A Re-Examination of Double Grafting[1]

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ABSTRACT

Double grafting did not produce heavier queens than did single grafting. If weight is a good indicator of queen quality, this study shows that double grafting is not worth the extra effort. Additionally, priming of cell cups before grafting into them did not improve weight of queens, but it did improve cell acceptance in nurse colonies.

INTRODUCTION

THE QUALITY of queens is not only genetically controlled, but also depends on the conditions in which they grow as larvae. Therefore, queen producers want to provide optimum conditions for larvae as they develop. Unfortunately, we don't always know which management procedures enhance queen quality.

Double grafting and cell priming are procedures that were designed to improve queen quality. In double grafting, a larva is grafted into a cell cup in which another larva (now discarded) had been reared for the previous 24 hours. This gives the second larva an immediate, large supply of food and, supposedly, a chance to become a superior queen. Cell priming is the placement of a drop of dilute royal jelly into a cell cup before a larva is grafted into it. This simplifies grafting and reduces dehydration and injury to larvae.

The scientific literature on double grafting is conflicting. Montagner (1962) found that double grafted queens were heavier and had more ovarioles than did single grafted queens. Örösi Pál (1963) said that double grafted larvae received "better treatment" from nurse bees. However, Weiss (1974) found that double grafted queens were not heavier than single grafted queens when both types of cells were reared in the same colony, thereby presenting the nurse bees with a choice of larvae.

Although Weiss's results are convincing, the advantages of double grafted larvae may have been diminished by placing them in a colony with single grafted larvae. Moreover, there are two possible types of single grafted larvae that should be compared with double grafted larvae: namely, those grafted on the first day (those usually discarded) and those of the same age as the double grafted larvae.

In light of the novel biological problems facing American beekeepers, it is important to fully understand the breeding tools at our disposal. Therefore, we ran two tests to compare the effects of double versus single grafting while controlling for nurse bee exposure to different types of larvae. Additionally, we here report a test on the effects of priming versus dry grafting.

Material and Methods

General:

Six single chamber, queenless, nurse colonies in deep Langstroth equipment were used. Each colony had six combs, and each colony was given fairly equal amounts of honey, pollen, and brood of all ages. Bees were collected from several colonies, stored in a common cage, then distributed from the cage into each hive (after Harbo 1983). About 17,000 bees were put in each hive. Colonies were fed sugar syrup and pollen supplement.

Altogether, 259 queen pupae were reared. We used standard wax cell cups in wooden bases, and each cup was primed with 5-10 mg royal jelly diluted 1:1 with water. Weekly, and before new cups were given to them, each colony was given more brood (sealed and unsealed), and unwanted queen cells on combs were destroyed. All grafted larvae for each test were less than 12 hours old, and only one colony was used as a source for larvae. Except for the test of cell priming, a nurse colony received 20 grafted larvae on two bars (10 cups per bar) in one frame. Queen pupae were weighed to the nearest mg on the eighth day after they were grafted.

Single Versus Double Grafting Choice Test

We compared the weight of queen pupae that had been single grafted with those that had been double grafted. Nurse bees were presented with both types of larvae at the same time. Each of four nurse colonies randomly received one of two groups of larvae (two colonies per group).

The first group was a combination of three larval treatments: single grafted on day 1, single grafted on day 2, and double grafted. All cups were single grafted on day 1, then on day 2 about ⅓ of the cups were replaced with new cups and larvae (single grafted on day 2), and another ⅓ of the larvae were replaced with newly hatched larvae (double grafted); therefore, the double grafted larvae had two controls.

The second group was a combination of only larvae single grafted on day 2 and double grafted larvae. All cups were single grafted on day 1, then on day 2 each cup was either double grafted or replaced with a new cup and larva.

Single Versus Double Grafting in Separate Colonies

We compared the weight of queen pupae that had been single grafted with those that had been double grafted, but this time the larval treatments were in separate colonies. Each of six colonies randomly received one of three larval treatments (two colonies per treatment). The first treatment had only larvae single grafted on day 1. The second treatment had larvae single grafted on day 2. The third treatment had double grafted larvae, in which all cups were single grafted on day 1 then replaced with newly hatched larvae on day 2.

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Priming Versus Dry Grafting:

We compared the weight of queen pupae that had been primed with those that had been dry grafted. Eighty-one queen pupae were reared in four single chamber nurse colonies. Each colony received 24 cups with larvae divided between two treatments: (1) priming the cups with a 1:1 water:royal jelly droplet, and (2) dry grafting by simply placing a larva on the bottom of a wax cup. In each colony the cups were randomly distributed between two cell bars. Queen pupae were weighed seven days after grafting. We also measured the effect of cell priming on acceptance of the cells by workers.

Statistical Analyses:

A randomized design analysis of variance and LSD mean separation were used to find treatment differences. Treatments were replicated with different colonies, but inter-colony variances were never different within a treatment. Therefore, data for each treatment were pooled. If colony effects were present, they were minimized because colonies were set up from a common pool of bees, made as similar as possible, and randomly received treatments. Significance at the $\alpha = 0.05$ level was accepted as different. Acceptance of primed versus dry cells was compared in a 1-tailed 2x2 Chi Square analysis.

Results and Discussion

Single Versus Double Grafting in the Same Colony

None of the larval treatments produced significantly heavier queens in colonies with three treatments ($P = 0.1880$) or with two treatments ($P = 0.3870$). When presented with different groups of larvae, nurse bees did not prefer any group, regardless of its age or grafting treatment (Table 1). This supports the results of Weiss (1974) who also showed no preference by nurse bees in a choice situation.

TABLE 1 Single versus double grafting choice test. Nurse bees were presented with a choice of larvae to rear. Average weights within each column are not significantly different among themselves.

Larval treatment	Avg. weight (± SD) of queen pupae (mg)	
	Colony given all 3 treatments	Colony given 2 treatments
single grafted day 1	261 ± 19 (n = 15)	•
single grafted day 2	255 ± 15 (n = 13)	252 ± 11 (n = 18)
double grafted	248 ± 11 (n = 8)	256 ± 15 (n = 21)

TABLE 2 Single versus double grafting in separate colonies. Each nurse colony had only one of these larval treatments. Average weights with the same letter are not different at the a = 0.05 level.

Larval treatment	Avg. weight (± SD) of queen pupae (mg)	
single grafted day 1	281 ± 16 a	(n = 36)
double grafted	275 ± 14 a b	(n = 34)
single grafted day 2	270 ± 17 b	(n = 33)

Single Versus Double Grafting in Separate Colonies

There were differences among treatments ($P = 0.0133$, Table 2), but the double grafted pupae were not significantly different from either of the other groups. The data suggest that conditions on day 1 may have been better than on day 2 or that there was a colony effect.

Double grafting did not improve the weight of queen pupae. This non-choice test reflects how queens are normally reared (only one type of grafting method per colony), and again it seems that double grafting is not worthwhile.

Priming Versus Dry Grafting:

Priming cells before grafting into them did not improve the weight of queen pupae. Mean weight was 267 mg for primed pupae, and 268 mg for dry grafted pupae. Although Weiss (1974) had dry grafted his larvae, we primed our cups in all our other experiments. Our results showed that this difference in grafting method does not affect the weight of queen pupae, nor invalidate comparisons of our data with those of Weiss.

However, priming improved acceptance of queen larvae by workers ($P < 0.05$). After pooling the results of all 4 test colonies, the number of successfully reared cells was 44 per 48 (92%) for the primed treatment, and 37 per 48 (77%) for the dry grafted treatment.

Conclusions

Double grafting did not improve the weight of queen pupae, and since heavier queens have more ovarioles (Hoopingarner & Farrar, 1959), weight is probably a good criterion for queen selection. Although double grafting may be beneficial in some queen rearing systems, it was not in ours nor in Weiss's (1974). This enhances earlier work by Weiss (1974) by including a test where only one grafting method was used per colony. Additionally, priming of cell cups improved cell acceptance by workers.

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