

THE EFFECT OF DIFFERENT DILUENTS ON INSEMINATION SUCCESS IN THE HONEYBEE USING MIXED SEMEN

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Summary

Homogeneous mixing of honeybee (*Apis mellifera*) semen by centrifugation can be an important tool in breeding programmes with honeybees. The effects of different semen diluents on the onset of oviposition, brood pattern and colony development of queens inseminated with mixed semen were tested. Insemination success depended on the type of diluent. A tris-buffer diluent containing arginine and lysine proved to be feasible for use with the centrifugation method.

Introduction

The insemination of a single queen by a large number of drones, which supply genetically effective semen, will be an important tool in breeding and selection schemes with the honeybee. The effective population size (N_e) could be kept at a maximum level depending on the number of drone mothers. Unfavourable inbreeding effects such as inbreeding depression and homozygosity at the sex locus can be efficiently limited (Page & Laidlaw, 1982a, 1982b; Moran, 1984; Moritz, 1984). Because of the large number of effective males per queen, a method is required for homogeneous mixing of a semen sample. Kaftanoglu and Peng (1980) proposed the so-called washing technique as an easy method for collecting honeybee semen. The semen of the drone is washed in diluent and then re-concentrated by centrifugation at 2500 rpm. In experiments with marker genes, Moritz (1983) showed that the centrifugation procedure leads to a homogeneous mixing of the semen when each drone contributes equally to the semen pool. In the classical insemination technique, as proposed by Mackensen and Tucker (1970) or Ruttner (1975), the semen of different drones is not represented in equal proportions in the spermatheca of the inseminated queen.

In spite of its great potential for applied breeding, the centrifugation technique still is in an experimental stage. Kaftanoglu and Peng (1980) found in several cases that queens inseminated with mixed semen became partially drone-layers. Also there is a significant delay in onset of oviposition when using this technique (Kaftanoglu & Peng, 1980, 1982). Therefore it is of interest to improve the method and make it feasible for practical applications.

As shown by various authors (Jaycox, 1960; Lensky & Schindler, 1967; Poole & Taber, 1969; Taber & Blum, 1960; Camargo, 1975; Ruttner, 1975; Verma, 1978) the success of artificial insemination depends on the diluent. Accordingly, several common diluents were tested for their feasibility with the centrifugation technique.

Material and Methods

Fifty queens were reared from a single breeder colony of Carniolan honeybees and kept in mating nuclei (c. 2000 worker bees) with queen excluders at the flight entrance to prevent natural matings. On the 5th and 7th days after emergence the queens were anaesthetized for 10 min with carbon dioxide. All queens were inseminated early in April 1983, a week after the CO₂ treatment. During insemination they were given a second exposure to CO₂. Thus all experiments were run simultaneously in order to keep seasonal effects small. The control group consisted of 20 queens, inseminated using the classical technique (8 µl undiluted semen per queen and Hyes solution as stopper fluid as described by Ruttner, 1975). In the treatment groups, semen samples of 30 µl were collected with an insemination syringe (Ruttner, 1975). This semen pool was diluted in 300 µl of the diluent and centrifuged at 10 000 G for 10 min in a capillary centrifuge (Moritz, 1983). Each independent sample provided semen for 2 queens. Each queen was inseminated with 8 µl of either the upper or the lower fraction of the semen sample, in order to test the effect of the more highly diluted semen in the upper fraction.

Three diluents were tested with 10 queens each. They were constituted as follows:

1. Tris-buffer diluent (Verma, 1973, 1978; Williams & Harbo, 1982): NaCl (1.1%), glucose (0.1%), L-arginine-HCl (0.01%) and L-lysine (0.01%) dissolved in 0.05 tris (hydroxy methyl)-amino-methane buffer at pH 8.7.
2. Hyes diluent (Ruttner, 1975): NaCl (0.9%), CaCl₂ (0.02%), KCl (0.02%) and NaHCO₃ (0.01%) in distilled water, at pH 8.5.
3. Kiev diluent (Paufler, 1974): trisodium citrate-2-hydrate (2.43%), D(+) glucose monohydrate (0.3%), NaHCO₃ (0.21%), sulphanilamide (0.3%), and KCl in distilled water, at pH 8.8.

Penicillin G sodium (0.1-m) and dihydrostreptomycin sulphate (0.1-m) were added to each of these diluents as antibiotics. Diluents were sterile-filtered before use. After insemination the queens were replaced in the nuclei and checked after 7 days. The day of first oviposition was determined according to the age of the oldest brood present in the colony.

Results

Not all the diluents tested seemed to be suitable for use with the centrifugation technique in artificial insemination. The onset of oviposition was much earlier with the tris-buffer diluent than with the other 2 diluents (Fig. 1). Analysis of variance showed a significant effect of diluent on the onset of oviposition of the inseminated queen ($F = 10.12$; $P \leq 0.05$).

There was a significant difference in the time between insemination and oviposition when Hyes solution was used with the centrifugation and the classical insemination techniques. With the centrifuge method the average time was 5.1 days, but with the classical method only 4.35 days.

There was no significant difference in the occurrence of drone layers between queens inseminated with the upper fraction and those inseminated with the lower fraction of semen

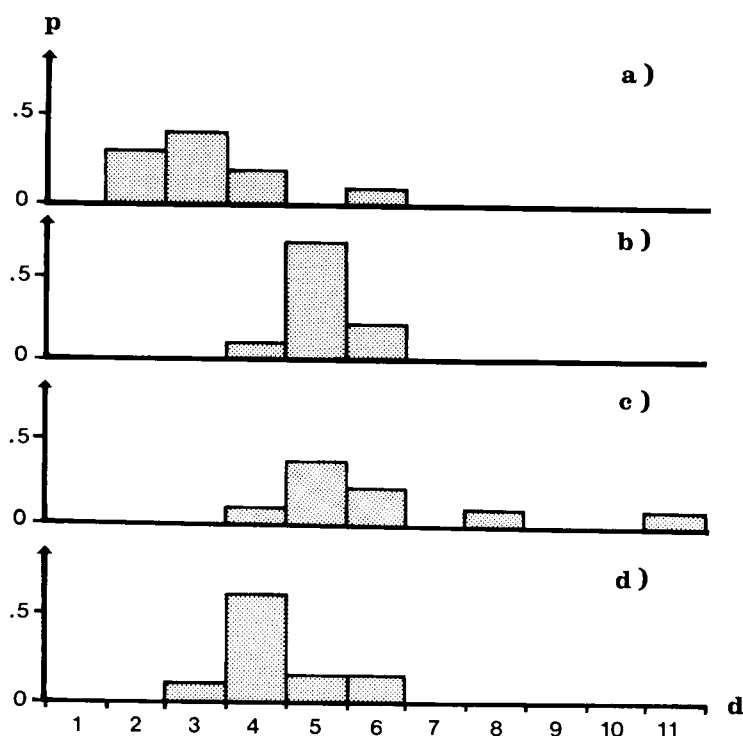


FIG. 1. Frequency distribution for time of oviposition by instrumentally inseminated queen honeybees. The centrifugation technique was used with semen diluted with (a) tris buffer, (b) Hyes diluent or (c) Kiev diluent, and the classical technique (control) with undiluted semen using Hyes solution as a stopper fluid (d). Sample number was 10 for each of (a), (b) and (c), and 20 for (d). The abscissa records number of days to oviposition after insemination and the ordinate indicates frequency.

isolated in the collecting capillary. Two out of 15 queens inseminated with the former and 3 out of 15 inseminated with the latter became drone layers ($\chi^2 = 0.24$). There was a significant effect of diluent, however ($\chi^2 = 12.52$, $P \leq 0.05$); 5 out of 10 queens inseminated with the Kiev diluent produced 30–60% drone brood. The case of the queen that died at the 12th day after insemination was treated as an unsuccessful insemination. Queens inseminated with the other 2 diluents showed a regular brood pattern, not differing from the control (Table 1).

In those colonies in which no drone brood was found, colony development was similar to that of the control group. The number of brood cells per colony (estimated by the area of sealed cells) increased at the same rate and there were no significant differences (Table 2). At the end of the observation period, 6 months after insemination, all queens (drone-layers excluded) still showed a regular brood pattern.

Discussion

Apparently the action of the solution added to the semen was different for the centrifugation and the classical insemination techniques. Though the Kiev diluent gives fine results in the classical technique (Ruttner, 1975), it seems to be unsuitable for the centrifugation technique. The delay of initiation of oviposition and the large number of drone-laying queens resulting from the use of this diluent makes it less valuable for practical use. These results agree well with the observations of Kaftanoglu and Peng (1980, 1982).

The Hyes solution, when used as a stopper fluid with the classical method, produced a shorter interval between insemination of the queen and oviposition than when used as a diluent for centrifugation. In neither case was laying of drone eggs observed. Centrifugation has a retarding effect on the onset of oviposition in both cases. Kaftanoglu and Peng (1980) hypothesize that the dissolution of the seminal plasma during centrifugation may delay oogenesis of inseminated queens. The good results obtained with the tris-buffer diluent is consistent with this hypothesis. This diluent contains the amino acids arginine and lysine, which show a high concentration in the seminal plasma of drone honeybee (Novak et al., 1960). Another explanation could be that the Hyes, and especially the Kiev, diluents contain substances which delay the initiation of oviposition and may have a negative effect on the fertility of the semen (in the case of the Kiev diluent). Williams (1983) showed that the osmolarity of the diluent also may affect the success of instrumental insemination.

In contrast to the data of Kaftanoglu and Peng (1980), all queens which produced some drone brood remained drone layers throughout the season. There was no correlation between laying of drone eggs and the semen fraction (upper or lower) used for insemination. Thus

TABLE 1. Number of successful inseminations of queen honeybees for each of 3 diluents and 2 techniques. Details as for Fig. 1.

Insemination evaluation	Treatment			
	Tris	Hyes	Kiev	Control
Successful	10	10	4	20
Unsuccessful	0	0	6	0

TABLE 2. Number of brood cells resulting from instrumental insemination of queen honeybees using the centrifuge and classical procedures.

Number of cells (\pm SE) was estimated from area of sealed brood. Reduction in number at 24 weeks reflect the end of the season.

Procedure	No. brood cells at:			
	2 weeks	4 weeks	12 weeks	24 weeks
Centrifuge	720 \pm 86	1488 \pm 384	1824 \pm 388	816 \pm 186
Classical	672 \pm 76	1536 \pm 388	1828 \pm 480	846 \pm 169

queens inseminated with the more dilute semen from the top of the collection tube did not become drone layers more often. In all cases at least 1 of the 2 fractions of each sample produced laying of fertilized eggs by queens. Thus there was no sample effect. In conclusion, the centrifugation technique seems to have no unfavourable effects on the quality of the inseminated queens, as long as the appropriate diluent is used. Its use produces similar results to those of the classical technique with respect to both time of oviposition and brood production.

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