Apidologie (1994) 25, 31-37

Original article

Influence of the presence of adult drones on the further production of drones in honey bee (*Apis mellifera* L) colonies

CE Henderson *

Department of Entomology, Cornell University, Ithaca, NY 14853, USA

(Received 21 January 1993; accepted 28 May 1993)

Summary — Adult drones were removed from experimental colonies and the number of drones reared was compared with that of control colonies containing natural numbers of drones. No differences were found in the number of drones reared in the experimental and control colonies in 3 samplings taken at 12, 24 and 36 d post-treatment.

drone / sex ratio

INTRODUCTION

How honey bees regulate the number of males produced in their colonies throughout the spring and summer seasons has received little attention (Allen, 1965; Free and Williams, 1975; Page, 1981; Page and Metcalf, 1984; Henderson, 1991), and consequently very little is known about how drone-to-worker ratios are maintained.

Drone production may be controlled in part by the workers which regulate the amount of drone comb built and feed the developing larvae within the colony, and in part by the queen who lays drone or worker eggs in the wax cells provided by the workers (Henderson, 1991). In addition, the influence on further drone production by the presence of adult drones (Rinderer *et al,* 1985; Omholt, 1988) or immature drones (Free and Williams, 1975) has been suggested. These studies suggest that the presence of adult or immature males, or both, limit the further production of males by means of a negative feedback mechanism.

Adult drones have been reported to have a depressing value of about –0.65 drones (inverse linear relationship of adult

* Present address: 213 Monte Vista Drive, Aptos, CA 95003-4014, USA

drones introduced into the colony and the number of drones reared, with slope = –0.648 ± 0.287) (Rinderer *et al*, 1985). This means that the loss of an adult drone stimulates the additional production of 0.65 drones. The mechanism by which this feedback may occur was suggested by Omholt (1988) who hypothesized that adult drones emit a long-lasting pheromone which has an inhibitory effect upon further drone rearing in honey-bee colonies. The empirical data for this hypothesis came from Rinderer *et al* (1985) who introduced large numbers (up to 2 000) adult drones into experimental colonies, and restricted their exit at the entrance of the colony. Their results indicated that colonies receiving the larger numbers of foreign drones reared fewer drones of their own. No information was provided as to how many adult drones those colonies contained initially.

The purpose of this experiment was to re-examine the adult feedback hypothesis by manipulating experimental colonies such that they could be depleted of adult drones, while control colonies would be left with their normal adult drone population. This method would avoid the unnatural situation resulting from the introduction of large numbers of foreign drones into treatment colonies. It would also allow the monitoring of the total number of adult males in the experimental colonies. The interest lay in determining whether the natural range of adult drone numbers within colonies played a role in the control of further drone production.

METHODS

This study was conducted from February through April 1990 at the Archbold Biological Station in Lake Placid, Florida. Ten 3-story hives containing 8-frame, medium depth supers were used. Colonies had 2 lower brood chambers (30 l each), and an upper chamber initially containing empty comb, but which slowly filled with honey as the experiment progressed. All colonies were located in a single beeyard, were equipped with queen excluders restricting the queens to the 2 lower chambers, and were queenright throughout the study. The entrances of immediately neighboring colonies were oriented 180° from each other to minimize drifting.

On 22 February, worker and drone brood were sampled in all colonies, and drone comb quantities were equalized, providing each brood chamber with approximately 17% drone comb area. This is the mean amount of drone comb found in feral colonies aged 1–4 yr (Seeley and Morse, 1976). The drone and worker pupae were counted by using a Plexiglas grid containing 72 sections, that provided an estimate accurate within 10% (Ratnieks, 1986). Each section on the grid was 29 mm wide by 31 mm tall. This measurement was done to assess the number of drones being reared in the colonies before any drone comb equalization had been done. The drone brood counted in this phase of the experiment was named "pre-treatment" data and will be referred to as such in the results.

Colonies were not disturbed for 12 d, allowing them to adjust their drone rearing to the new quantity of comb within their hives. On 6 March the colonies were randomly allocated to 2 groups: 1) 5 drone-depleted colonies (experimental); and 2) 5 drone-containing colonies (control). The 5 control colonies were left with open entrances that allowed the free flight of drones, whereas the experimental colonies had drone traps attached to their entrances. Traps are designed to hold the drones in a separate compartment, and not allow their exit from or re-entrance to the hive. The drone traps did not hinder the entrance of pollen to the hive. Each colony was then re-sampled to determine the number of drone and worker pupae. This measurement was named the "onset" data and will be referred to as such in the results.

Starting 7 March, all the drones that had been trapped while attempting to exit the experimental colonies on the previous day were removed each morning and counted. To further deplete these colonies of younger drones that were not yet attempting flight (Howell and Usinger, 1993), every 3rd d colonies were opened and drones were manually removed from the brood chamber. This procedure was performed before drone flight time, avoiding their entrance or exit from the opened colonies. Control colonies (open entrance) were also opened and the brood chambers were inspected to obtain an estimate of the number of adult drones present at 3 times during the experiment (21 March, 4 April, 14 April). Hence, control colonies were disturbed less than the treatment colonies that were opened every 3 d. Every brood frame was searched on 1 side (the west side) and, with the use of the grid, all drones on that side were counted. Total numbers were multiplied by 2 to obtain an estimate of total drones within the control colonies.

All colonies were subsequently sampled for worker and drone pupae 3 times at a 12-d interval (18 March, 30 March and 11 April). These measurements were named "post-treatment" data and will be referred to as such in the results. Worker pupal developmental time is 12 d, therefore every pupa would be counted only once. However, 12-d brood counts tend to overestimate the number of drones reared by a colony since they spend 14 d in the pupal stage (some would be counted twice). To correct for this, the numbers of drone pupae counted on 30 March and 11 April (2nd and 3rd sample dates) were multiplied by 12/14.

If a change in the number of drones reared post-treatment were to occur, it should be evident within 24 d of the onset of the treatment (after 2–12-d interval samplings), as it is nearly 2 generation times since the initiation of the experiment. However, data were collected a 3rd time (36 d post-onset), to avoid missing a phenomenon of differential drone production due to a possible slow feedback mechanism in the colonies. Means were taken for the 3 post-treatment dates (18 March, 30 March, 11 April).

Means of number of drone brood reared in experimental and control groups were statistically compared by using *T*-tests, and significance was determined at the 0.05 alpha level. When comparing percentages, arcsin square-root transformations were performed to account for the heterogeneity of variances.

RESULTS

There was no significant difference between the number or percentage of drones reared in the experimental and control colonies (t = 1.08, p = 0.3; and t = 2.05, p =

Table I. Number of drones reared in drone-depleted (treatment) and drone-containing (control) colonies.

	Pre-treatment	*Onset*		*Post-treatment*		*Mean*
	22 Feb	*6 Mar*	*18 Mar*	*30 Mar*	*11 Apr*	
Treatment colony number						
1	750	1 588	2 050	1 900	1 450	1 800
3	500	635	1 350	1 250	1 100	1 233
5	1 050	699	1 050	1 000	50	700
7	600	64	400	850	400	550
9	250	508	500	450	460	470
Mean	630	699	1 070	1 090	692	
Control colony number						
2	100	1 016	1 600	850	500	983
4	1 300	1 397	2 800	2 600	1 550	2 317
6	550	508	750	700	1 600	1 017
8	850	191	2 300	1 200	1 100	1 533
10	400	889	1 300	800	850	983
Mean	640	800	1 750	1 230	1 120	

0.06, respectively) (table I). Experimental colonies (drones removed) reared fewer drones at all stages of the experiment, and although this difference became larger after treatment, differences were never statistically significant (fig 1). The difference in drone production between experimental groups is even larger when computed as the percent drones of the total brood reared, but again, the differences are not statistically significant (fig 2). The number of workers reared in both the drone-depleted and control colonies are shown as an indication of the population growth and general colony condition (table II). The mean number of adult drones counted in the control colonies on the 3 post-treatment sample dates was 3 178 (± SE 678). The experimental colonies had a total of 2 132 (± SE 607) drones removed from them.

DISCUSSION

There was no evidence that the low number of adult drones in the experimental colonies stimulated further rearing of drones in those colonies. The number of drones reared in both groups was not statistically different, but the number and percentage of drones reared in the control colonies, which contained their natural population of adult drones, was somewhat higher than the number of drones reared in the control colonies. These results are inconsistent with those of Rinderer *et al* (1985) who concluded that the presence of increasing numbers of adult drones inhibits the further production of males within honey bee colonies in a linear fashion. One possible explanation of this discrepancy could be that in order for that feedback mechanism to function, the number of young adult drones would have to be very high, superior to the number that were present in control colonies reported here. This explanation seems unlikely, however, because if the phenomenon exists, it should be sensitive to the levels of naturally occurring drone numbers within the colony. Rinderer *et al* (1985) introduced from 500 to 2 000 drones (1 to 6 d of age) into the experimental colonies. Perhaps these high numbers induced the feedback phenomenon. However, we do not know what the total numbers of drones in the Rinderer *et al*

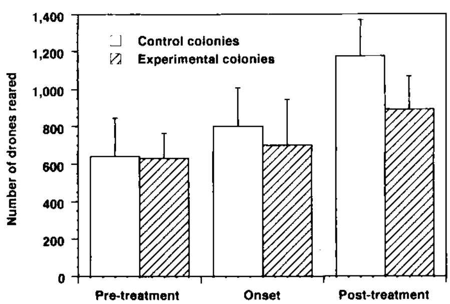

Fig 1. Mean number of drones reared by treatment colonies and control colonies before any manipulation (pre-treatment); after drone comb equalization and prior to drone depletion (onset); and after drone depletion of experimental colonies (post-treatment). Bars indicate standard errors. ☐: control colonies; ▨: experimental colonies.

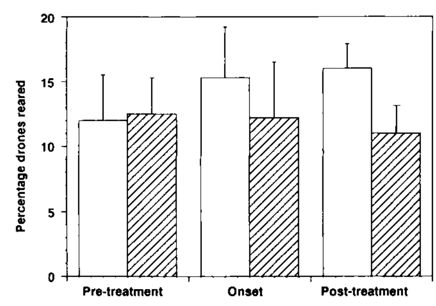

Fig 2. Mean percent of drones relative to total brood reared by treatment and control colonies before any manipulation (pre-treatment); after drone comb equalization and prior to drone depletion (onset); and after drone depletion of experimental colonies (post-treatment). Bars indicate standard errors. ☐: control colonies; ▨ : experimental colonies.

Table II. Number of workers reared in drone-depleted (treatment) and drone-containing (control) colonies.

	Pre-treatment	*Onset*	*Post-treatment*			*Mean*
	22 Feb	*6 Mar*	*18 Mar*	*30 Mar*	*11 Apr*	
Treatment colony number						
1	7 344	5 886	12 312	11 232	13 176	12 240
3	6 984	6 671	13 752	12 528	11 448	12 576
5	5 472	8 476	8 280	7 560	2 304	6 048
7	5 400	5 964	9 864	10 296	8 712	9 624
9	6 768	7 534	11 520	10 584	11 052	11 052
Mean	6 394	6 906	11 146	10 440	9 338	
Control colony number						
2	5 400	5 101	9 792	9 144	5 400	8 112
4	6 336	5 964	9 864	9 000	9 432	9 432
6	7 128	6 278	8 064	7 416	12 672	9 384
8	6 192	6 671	9 360	8 712	4 968	7 680
10	6 480	7 377	8 928	7 560	9 576	8 688
Mean	6 307	6 278	9 202	8 366	8 410	

(1985) experimental colonies were, as those adults already present before the experimental manipulation were not counted. Colonies in the present study were provided with natural quantities of drone comb, allowing the colonies to rear drones at a level close to that of feral colonies. In addition, judging by the numbers of drones counted in the control colonies at the onset of the experiment and after 1 month, the number of drones reared by these colonies was not low (table I). Sixteen percent drone production is comparable to the percentage of drones reared in undisturbed colonies (Page and Metcalf, 1984; Henderson, 1991).

A second explanation for the discrepancy in the data presented here and those of Rinderer *et al* (1985) is that perhaps the artificial introduction of foreign adult drones of equal age into colonies created a disturbance that limited further brood production by their colonies. Levels of worker brood production were not reported.

Season may be important in the detection of a potential drone feedback mechanism. The Rinderer *et al* (1985) study does not report the season in which the experiment was conducted. The present experiment was performed in early spring, a time when colonies are naturally rearing large numbers of drones. These numbers decreased in all but 1 colony by the latest sample date (April 11) (table I). Possibly a negative feedback of adult drones could be offset at times of maximum drone production. This seems unlikely, however, because if a feedback mechanism occurs, it should be tuned to work best at a time when drone rearing is at its maximum, especially if it involves a pheromone.

Experimental colonies produced somewhat fewer drones than the control colonies. This may have been an artifact of manipulation. Control colonies did not have traps attached to their entrances nor were they opened as frequently as the experimental ones. The disturbance, however, did not seem to affect the rearing of workers. Control colonies reared slightly fewer workers than the manipulated treatment colonies (table II), and, although similar to the findings of Rinderer *et al* (1985), the more disturbed colonies reared fewer drones. To determine whether these results were related to an artifact of experimental design would require further investigation. Nevertheless, the hypothesis of the presence of a long-lasting inhibitory adult drone pheromone (Omholt, 1988) seems premature, in light of the conflicting empirical date available to date.

ACKNOWLEDGMENTS

I thank A Brew for loaning honeybee colonies, and Archbold Biological Station in Florida for hosting me. RA Morse, TD Seeley and GC Eickwort from Cornell University reviewed this manuscript.

Résumé — Influence de la présence de mâles adultes sur la production d'autres mâles dans la colonie d'abeilles (*Apis mellifera* L). On a utilisé 10 ruches comportant 3 corps de ruche et renfermant toutes 17% de rayons de mâles, quantité moyenne présente dans les colonies sauvages. Cinq colonies ont été munies de pièges à mâles conçus pour capturer tous les mâles adultes quittant la colonie. On a démuni ces colonies de leurs jeunes mâles en les ôtant à la main du corps de ruche tous les 3 j. À 3 reprises, à 12 j d'intervalle, les 10 colonies ont été ouvertes et tous les mâles au cours de développement ont été comptés au moyen d'une grille sur une face de chaque cadre de couvain. Dans les colonies témoins, les mâles étaient libres d'entrer et de sortir. On n'a pas trouvé de différence significative entre le nombre ou le pourcentage de mâles élevés dans les colonies expérimentales et ceux élevés dans les colonies témoins (t = 1,08; p = 0,3 et t = 2,05; p = 0,06 respectivement). Il n'y a donc pas de preuve qu'un faible effectif de mâles dans une colonie stimule l'élevage d'autres mâles.

mâle / sex-ratio

Zusammenfassung — Einfluß der Anwesenheit erwachsener Drohnen auf die Aufzucht weiterer Drohnen im Bienenvolk (*Apis mellifera* L). Es wurden 10 Völker mit je drei Räumen (zwei Bruträume) benutzt. Alle Völker enthielten 17% Drohnenbau, entsprechend der mittleren, in Wildvölkern vorhandenen Menge. Fünf Völker waren mit Drohnenfallen ausgestattet, welche alle Drohnen beim Verlassen des Volkes abfingen. Diese Völker wurden durch manuelle Entfernung der jungen Drohnen aus dem Brutraum jeden dritten Tag noch weiter ihrer Drohnen beraubt. In den Kontrollvölkern konnten die Drohnen frei ein- und ausfliegen. An drei Terminen, in 12-tägigem Intervall, wurden alle 10 Völker geöffnet und die sich entwickelnden Drohnen auf einer Seite jeder Brutwabe mit Hilfe eines Gitters gezählt.

Es bestand kein signifikanter Unterschied zwischen Zahl oder Prozentsatz der in den Versuchs- und den Kontrollvölkern aufgezogenen Drohnen (t = 1,08, p = 0,3, bzw t = 2,05, p =0,06; Tabelle I und Abb 1). Es wurde kein Beweis für die Annahme gefunden, daß niedrige Zahlen erwachsener Drohnen in den Völkern die Aufzucht weiterer Drohnen anregen.

Drohnen / Geschlechtsverhältnis

REFERENCES

Allen MD (1965) The effect of a plentiful supply of comb on colonies of honeybees. *J Apic Res* 4, 109-119

Free JB, Williams IH (1975) Factors determining the rearing and rejection of drones by the honeybee colony. *Anim Behav* 23, 650-675

Henderson CE (1991) Reproductive investment in drones in honey-bee *(Apis mellifera)* colonies. PhD Thesis, Cornell University, Ithaca, NY, USA

Howell DE, Usinger RL (1933) Observations on the flight and length of life of drone bees. *Ann Entomol Soc Am* 62, 239-246

Omholt SW (1988) Drone production in honeybee colonies: Controlled by a long-lasting inhibitory pheromone from the drones? *J Theor Biol* 134, 309-318

Page RE Jr (1981) Protandrous reproduction in honey bees. *Environ Entomol* 10, 359-362

Page RE Jr, Metcalf RA (1984) A population investment sex ratio for the honey bee (*Apis mellifera* L). *Am Nat* 124, 680-702

Ratnieks FLW (1986) The effect of colony population size on the efficiency of nectar collection and honey production in honey bee *(Apis mellifera)* colonies. MS Thesis, Cornell University, Ithaca, NY, USA

Rinderer TE, Hellmich RL, Danka RG, Collins AM (1985) Male reproductive parasitism: a factor in the Africanization of European honey-bee populations. *Science* 228, 1119-1121

Seeley TD, Morse RA (1976) The nest of the honey bee (*Apis mellifera* L). *Insectes Soc* 23, 495-512