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Original article

Seasonal constraints on mating and insemination of queen honey bees in a continental climate

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Summary — Mating frequency and quality of insemination of queen honey bees were examined during spring 1984 to 1986 and summer 1984 at Madison, WI, USA. Mating frequency and sperm counts were highly variable among queens reared and mated during spring. During summer 1984, results were comparable to that reported for commercial queen rearers in the United States.

Apis mellifera **— mating — insemination**

Résumé — Contraintes saisonnières pour l'accouplement et la fécondation des reines d'abeille en climat continental. *On a examiné la fréquence d'accouplement des reines d'abeille (*Apis mellifica *L.) au cours des printemps 1984 à 1986 et durant l'été 1984 à Madison, Wisconsin (USA). Les larves ont été greffées chaque semaine à partir de la mi-mars ou la fin de mars de chaque saison. Des cellules royales individuelles ont été placées dans les nuclei de fécondation le dixième jour après le greffage. Sept jours après l'introduction des cellules, on a vérifié l'émergence des reines et on les a retirées des nucléi 7 jours plus tard. On a calculé pour chaque semaine le pourcentage des cellules qui avaient donné naissance à une reine et le pourcentage des cellules dont les reines s'étaient ou non accouplées. Afin d'évaluer les chances de réussite de la fécondation, on a dénombré les spermatozoïdes présents dans la spermathèque d'environ 10 reines par semaine.*

Les reines issues des larves greffées la première semaine d'avril se sont accouplées au cours des 3 années de l'étude. La fréquence d'accouplement des reines issues de larves greffées avant le 24 avril a énormément varié à l'intérieur d'une même année et d'une année à l'autre et un fort pourcentage de reines ne s'est pas accouplé. Sur les 3 années étudiées, 41% (33 sur 81) des reines examinées provenant de cette période de greffage possédaient au moins 4,5 millions de spermatozoïdes.

La fréquence d'accouplement des reines issues de larves greffées entre le 24 avril et le 15 mai a varié de 50 à 75% (Fig. 1). Le nombre moyen de spermatozoïdes était généralement > 4 millions (Fig. 2). Les teneurs en spermatozoïdes de la spermathèque des reines issues de cette période de greffage ont été comparables à celles des reines produites dans le sud et l'ouest des Etats-Unis (au climat subtropical et méditerranéen, respectivement) par les éleveurs professionnels de reines, au printemps 1985 (Fig. 3). La majorité de ces reines ont été suffisamment fécondées pour être utilisées par les apiculteurs : 60% (66 sur 110) des reines avaient au moins 4,5 millions de spermatozoïdes contre 70% (35 sur 50) des reines produites dans le sud et l'ouest des Etat-Unis.

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La fréquence d'accouplement des reines issues de larves greffées entre le 25 mai et le 11 septembre 1984 a varié de 45 à 90% (Fig. 4A), et le nombre moyen de spermatozoïdes de 5,0 à 6,5 millions (Fig. 4B). La probabilité était très élevée (P=0,95) d'obtenir des reines fécondées ayant un nombre minimum de spermatozoïdes d'au moins 4,0 millions.

La fréquence d'accouplement a été largement déterminée par l'émergence des reines et leur acceptation dans les nuclei : l'émergence s'est produite dans 50 à 95% des cas (Fig. 1 et 4A) (70,6% sur l'ensemble de l'étude). Seulement 8,5% des reines ont été perdues à la suite du premier examen après l'émergence.

Apis mellifera — *accouplement — insemination*

Zusammenfassung — Saisonale Einschränkungen des Begattungs- und Besamungserfolges bei Königinnen der Honigbiene im Kontinentalklima. *Der Begattungserfolg von Honigbienen wurde in den Frühjahren 1984 bis 1986 und im Sommer 1984 in Madison, Wisconsin, USA untersucht. Dazu wurden ab Mitte bis Ende März wöchentlich Larven umgesetzt. Die Weiselzellen wurden 10 Tage nach dem Umlarven einzeln in Begattungskästchen gehängt. Die Kästchen wurden 7 Tage später auf Anwesenheit der Königin durchgesehen und noch einmal 7 Tage später wieder entweiselt. Der Prozentsatz an geschlüpften Zellen und an begatteten und unbegatteten Königinnen wurde wöchentlich protokolliert. Um den Besamungserfolg zu beurteilen, wurden wöchentlich die Spermien in der Spermatheka von ≈ 10 Königinnen ausgezählt.*

In allen drei Jahren wurden auch Königinnen aus Zuchten der ersten Aprilwoche begattet. Der Begattungserfolg von Königinnen, die vor dem 24. April gezüchtet wurden, unterschied sich jedoch deutlich in den einzelnen Jahren und ein hoher Prozentsatz an Königinnen wurde nicht begattet (Abb. 1). 41% (33 von 81) aller Königinnen, die in diesem Zeitraum in den drei Jahren gezüchtet wurden, hatten mehr als 4.5 Mio. Spermien in ihrer Spermatheka.

Der Paarungserfolg von Königinnen, die zwischen dem 24. April und 15. Mai zur Zucht kamen, variierte von etwa 50 bis 75% (Abb. 1). Die mittlere Spermienanzahl war im allgemeinen größer als 4 Mio (Abb. 2). Der Spermiengehalt der Spermatheken der Königinnen aus dieser Zuchtperiode war in etwa vergleichbar mit dem der Königinnen, die von kommerziellen Züchtern in den Süd- und Weststaaten (subtropisches bzw. mediterranes Klima) im Frühjahr 1985 angeboten wurden (Abb. 3). Die Mehrzahl der Königinnen war also für den Einsatz in der Imkerei ausreichend besamt : 60% (66 von 110) hatten mehr als 4.5 Mio Spermien in ihrer Spermatheka im Vergleich zu 70% (35 von 50) der Könginnen aus den Süd- und Weststaaten.

Der Begattungserfolg von Königinnen, die zwichen dem 22. Mai und 11. September 1984 aufgezogen wurden, schwankte zwischen 45 und 90% (Abb. 4A). Die mittlere Spermienzahl variierte von 5.0 bis 6.5 Mio (Abb. 4B). Zu dieser Zeit war die Wahrscheinlichkeit, eine begattete Königin mit einem Minimum an Spermien von 4.0 Mio zu erhalten, sehr groß (P=0.95).

Der Begattungserfolg wurde vor allem durch den erfolgreichen Schlupf der Königin und deren Annahme durch das Volk bestimmt : die Schlupfrate variierte von 50 bis 95% (Abb. 1, 4A) (70.6% des gesamten Versuchs). Nur 8.5% der Königinnen verschwanden nach der ersten Durchsicht nach dem Schlupf.

Apis mellifera — *Begattung — Besamung*

Introduction

The frequency of queen loss in feral and commercial honey bee colonies is influenced by spermathecal sperm depletion, disease, or other catastrophic events; colony survival requires successful rearing and mating of replacement queens. Observations of 39 commercially managed colonies in Wisconsin, USA over 2 consecutive seasons (Severson and Erickson, 1984) indicate that queen loss is a common occurrence : 5 colonies (12.8%) were not successful in replacing their queens and remained queenless, while 11 colonies (28.2%) successfully replaced (superseded) their queens. Harbo and Szabo (1984) reported similar results : 42% (25 of 59) of naturally mated

queens in commercially managed colonies did not survive 12 months. Beekeepers in the northern United States and Canada frequently order replacement queens from queen breeders in the southern United States where early spring weather conditions are conducive to successful matings. However, the continued availability of these replacement queens is questionable : an invasion of Africanized honey bees into the southern United States by as early as late 1988 (Taylor, 1977, 1985) may result in implementation of inter-state quarantines. In a worst-case scenario (Taylor, 1985), beekeepers in the northern United States and Canada would have no other outlet for replacement queens except to rear them locally.

Queens mate in flight on warm sunny afternoons about 6—8 days after emergence from the queen cell (Ruttner, 1956). Queen mating flights are often restricted if cool, windy or cloudy conditions prevail during this period (Lensky and Demter, 1985). The cool and irregular weather characteristic of spring in continental climates would appear to limit successful early matings. However, the reproductive ecology of honey bees in continental climates has not been investigated. In this study, our objectives were to conduct preliminary examinations of : 1) seasonal variation in mating frequency, with an emphasis on spring mating frequency; and 2) the quality of insemination of newly mated queens in a continental climate (Wisconsin, USA) under commercial queen rearing conditions.

Materials and Methods

This study was conducted at Madison, WI, USA from 1984 to 1986. Each year in mid-February, colonies to be used as queen cell rearers were fed a pollen supplement to stimulate brood rearing. The supplement was provided continuously until fresh pollen was readily available in the field. Queen mother colonies were fed pollen supplement during early March to ensure the presence of suitable age larvae for grafting. During mid to late March (13 March 1984, 12 March 1985, 25 March 1986) grafted larvae were introduced into the cell rearers using standard queen rearing procedures (Laidlaw, 1979). Larvae were grafted weekly throughout the spring and summer during 1984 and until about mid-May during 1985 and 1986.

Individual queen cells were placed in 4-frame nucleus colonies containing ≈ 0.45 kg of worker bees and one frame of brood on the 10th day after grafting (queens emerge ≈ 12 days after grafting). Nuclei were established in 2 apiaries. From 6-16 (usually 10) queen cells were introduced weekly in each apiary. Nuclei were examined for emerged queens 7 days after cell introduction and queens were removed from them 7 days later. The same nuclei were utilized throughout each year; queens were removed from them and another set of queen cells introduced on a biweekly basis. Brood and bees were added to the nuclei as necessary to maintain adequate populations. Queens that were laying after 2 weeks were considered to be inseminated; with non-laying queens the spermathecae were examined for the presence of spermatozoa.

A preliminary examination of the results indicated that queen mating frequency was similar in both mating apiaries; data analyses, therefore, represent combined data. The percentage of cells which emerged and the percentages of cells resulting in mated and unmated queens were calculated weekly. As a measure of insemination quality, spermathecal sperm counts were estimated (Mackensen and Tucker, 1970) from at least 5 queens per apiary per week, whenever possible. Mean spermathecal sperm counts and < 95% confidence limits (Sokal and Rohlf, 1981) were calculated weekly. To provide a comparison between queens reared in a continental climate and those from other areas, 5 queens from each of 10 commercial queen producers representing the major queen rearing areas of the United States, were examined for spermathecal sperm contents during spring 1985.

Results and Discussion

Queens from larvae grafted as early as the first week of April mated (queens mate ≈ 18—20 days after grafting) during all 3

years of this study, although the frequency was relatively low (Fig. 1). The earliest that mating was observed was with queens from a graft of 26 March 1985; no queens produced prior to this date mated. Mating frequency of queens from grafts made before about 24 April varied considerably within and between years and a large percentage of these queens did not mate (Fig. 1). Mating frequency of queens from grafts made from about 24 April—15 May varied from ≈ 50—75% and fewer unmated queens were observed relative to this grafting period (Fig. 1). Since mating of aged virgin queens has been documented (Oertel, 1940; Zmarlicki and Morse, 1963), it is possible that a higher percentage of matings would have been observed if the queens had remained in the nuclei for longer periods. The present study, however, specifically examined mating frequency under standard commercial queen rearing practices (Laidlaw, 1979). Spring mating frequency obviously varied between years in relation to weather conditions : the lowest mating frequency was observed in 1984 where the maximum daily temperature was generally < 18°C (Table I).

Mean sperm counts of mated queens from grafts made before about 24 April were highly variable and generally <- 4 million (Fig. 2). However, over the 3 years of this study, 41% (33 of 81) of these queens had sperm counts of at least 4.5 million. These queens were, therefore, adequately inseminated : sperm counts of 4.5 million are comparable to those reported for queens mated under commercial conditions in a major queen rearing area (California) of the United States (Harizanis and Gary, 1984). Determination that such a high percentage of the queens from grafts made before 24 April were adequately inseminated suggests that a primary limiting factor is drone availability. Since individual queens mate with up to 18 drones (Adams *et al.*, 1977), colony growth during spring in continental climates may be insufficient for adequate drone production.

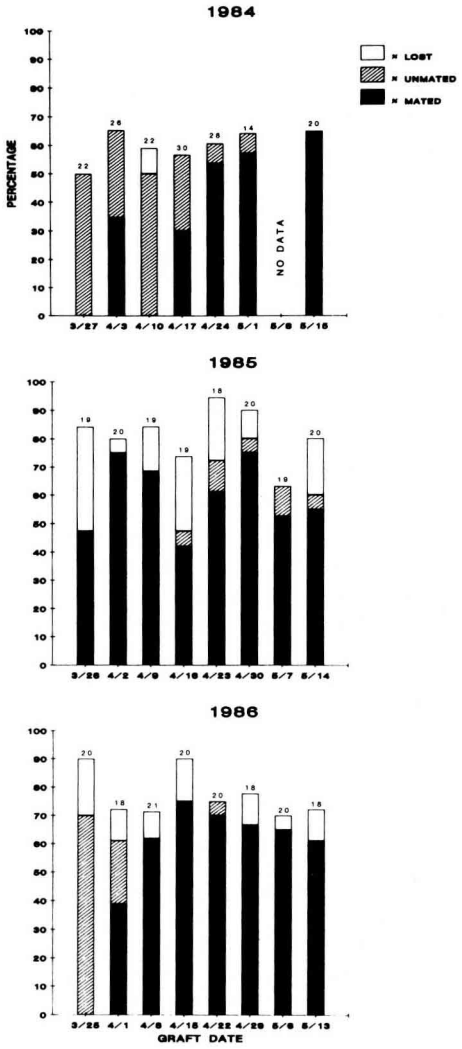

Fig. 1. Percentages of queen honey bees that emerged (entire bar), mated, did not mate, or were lost following a post-emergence examination relative to graft date during spring 1984—1986. Percentages are relative to total number of queen cells introduced weekly to nucleus colonies. Number of queen cells introduced per week is indicated in the figure.

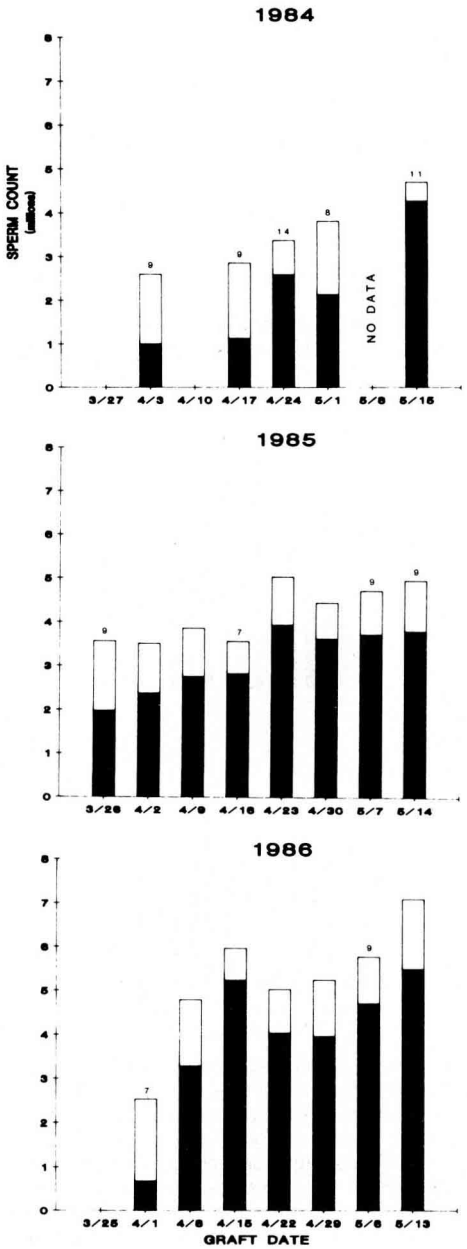

Fig. 2. Mean spermathecal sperm counts (entire bar) and lower 95% confidence limits (solid portion) for mated queen honey bees relative to graft date during spring 1984—1986. Sperm counts were estimated from 10 queens weekly, except where indicated in the figure.

Mean sperm counts of mated queens grafted from about 24 April—15 May were generally > 4 million (Fig. 2). Spermathecal sperm contents of queens from grafts made during late April to mid-May in a continental climate are, therefore, similar to or above previously reported values for naturally mated queens (Woyke, 1964; Kaftanoglu and Peng, 1982; Harizanis and Gary, 1984). Spermathecal sperm contents of mated queens from grafts during this period are also comparable to those of queens reared and mated in the southern and western United States (subtropical and Mediterranean climates, respectively) by commercial queen producers during spring 1985 (Fig. 3). Although sperm counts were slightly more variable among queens produced in a continental climate, the majority of such queens were adequately inseminated for utilization by beekeepers : 60% (66 of 110) of the queens had sperm counts of at least 4.5 million compared to 70% (35 of 50) of the queens produced in the southern and western United States.

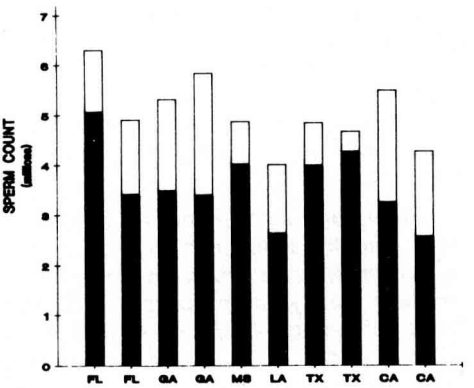

Fig. 3. Mean (N = 5) spermathecal sperm counts (entire bar) and lower 95% confidence limits (solid portion) for mated queen honey bees obtained from 10 commercial queen producers in the southern and western United States during spring 1985. State of origin is indicated : FL = Florida; GA = Georgia; MS = Mississippi; LA = Louisiana; TX = Texas; CA = California.

Table I. Number of days in which the maximum daily temperature exceeded 18°C during April and May, 1984-1986.

	April	*May*
1984	3	15
1985	13	27
1986	8	21

Mating frequency of queens from grafts between 22 May to 11 September, 1984 varied from ≈ 45—90% (Fig. 4**A**). Commercial queen mating frequency typically varies from ≈ 30—95% (Laidlaw, 1979). Mean sperm counts varied from ≈ 5.0—6.5 million, and more importantly, there was a high probability (P=0.95) of obtaining mated queens with a minimum sperm count of at least 4.0 million (Fig. 4**B**). Mating frequency did, however, decrease markedly with queens from grafts after 14 August : a large percentage of these queens did not mate (Fig. 4**A**), while sperm counts among queens that did mate became highly variable (Fig. 4**B**). Although late summer weather conditions undoubtedly influence mating success, drone availability may also be a limiting factor : drone rearing generally decreases while mature and emerging drones are often ejected from colonies in late summer (Morse *et al.*, 1967).

Spring and summer mating frequency was largely determined by successful queen emergence and acceptance by the nuclei : emergence success varied from ≈ 50—95% (Figs. 1, 4**A**). Over the 3 years of this study, 70.6% queen cell emergence and virgin queen acceptance by the nuclei was observed (Table II). Nearly all queens which were observed in the nuclei one week after cell introduction were successful in mating or did not mate (Figs. 1, 4**A**). Only 8.5% of the queens were lost following the post-emergence examination (Table II).

Our results indicate that queen honey bees can mate successfully throughout

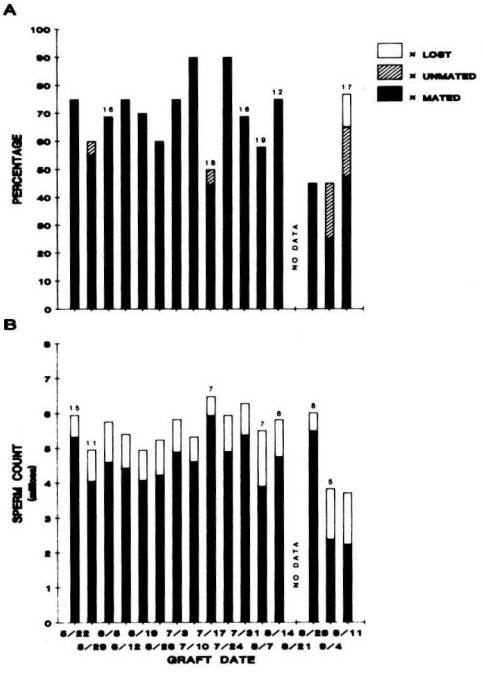

Fig. 4. A, Percentages of queen honey bees that emerged (entire bar), mated, did not mate, or were lost following a post-emergence examination relative to graft date during summer 1984. Percentages are relative to total number of queen cells introduced weekly to nucleus colonies (N = 20 per week, except where indicated in the figure). **B,** Mean spermathecal sperm counts (entire bar) and lower 95% confidence limits (solid portion) for mated queen honey bees relative to graft date during summer 1984. Sperm counts were estimated from 10 queens weekly, except where indicated in the figure.

Table II. Summary of queen cell introductions, 1984—1986 [a].

	N	%
Cells introduced	769	—
Cells emerged	543	70.6
Mated queens	421	54.7
Unmated queens	76	9.9
Post-emergence loss	46	8.5

[a] Summary does not include data from grafts prior to 27 March of each year.

the spring and summer in continental climates and also provide a baseline for efforts to increase spring mating success.

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