure for comb building activity.

In order to measure ovary and hypopharyngeal gland development, 3 to 5 bees were removed from each cage on days 0, 7, 14, and 21 and frozen at –16°C. At day 26, all remaining bees were frozen. They were later dissected and their ovaries and hypopharyngeal glands rated. For the ovaries, we distinguished three classes, as described by Velthuis (1970). Ovaries of stage 1



A. Piece of drone comb, inserted in a 2cm-long section of plastic tube (diameter 8cm)
B. Block of foamed plastic material
C. Acrylic glass lid with holes for the introduction of bees and the water dispenser
D. Acrylic glass pane
E. Trap
F. Cut-out for the insertion of a piece of foundation, mounted on a lath
G. Fitting for food trays
H. Wire mesh bottom
Outer measurements of the cage (trap excluded): length x depth x largeness = 18.8 x 7.3 x 12.6 cm
To change the comb in the presence of bees, a plastic screen is inserted between guide bars on the outer side of the back wall, between comb and cage.

Fig. 1. **Experimental cage for the control of egg laying.**

have resting or only slightly developed ovarioles, in which no eggs can be discerned. In stage 2, ovarioles are swollen and round to bean-shaped eggs are visible. In stage 3, ovarioles are swollen and the eggs are sausage-shaped. Hypopharyngeal glands were classified according to a scale introduced by Hess (1942), reaching from 1 (undeveloped, glandular lobes flat and translucent) to 4 (fully developed, spherical lobes containing many vesicles).

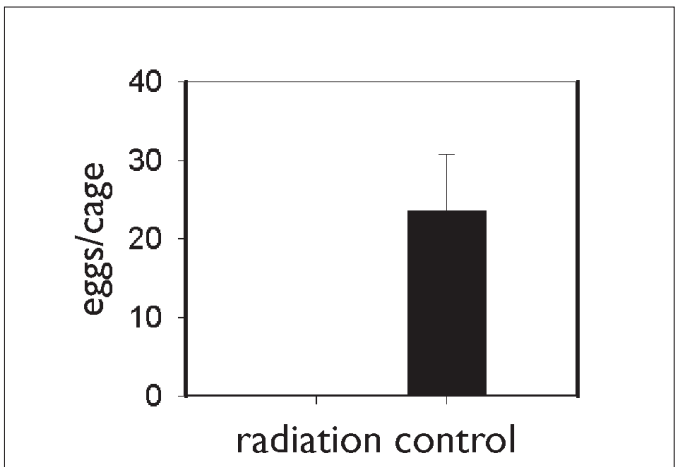
Results

Emergence from both the irradiated and control combs was almost complete. The last bees hatched 12–14 days after the removal of the combs from the hives of origin. Approximately 500–600 unhatched cells remained on the 6 irradiated combs, against 200 on the two control combs. The number of brood cells originally present on each comb is not known. Unhatched cells contained mainly fully formed pupae that had completed pigmentation. There were no visible differences between irradiated and control combs as to the quantity or appearance of dead brood.

Worker fertility

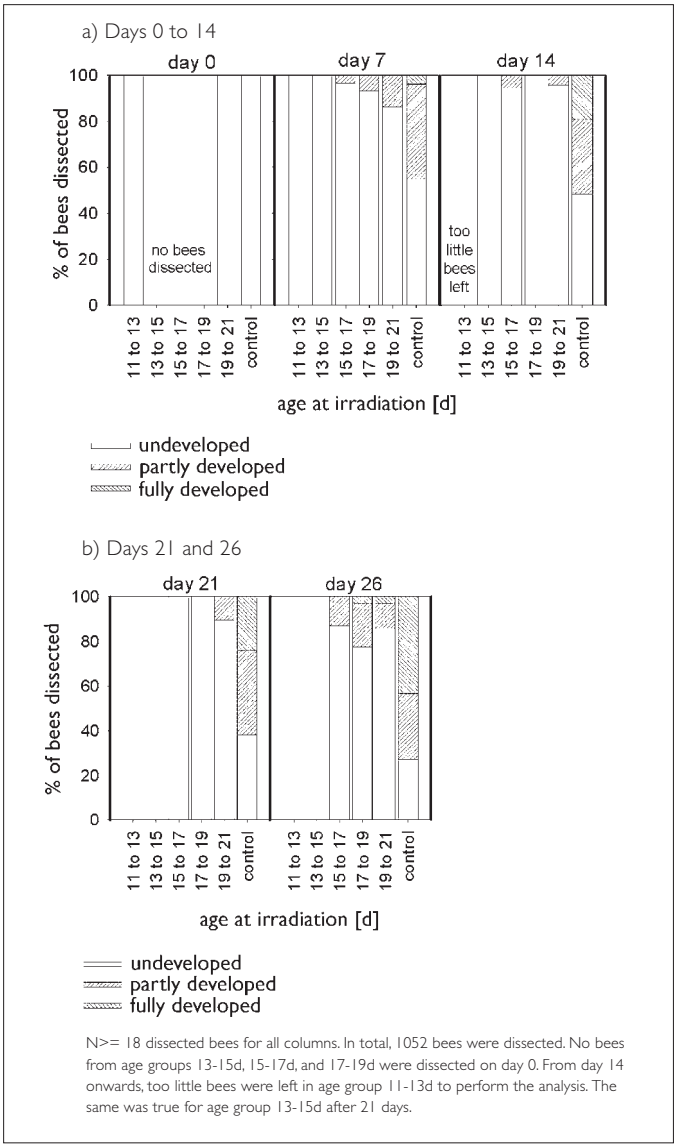
No eggs were found in the cages of the irradiated bees of any age group during the whole period of observation (figure 2). An average of 23.6 ± s.e. 7.13 eggs per cage were laid by the controls. At the end of the observations, egg production had passed its peak but was still at a high level.

For figure 3, the ovaries of 1052 bees were classified. The control cages of all age groups were grouped for this graph. They did not differ from each other at any date (p=0.106 to 0.988; df=8 in each case; X^2=1.695 to 13.183). No newly emerged bees were dissected from age groups 17–19d to 13–15d. Irradiated bees of group 11–13d showed no ovary development until day 7, and after this date mortality was too high to make any statement. Ovary development of the irradiated fraction of group 13–15d was equally low until day 14 and could not be assessed any more



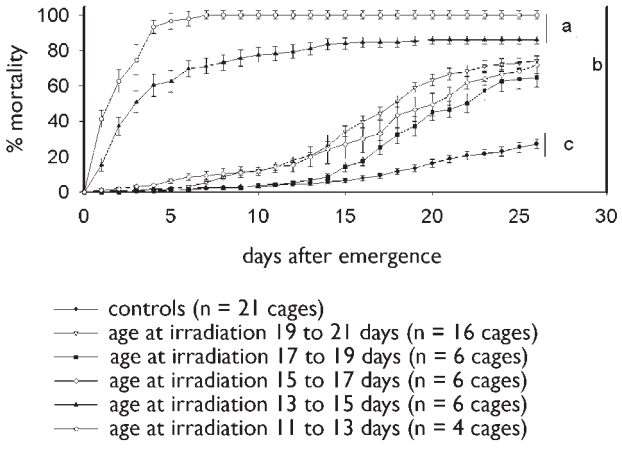
Number of eggs found per cage over the whole duration of the experiment (arithmetic mean ± s.e.). No eggs were found in the cages of the irradated bees.
Irradiated groups: n = 38 cages
Controls: n = 21 cages

Fig. 2. Egg production in irradiated and control cages.



N>= 18 dissected bees for all columns. In total, 1052 bees were dissected. No bees from age groups 13-15d, 15-17d, and 17-19d were dissected on day 0. From day 14 onwards, too little bees were left in age group 11-13d to perform the analysis. The same was true for age group 13-15d after 21 days.

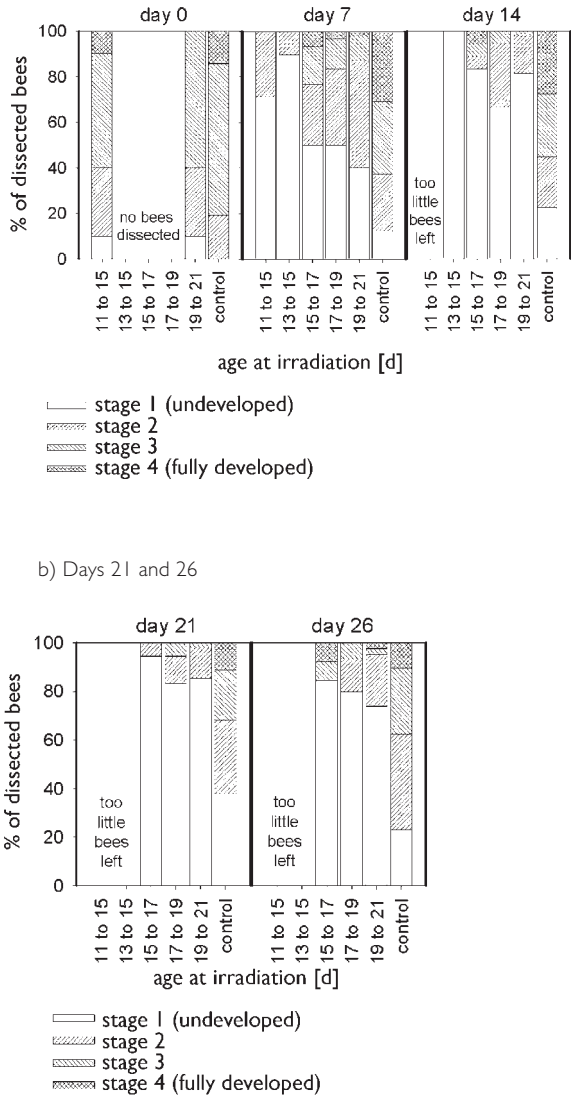
Fig. 3. Ovary development in irradiated and untreated workers.



Arithmetic mean of mortalities ± s.e.. Data were corrected for the removal of bees for dissection during the experiment.
Controls of all age groups are presented together.
Letters 'a', 'b', and 'c' designate significance of differences between mortalities at the end of the experiment. Groups with the smae letter are not significantly different.

Fig. 4. Mortalities of irradiated and control groups as a function of time.

afterwards. Cages of irradiated animals from groups 19–21d to 15–17d contained some individuals with stage 2 – ovaries already at day 7. On day 26, some irradiated bees from groups 19–21d and 17–19d had fully developed (stage 3) ovaries (3.3 % in group 19–21d, 3.2 % in group 17–19d). However, we never found any ready-to-lay-eggs in the vagina of an irradiated bee. Such eggs could be seen relatively frequently in the controls, where developed ovaries (stage 3) occurred from day 7 onwards. At day 26, stage 3 – ovaries could be found in 43.4 % of the untreated animals.



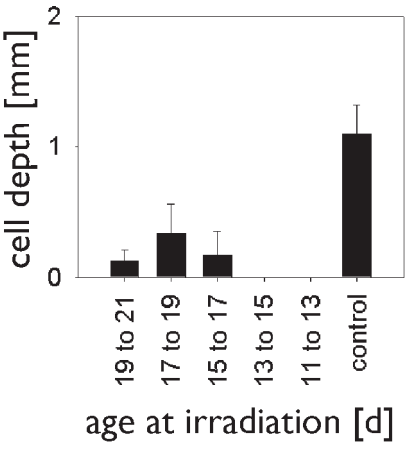
N>= 16 dissected bees for all columns. In total, 758 pairs of glands were rated.
No bees from age groups 13-15d, 15-17d, and 17-19d were dissected on day 0. From day 14 onwards, too little bees were left in age group 11-13d to perform the analysis. The same was true for age group 13-15d after 21 days.

Fig. 5. Development of hypopharyngeal glands of irradiated and control bees.

Somatic damage

Figure 4 shows the mortalities in the cages of irradiated and control bees. The results from the controls of the five age groups are again grouped. All irradiated bees from group 11–13d had died by day 13. The fact that there is still variation among the mortalities from different cages after this date is an artefact probably due to small errors committed while counting the bees introduced into each cage. In the treated cages of age group 13–15d, mortality reached a plateau at approximately 82%, with only little mortality occurring after day 15. The mortality curves of irradiated bees from groups 19–21d to 15–17d were very similar to each other. They differed from those of groups 13–15d and 11–13d in that mortality was more stretched out in time and did not reach a stable point during the 26 days of observation. Mortality in the controls was low, reaching only 23% after 26 days of caging. When applying the Scheffé-test for multiple comparisons to the cumulated mortalities at day 26, the controls differ from all of the treated groups ($p<0.001$ for each comparison; total df=61; mean difference= –72.77 to –34.48). Irradiated groups 19–21d to 15–17d and 13–15d to 11–13d form two homogenous blocks, although groups 19–21d and 13–15d appear in both of them.

Figure 5 shows the result of the dissection of 758 pairs of hypopharyngeal glands. At day 0, there were no significant differences between the controls and irradiated bees of either group 19–21d ($p=0.269$; df=3; $X^2=3.935$; X^2-test) or 11–13d ($p=0.413$; df=3; $X^2=2.863$). No newly – emerged bees from groups 17–19d and 15–17d were dissected. On day 7, all treated groups differed from the controls ($p<0.01$ in all cases, df=3 at each time, minimum $X^2=18.45$; X^2-test) and this stayed the



II treatments differ significantly from the grouped controls (multiple chi^2-test, α = 0,05). Data for groups 13-15d and 11-13d are not missing, but zero.

Fig. 6. Cell building in the cages.

case until the end of the observations on day 26. On day 0, more than 90% of bees from all three groups (irradiated and controls) examined had glands that reached at least stage 2. From then on, the glands degenerated faster in the irradiated bees than in the bees from the control cages, so that on day 26, the remaining irradiated groups had between 15.38 (group 15–17d) and 26.3 % (group 19–21d) of glands classified stage 2 and above, against 77.1 % in the controls.

The results concerning cell-building activity are given in figure 6. No building occurred among irradiated bees of groups 13–15d – 11–13d, and only little among those of groups 19–21d to 15–17d. In contrast, 14 out of the 21 controls had at least initiated the construction of some cells. In a number of cages, storage of honey in the cells built was initiated, although the cells never reached the full depth of ordinary worker cells.

Defecation was only observed in age groups 19–21d to 13–15d, the bees from group 11–13d mostly dying before any faeces appeared in the cages. Defaecation started after an average of 13.19 days ± s.e. 0.69 in the control cages. The treatment with the latest onset was age group 15–17d (16.60 days ± s.e. 1.17), that with the earliest group 13–15d (10.50 days ± s.e. 2.06). In none of the irradiated groups, the time of onset of defecation differed significantly from that of the controls ($p=0.067$ to 0.905; $n=4$ to 25; $U=1.00$ to 8.50; Mann-Whitney U-tests).

Discussion

A radiation dose of 20 Gy, applied to capped brood of different ages, successfully suppressed egg-laying by the irradiated workers. Mortality was highest in the groups irradiated shortly after capping, but bees emerging during the first two days after irradiation also showed greater mortality than non-irradiated bees. Development of hypopharyngeal glands and cell-building activity were reduced by irradiation.

Can irradiated bees be used to assist in the development of the fertility of a single non-irradiated bee that is kept in the same cage or colony? The fact that some bees in age groups 17–19d and 19–21d had stage 3-ovaries is encouraging, since it shows that the general fitness of the bees is not altogether insufficient to support ovary development. Stage 3-ovaries occurred 19 days later in the irradiated groups than in the controls. Even if some irradiated animals should be physiologically able to complete egg formation, non-irradiated bees placed in their company may have a sufficient advance on them to establish reproductive dominance.

On the other hand, all irradiated bees suffered severe somatic damage, as can be seen from their high mortalities and low production of wax. Moreover, the poor development of their hypopharyngeal glands indicates that jelly production by irradiated bees is probably low. Many studies have shown that the quantity of jelly a worker receives from fellow workers is an important factor for ovary development (Müssbichler, 1952; Dreischer, 1956; Velthuis, 1985; Lin and Winston, 1998). Crailsheim (1992) estimated that between one third and one half of the jelly produced by workers is fed to other adult bees (workers, drones, queen). Therefore, it seems doubtful whether irradiated workers can support the development of fertility of non-irradiated workers placed in their company.

We can only speculate about the mechanism involved in the suppression of worker fertility. It was said above that radiation damage mostly concerns dividing cells. Yet it is not clear whether any divisions occur among the reproductive cells of honey bee workers during pupation, since their ovaries suffer reduction during the late larval and early pupal stages and apparently are inactive still at emergence. According to Reginato and da Cruz-Landim (2002), germinative cells form before larval stage 3, but oogenesis takes place later in the development. The fact that in some age groups, ovary development was not completely suppressed but only retarded may indicate that cells at the beginning of the germ line are not very radiosensitive, while some later stages are. Support for this hypothesis comes from an experiment by Pehani (1963). When he irradiated fertile honey bee queens, they laid unfertile eggs for some days before egg production stopped completely. It resumed after 8–12 days, when fertile eggs were once again laid. Avetisian and Manuilova (1968) report similar results with lower doses. Variable radio sensitivity during different phases of mitotic as well as meiotic activity is also a well-known phenomenon in human radiobiology (McBride and Withers, 2004).

As in many studies on insect irradiation, the precise cause of premature death among the irradiated individuals is hard to assess. Some authors report that damage to epithelial stem cells of the midgut can be a major cause of death of irradiated insects (Riemann and Flint, 1967; Bakri *et al*., 2005). In this case, the insects are unable to digest and stop feeding. In our study, however, irradiated bees defecated as early as bees from the controls, so at least some degree of ingestion must have taken place.

Nearly all the parameters measured in our study (mortality, ovary and hypopharyngeal gland development, cell building activity) indicate a major change in radiosensibility somewhere around 110 hours after capping, approximately 13–14 days after the egg is laid. According to a very detailed histological study by Oertel (1930), at his time, replacement of larval tissues by imaginal ones is complete in most parts of the body, although the differentiation of the produced cells is still far from complete. So it appears that the observed shift in sensitiveness coincides with a stage in which mitotic division is decreasing.

In conclusion, egg laying by worker honey bees can be suppressed by irradiation, although it is not certain whether this is the result of a specific action on dividing germ cells. The irradiated animals show signs of somatic damage. This as well as the low degree of development of their hypopharyngeal glands puts into doubt their usefulness for the production of worker-derived drones from other workers.

Acknowledgements

The work of J. Wegener was made possible by a grant from the Friedrich Schaumann Stiftung. We thank Christoph Jankhöfer for preliminary work on radiation dosage, and Andrea Jäkisch as well as the team of beekeepers of the Länderinstitut for their assistance. We are also very grateful for the useful critics of two anonymous referees.

References

AVETISIAN, G. A.; MANUILOWA, A. C. (1968) Effect of different doses of gamma-radiation on the honey bee (Russian). *Ptchelovodstvo* 48(12): 21.

BAKRI, A.; HEATHER, N.; HENDRICHS, J.; FERRIS, I. (2005) Fifty years of radiation biology in entomology: Lessons learned from IDIDAS. *Annals of the Entomological Society of America* 98(1): 1–12.

BLOM, J. V. D. (1991) Social regulation of egg-laying by queenless honey bee workers (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29(5): 341–346.

BÖGER, K. (1969) Zur Selektion von Bienenvölkern auf Sammelleistung. *Zeitschrift für Bienenforschung* 9(11–12): 545–564.

CRAILSHEIM, K. (1992) The flow of jelly within a honey bee colony. *Journal of Comparative Physiology* B. 162(8): 681–689.

DREISCHER, H. (1956) Untersuchungen über die Arbeitstätigkeit und Drüsenentwicklung altersbestimmter Bienen im weisellosen Volk (German). *Zoologische Jahrbücher* (Physiologie) 66: 429–472.

HARRIS, J. W.; HARBO, J. R. (1991) Producing eggs from a single worker honey bee (Hymenoptera: Apidae). *Journal of Economic Entomology* 84(3): 818–824.

HESS, G. (1942) Über den Einfluss der Weisellosigkeit und des Fruchtbarkeitsvitamins E. auf die Ovarien der Bienenarbeiterin. Beiheft der *Schweizerischen Bienenzeitung* 1: 33–109.

LIN, H.; WINSTON, M. (1998) The role of nutrition and temperature in the ovarian development of the worker honey bee (Apis mellifera). *Canadian Entomologist* 130(6): 883–891.

LINDQUIST, A. W. (1963) *Insect population control by the sterile male technique: scientifically edited comprehensive report of a panel held in Vienna, Oct 16–19 1962*. International Atomic Energy Agency; Vienna, Austria, 59 pp.

MCBRIDE, W. H.; WITHERS, H. R. (2004) Biologic basis of radiation therapy. *In* PEREZ, C. A.; BRADY, L. W.; HALPERIN, E. C.; SCHMIDT-ULLRICH, R. K. (eds) *Principles and practice of radiation oncology*. Lippincott-Williams and Wilkins; Philadelphia, USA; pp 97–136 (4th edition).

MÜSSBICHLER, A. (1952) Die Bedeutung äußerer Einflüsse und der Corpora allata bei der Afterweiselentstehung von *Apis mellifica*. *Zeitschrift für vergleichende Physiologie* 34: 207–221.

O'BRIEN, R. D.; WOLFE, L. D. (1964) *Radiation, radioactivity and insects*. Academic Press; New York, USA, 211 pp.

OERTEL, E. (1930) Metamorphosis in the honey bee. *Journal of Morphology and Physiology* 50(2): 295–232.

PAGE, R. E.; ERICKSON, E. H. (1988) Reproduction by worker honey bees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 23(2): 117–126.

PEHANI, H. (1963) Einige Beobachtungen bei der Bestrahlung von Bienen verschiedener Entwicklungsstadien. *Proceedings of the XIXth Congress of the APIMONDIA held in Prague*, APIMONDIA publishing house; Bukarest, Bulgaria; pp. 422–424 (part 2 of German edition).

REGINATO, R. D.; CRUZ-LANDIM, C. (2002) Morphological characterization of cell death during the ovary differentiation in worker honey bee. *Cell Biology International* 26(3): 243–251.

RIEMANN, J. G.; FLINT, H. M. (1967) Irradiation effects on midguts and testes of the adult boll weevils, *Anthonomus grandis*, determined by histological and shielding studies. *Annals of the Entomological Society of America* 60(2): 289–308.

RUTTNER, F.; HESSE, B. (1981) Rassenspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L. *Apidologie* 12(2): 159–183.

VELTHUIS, H. H. W. (1970) Ovarian development in Apis mellifera worker bees. *Entomologia Experimentalis et Applicata* 13(4): 377–394.

VELTHUIS, H. H W. (1985) The honey bee queen and the social organisation of her colony. *Fortschritte der Zoologie* 31(1): 343–357.