Effects of male to female ratio and number of females per nesting tunnel on sex ratio and number of progeny of the alfalfa leafcutter bee *Megachile rotundata* (Hymenoptera:Megachilidae)

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ABSTRACT

This paper reports the results of two 3-year studies on the effects of: 1) the ratio of males to females in the parental generation on percent female progeny; and 2) the ratio of females to nesting tunnels on percent female progeny. In study 1): there were no significant differences between treatments in percent females in the progeny or total number of nesting cells produced each year. The average percentages of females were 22% in 1990, 27% in 1991, and 28% in 1992. In study 2): there were significantly fewer female progeny with 2 females per tunnel in 1991, and in all years, significantly fewer progeny when the females were crowded. The total number of cells produced was inversely related to crowding with ratios of females per tunnel of 1:2, 1:1 and 2:1.

Key words: alfalfa leafcutter bee, *Megachile rotundata*, alfalfa seed

INTRODUCTION

The alfalfa leafcutter bee, *Megachile rotundata* (F.), is the primary pollinator of commercial alfalfa seed in the Pacific Northwest of the United States and southwestern Canada (Richards 1984; Mayer *et al.* 1990). Alfalfa seed growers in the U.S. often purchase leafcutter bees by the gallon from producers in Canada. A gallon of bees contains about 10,000 cells, although the percent females in a gallon may vary considerably. Consumers (alfalfa seed growers) prefer the highest possible percent females because females, not males, pollinate the seed crop.

Variable sex ratios have been reported for *M. rotundata* (Osgood 1964, Stephen and Osgood 1965, Waters 1969, Maki and Moffett 1986). Basal cells in the nesting tunnel tend to contain female bees, whereas apical cells contain males (Rothschild 1979).

Some studies show that tunnel diameter affects sex ratios (Stephen and Osgood 1965). In wider tunnels the percentage of females produced is greater than in narrower tunnels of the same depth. Klostermeyer *et al.* (1973) showed that the percent of females increased progressively in 4, 4.8 and 6.2 mm diameter holes. However, Rothschild (1979) found no differences in percent females using 6.35 and 7.14 mm diameter holes.

Gerber and Klostermeyer (1970) showed sex determination to be a voluntary act for *M. rotundata* concluding that the stimulus which induces the bee to lay fertilized, or female eggs is associated with tunnel depth. Later Gerber and Klostermeyer (1972) showed that altering the depth at which cells were completed changed the probability that a cell would receive a fertilized egg. Depths of 4, 8 and 16 cm showed a significant association with sex ratios although there was none with a 12 cm tunnel. Rothschild (1979) also found no association between sex and tunnel with an 11.7 cm tunnel. Stephen and Osgood (1965) found no correlation between sex ratio and tunnel depths of 5.0, 7.5 and 15.0 cm. Jay and Mohr (1987) found sex ratios (male to females) from 1.6:1 to 3:1 despite the use of tunnels of standard length and diameter. They also concluded that there were no significant increases in females produced in replacement nests. Tepedino *et al.* (1994) in a one-year study showed that intertunnel distance had a significant effect on the percentage of female progeny.

Tepedino and Parker (1988) found that the sex ratio of emergent second-generation bees was strongly biased toward females and the sex ratio of diapausing bees was strongly biased toward males: only 35.5% of second generation bees were males compared with 61.7% of those that entered diapause. Parker and Tepedino had reported limited evidence of alternation of sex ratio in 1982.

Gosek *et al.* (1988) reported percentages of females in different breeding lines from 0 to 87%, with a mean of 48%; in wild populations it was 45%. An over-abundance of males led to a small number of females in the next generation (one-quarter to one-fifth of normal). The highest proportion of females (50% above normal) was obtained when the parental generation had only a low number of males. They concluded that the optimal number of males in isolated mating cages is about 15% of the population.

Gerber and Klostermeyer (1972) and Rothschild (1979) concluded that females act as though they know beforehand the number of cells they will provision per tunnel. They could 'know' the number of eggs they will fertilize.

I report here results of 2 studies: 1) the effect of the ratio of males to females in the parental generation on percent female progeny; and 2) the effect of the ratio of females to nesting tunnels on percent female progeny.

MATERIALS AND METHODS

Each year, new nest blocks were prepared by taping laminate wood pieces (1cm x 13cm x 12cm) together with strapping tape to form small blocks with 104 nesting tunnels, and covering the back of each block with aluminum foil. Tunnels were 5mm in diameter and 12cm deep. One nest block was placed in each cage.

Each year, loose bee cells were obtained from Mr Pollination Services in Canada during the winter and stored at 3° C for about 36 weeks. In the spring, the cells were removed from storage and incubated at 28-29° C. Adults that emerged after about 19-21 days were allowed to fly

Table 1

Effect of the ratio of male to female leafcutter bees on percent female progeny and total cells produced. Prosser, WA.

	1990		1991		1992	
Males:Females	% Females	Total	% Females	Total	% Females	Total
6:1	24a	684a	28a	576a	32a	589a
3:1	19a	683a	24a	664a	30a	661a
2:1	26a	726a	31a	660a	29a	694a
1:5	18a	606a	29a	867a	21a	588a

Means within a column followed by the same letter are not significantly different at the p=0.05 level, Newman-Keuls studentized range test.

Table 2

Effect of the ratio of female leafcutter bees to tunnels on percent female progeny and total cells produced. Prosser, WA.

	1990		1991		1992	
Females:Tunnels	% Females	Total	% Females	Total	% Females	Total
1:2	18a	566a	33a	421a	30a	505a
1:1	23a	496a	30a	303b	34a	483a
2:1	22a	181b	19b	300b	28a	286b

Means within a column followed by the same letter are not significantly different at the p=0.05 level, Newman-Keuls studentized range test.

in the laboratory and males and females were counted and collected into separate vials off the windows. The adults were then released into the cages containing blooming alfalfa and the nest blocks.

For the male-to-female ratio studies, 16 cages (6 x 6 x 1.8 m) and for the females-per-tunnel studies, 12 similar cages were erected over different plots of blooming alfalfa each year at Prosser, WA. For male-to-female ratio studies, 80 females were put in each cage and then males added to obtain a 6:1 ratio in 4 cages, 3:1 in 4 cages, 2:1 in 4 cages, and 1:5 in 4 cages. For female-per-tunnel studies, 52 females were put in 4 cages (1 female:2 tunnels), 104 females were put in 4 cages (1 female:1 tunnel) and 208 females in 4 cages (2 females:1 tunnel). Males were put in the cages at the same time and in equal number to females (1:1). In 1990, bees were put in the cages on 30 July; in 1991 on 5 July; and in 1992 15 July.

The bees foraged and constructed cells in the nest blocks during each season. At the end of the nesting season (August) the bee cells were extracted from the laminate boards, counted and put into cold storage at 3° C. After a chilling period of about 36 weeks, the cells were incubated at 28-29° C, the adults reared out and sexed to determine sex ratios.

The data were analyzed as a randomized complete block design by analysis of variance, with Newman-Keuls studentized range test for mean separations (Lund 1989).

RESULTS AND DISCUSSION

Male to Female Ratio

There were no significant differences between treatments in the percent females in the progeny in any year (Table 1), and no significant differences between treatments in the total number of cells produced each year. There were no significant differences in the mean percent females in different years (22% in 1990, 27% in 1991, and 28% in 1992).

In contrast to Gosek *et al.* (1988), I found that the ratio of males to females had no effect on percent females in the progeny. This difference could conceivably be attributed to different test conditions, methods, or even differences between races of the alfalfa leafcutter bee.

Females per Tunnel

In 1991, there was a significantly lower percentage of female progeny produced with 2 females per tunnel (Table 2). In all 3 years, there were significantly fewer progeny produced when the bees were crowded (2:1 ratio) than when uncrowded (1:2 ratio). The total number of cells produced was inversely related to crowding with ratios of females per tunnel of 1:2, 1:1 and 2:1. Large-scale research is needed to confirm these effects in the field.

CONCLUSION

The percentage of female progeny in any population of alfalfa leafcutter bees appears to depend on several, interrelated, factors. It was not affected by different ratios of male to female parents or the number of females released into large cages with a fixed number of wooden tunnels. Larger numbers of females made significantly fewer cells. The results were consistent over three years except that in one year the percentage of females was reduced in the most crowded condition.

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REFERENCES

Gerber, H.S. and E.C. Klostermeyer. 1970. Sex control by bees: a voluntary act of egg fertilization during oviposition. Science 167: 82-84.

Gerber, H.S. and E.C. Klostermeyer. 1972. Factors affecting the sex ratio and nesting behavior of the alfalfa leaf-cutter bee. Tech. Bull. Wash. Agric. Sta. 73. 11 pp.

Gosek, J., A. Ruszkowski, M. Bilinski and K. Kaczmarska. 1988. The percentage of females in the progeny of the lucerne leafcutter bee (*Megachile rotundata*) in relation to the percentage of females in the parent generation and to the breeding line and population density. Pszczelnicze Zeszyty Naukowe 32: 99-110. (In Polish).

Klostermeyer, E.C., S.J. Mech, Jr. and W.B. Rasmussen. 1973. Sex and weight of *Megachile rotundata* (Hymenoptera: Megachilidae) progeny associated with provision weights. J. Kans. Entomol. Soc. 46: 536-548.

Jay, S.C. and N. Mohr. 1987. The effect of nest replacement on the production of females of the alfalfa leaf-cutter bee *Megachile rotundata* (F). J. Apicult. Res. 26:69-72.

Lund, R.E. 1989. MSUSTAT statistical analysis package 4.12. Montana State University, Bozeman, MT. 133 pp.

Maki, D.L. and J.O. Moffett. 1986. Nesting cell position, sex ratios, and emergence times of the alfalfa leafcutting bee, *Megachile rotundata* (F.) in northwestern Oklahoma. Southwest Entomol. 11: 107-112.

Mayer, D.F., J.D. Lunden and E.R. Miliczky. 1990. Effects of fungicides on chalkbrood disease of alfalfa leafcutting bee. Appl. Agric. Res. 5: 223-226.

Osgood, C.E. 1964. Foraging and nesting behavior of the leafcutter bee *Megachile rotundata* (Hymenoptera: Megachilidae). Can. Entomol. 112: 555-558.

Parker F.D. and V.J. Tepedino. 1982. Maternal influence on diapause in the alfalfa leafcutting bee. Ann. Entomol. Soc. Amer. 75: 407-410.

Richards, K.W. 1984. Alfalfa leafcutter bee management in Western Canada. Agric. Can. Publ. 1495/E. 53 pp.

Rothschild, M. 1979. Factors influencing size and sex ratio in *Megachile rotundata* (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 53:392-401.

Stephen, W.P. and C.E. Osgood. 1865. Influence of the tunnel size and nesting material on the sex ratios in a leaf-cutter bee, *Megachile rotundata*. J. Econ. Entomol. 58:965-968.

Tepedino, V.J. and F.D. Parker. 1988. Alternation of sex ratio in a partially bivoltine bee, *Megachile rotundata* (Hymenoptera:Megachilidae). Ann. Entomol. Soc. Amer. 467-476.

Tepedino, V.J., D.R. Frohlich and C.R. Baird. 1994. Effect of intertunnel distance and nest-surface aspect on progeny production rate and sex ratio in the alfalfa leafcutting bee (Hymenoptera: Megachilidae). J. Econ. Entomol. 87: 27-30.

Waters, N.D. 1969. Alfalfa pollinators. Idaho Agric. Ext. Serv. Curr. Info. Ser. 106. 2pp.