Research article

Timing of production of winter bees in honey bee (*Apis mellifera*) colonies

H.R. Mattila[1], J.L. Harris[2] and G.W. Otis[1]

[1] *Department of Environmental Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1, e-mail: hmattila@uoguelph.ca, gotis@evb.uoguelph.ca*
[2] *2839 Lakeview Ave., Regina, SK, Canada, S4S 1G6*

Summary. Little is known about the development of the overwintering population of honey bees (*Apis mellifera*) colonies in temperate climates. Colonies were subjected to one of four requeening treatments: requeened in mid-summer with a mated, virgin or colony-reared queen, or left with the original queen (control). Worker survival in cohorts of newly emerged bees introduced to colonies in late summer and fall was followed until all marked bees had died. Winter bees were reared over a relatively similar length of time in all treatments, but they appeared earlier in control colonies compared to requeened colonies. The gradual increase in proportion of winter bees over time was similar among treatments, but requeened colonies lagged behind control colonies. The bulk of winter bees appeared much earlier in control colonies than in colonies that were requeened. This response demonstrates that cues within the colony (i.e., differences due to requeening) are perceived by workers as part of the conditions that influence summer bee or winter bee status.

Key words: Honey bees, *Apis mellifera*, winter bees, requeening, worker survival.

Introduction

At the colony level, honey bees (*Apis mellifera*) demonstrate a K-type life history strategy, which is characterised by low reproductive rate, long lifespan, high population stability, repeated reproduction, and a survivorship curve with mortality concentrated in older individuals (Sakagami and Fukuda, 1968; Seeley, 1985). The population demography of the perennial honey bee colony follows a seasonal cycle related to its needs throughout the year, and demonstrates unique adaptations to overcome the challenge of winter (Bodenheimer, 1937; Seeley and Visscher, 1985). In northern temperate climates, brood rearing typically begins in mid-winter and increases until the summer nectar flow, after which brood rearing slows until it ceases in late fall. The longevity of the

individual worker also varies with the season (Fukuda and Sekiguchi, 1966). Those workers which are reared from late winter to late summer are usually short-lived and participate in brood rearing and foraging. "Spring" bees have a mean longevity of 30–40 days, while "summer" bees working through the major nectar flows experience a reduced mean lifespan of 25–30 days (Fukuda and Sekiguchi, 1966). Workers that emerge in the fall may die before winter, but many survive the coldest months as "winter" bees. Winter bees have a markedly extended lifespan compared to their summer counterparts: a mean lifespan greater than 100 days depending on date of emergence, with a maximum between 212 and 252 days (Fukuda and Sekiguchi, 1966), and a reported record of 304 days (Southwick, 1991). Over the winter months, these bees regulate nest temperature and initiate mid-winter brood rearing to produce the new spring population. The seasonal changes in population age structure within a colony are a necessary adaptation for temperate honey bees due to the relatively short period of time for nectar and pollen collection and the need to maximise colony strength when these resources are available. For the purposes of this study, winter bees are defined as those bees reared in late summer and fall before brood rearing ceases and which have a low rate of mortality over the winter months.

Physiological changes accompany the increased longevity of winter bees. Juvenile hormone (JH) titres are typically low in overwintering bees (Huang and Robinson, 1995) compared to higher titres in summer bees (Fluri et al., 1982). JH regulates worker polyethic tasks: low titres are associated with in-hive tasks typically performed by young (or overwintering) bees and higher titres are found in older foragers (Jaycox, 1976; Huang and Robinson, 1995). Winter bees are also characterised by hypertrophied hypopharyngeal glands (Fluri et al., 1982) and enlarged fat-bodies (Fluri and Bogdanov, 1986).

What factors are associated with the transition from a summer bee to a winter bee population? Because the annual appearance of long-lived bees coincides with the onset of winter in temperate climates, signals external to colonies and

associated with the seasons have been examined as factors that could induce the appearance of winter bees within the colony. Experimentation has thus far been inconclusive. Changes in photoperiod had no effect on brood rearing, worker longevity or JH titres (Kefuss, 1978; Fluri and Bogdanov, 1987; Huang and Robinson, 1995). A temperature effect could be responsible for seasonal changes in JH titre (Bühler et al., 1983; Huang and Robinson, 1995), but this effect has not been definitively established in overwintering populations. Very little is known about the extent to which internal changes alter the transition from a summer bee to winter bee population. A few authors have generated worker survivorship curves over this period (Free and Spencer-Booth, 1959; Fukuda and Sekiguchi, 1966; Sakagami and Fukuda, 1968), but none have considered how differences between colonies might affect the timing of this change.

A data set can sometimes provide insight into a question for which it was not originally intended. The present study will reanalyse data collected in southern Manitoba, Canada (Harris, 1980), to investigate the effect of requeening on the timing of the fall transition from a summer bee to a winter bee population. If the nature of the transition differs between treatment groups, it can be inferred that internal changes within a colony associated with requeening influence the appearance of winter bees.

Methods

The experimental design

The fieldwork took place in 1976–1977 at an apiary in central southern Manitoba. The climate in this prairie region of North America is characterised by moderate/hot summers and long, cold winters. Colonies (n = 24) were initiated from 0.9 kg packages of honey bees, composed of approximately 7000 worker bees, on 22 April. A caged mated queen was placed in each colony and released after three days. To minimise drift, the colonies were placed in a circular pattern one meter apart from adjacent hives, with their entrances pointing outwards.

Every 12 days from 14 July until the end of fall brood rearing, a cohort of 100 newly emerged workers (identified by their downy appearance) was taken from each colony, marked and returned to its original colony. At this time, the presence of marked bees from previously introduced cohorts was recorded and sealed brood measurements were taken. The area of sealed brood was estimated by superimposing a grid with 2.54×2.54 cm squares over the brood area. Solid squares were counted directly and partial areas estimated. Counts were made in the early morning to avoid missing bees on foraging flights. This procedure was last used on 30 October, after which the bees became too densely clustered for accurate counts to be made, and colonies were moved into a dark overwintering room maintained at $6 \pm 2\,°C$. Here, sealed brood measurements continued until brood rearing ceased in all colonies (23 November), but no more cohorts were introduced. On 11 March, actual counts of brood area and bee survival recommenced. During the winter when colonies could not be examined, it was assumed that worker mortality was constant. Cohort survival was followed until 27 June when no more marked bees were observed.

Colonies that became queenless or swarmed were removed from the study. Requeening treatments were implemented on 26 July. Colonies (n = 22) received one of four treatments: group A (n = 4), the control with the original package queen; group B (n = 6), requeened with a mated queen; group C (n = 6), requeened with a virgin queen; and group D (n = 6), made queenless and allowed to rear a replacement queen. All colonies received similar management.

Analysis of the data

Cohort survival for each 12-day interval was calculated as the proportion of bees observed at time t_n remaining of the bees successfully introduced at t_0, where n is the age of individuals in the cohort. The number of successfully introduced bees at time t_0 was estimated by the y-intercept of a linear regression of the first three counts after introduction within each cohort's survivorship curve. A linear rate of mortality has been observed for bees after their emergence, the slope of which depends on the season (Harris, 1980; Fukuda and Sekiguchi, 1966). A mean survivorship curve was calculated for each cohort in a treatment group.

The mean longevity of bees in each cohort was calculated for every colony by the following:

$$\text{longevity} = \left(\sum_{n=0}^{n=a} [(\text{bees present at } t_n - \text{bees present at } t_{n+12}) \times (n+6)]\right)/\text{bees present at } t_0$$

where a is the age at which individuals are last observed in the cohort. This formula treats the bees lost in a 12 day interval as all having lived to the mid-point of that interval. The exception is between 30 October and 11 March when no live counts were made, and the interval was extended to 132 days. Summation of the numerator gives the total days lived by all individuals in a cohort; dividing by the number of bees introduced in that cohort gives mean worker longevity. The mean longevity of the cohorts introduced on the same day within a treatment was calculated.

Under normal conditions, mortality of sealed brood is very low (Sakagami and Fukuda, 1968); thus estimates of emerging adults can be made from sealed brood measurements. To examine the timing of winter brood production, the contribution of winter bees made at 12-day intervals to the total winter bee population was determined by the following:

$$\text{number of winter bees produced at } t_n = p\,[(b_{t(n)} + b_{t(n-12)})/2] \times 4.3$$

where p is the proportion of the cohort at t_n that are long-lived (inflection point from survivorship curves where rate of mortality changes dramatically), and $b_{t(n)}$ and $b_{t(n-12)}$ are the area of sealed brood (cm²) at t_n and t_{n-12}, respectively. The equation uses a factor of 4.3 cells/cm² to convert sealed brood measurement to actual number of sealed cells (Harris, 1980). The best estimate of the number of winter bees produced at t_n required an estimation of sealed brood area 6 days earlier, so that the brood that emerged over the following 12-day interval had a midpoint (t_n) which coincided with a date when measurements were taken to determine the proportion (p) of long-lived bees. The mean number of winter bees ($\pm$ S.E.) produced in each 12-day interval until brood rearing ceased was determined for each treatment.

Results

Figure 1 shows a gradual increase in the proportion of winter bees in successive cohorts in all treatments. The first 4 to 5 cohorts of bees introduced on and after 14 July consisted entirely of summer bees with a relatively high and constant rate of mortality. After this early period when cohorts consisted of only short-lived bees, there was a proportion of bees from each successive cohort that survived the winter months. For example, in group A (control), winter bees were first observed in the cohort introduced on 31 August: 60% of that cohort consisted of bees with a high mortality similar to the cohorts introduced between 14 July and 19 August, while 40% (winter bees) had a markedly reduced mortality rate (Fig. 1). Bees of the 19 August cohort were no longer observed
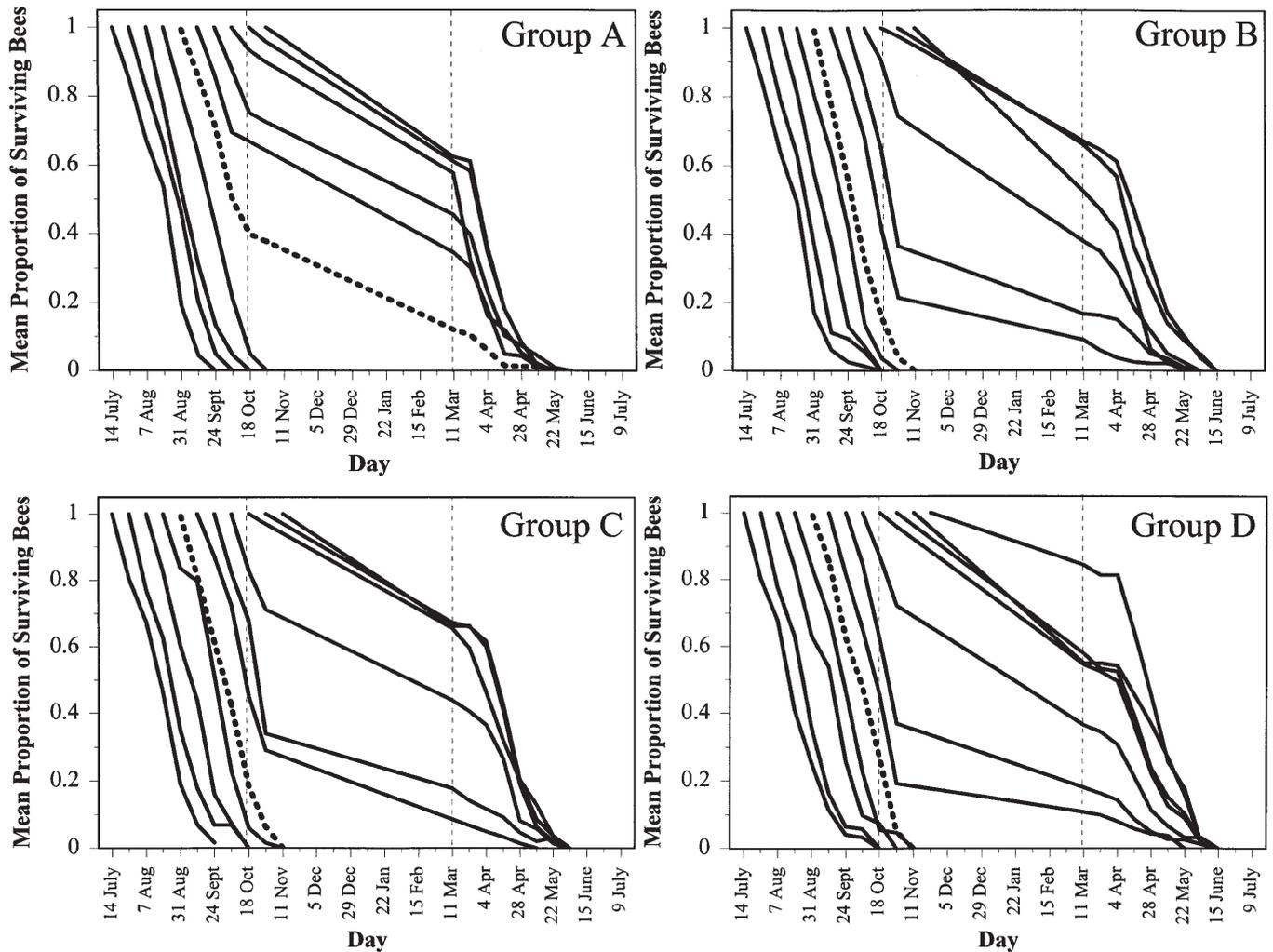
Figure 1. Mean survivorship curves for cohorts of bees introduced at 12-day intervals (from 14 July until fall brood rearing ceased) into colonies receiving one of the following queen treatments: A, original package queen (control, n = 4); B, requeened with mated queen (n = 6); C, requeened with virgin queen (n = 6); or D, made queenless and allowed to rear queen (n = 6). The survivorship curve for the cohort introduced on 31 August is dashed bold to facilitate comparison between treatments. Vertical lines bracket the period during which actual counts of marked bees were suspended due to cold temperatures and dense clustering; mortality was assumed to be constant for that period

by the end of October, compared to the last observed individuals of the 31 August cohort, which survived until the following May. Subsequent cohorts showed a steadily increasing proportion of winter bees at each 12-day interval until fall brood rearing ceased.

This gradual replacement of summer bees with winter bees also occurred in requeened colonies, however the first cohorts with individuals surviving the winter were introduced on 12 September, 12 days later than control colonies. The proportion of each cohort that was long-lived in requeened colonies consistently lagged behind that of the control colonies until cohorts had only long-lived bees. Brood rearing stopped on 11 November for group B and C, and 23 November for group D, but had already ceased in the control colonies on 30 October. Winter bees were observed in cohorts introduced over a 60-day period in the fall (before brood rearing ceased) in Groups A, B and C, and over a 72-day period in Group D.

Increases in mean longevity paralleled the increasing proportion of winter bees at subsequent times. Mean longevity of workers began to increase on 31 August in control colonies when cohorts first contained winter bees (Fig. 2). This increase did not occur in requeened colonies until 12 September, which reflects the 12-day delay in the appearance of winter bees in Figure 1. The pattern of change in mean longevity over time was similar for all treatment groups, but generally lagged by 12 days in requeened colonies.

By combining data on brood area with the proportions of each cohort comprised of winter bees, estimates of the number of winter bees produced over time were made (Fig. 3). Group A colonies produced 53 % of their wintering population in the first two cohorts containing winter bees. In all three requeened treatments, the bulk of winter bee production was delayed until the third and fourth cohorts containing winter bees, 36 days later, with 54, 56 and 64 % of the wintering population produced at that time in group B,
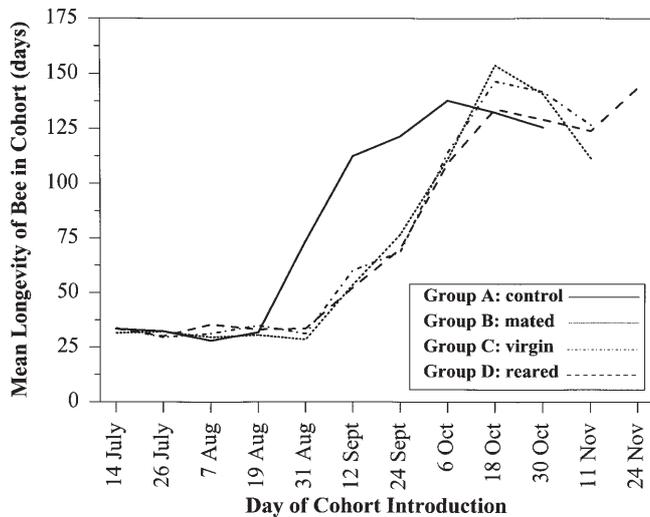
Figure 2. Mean longevity of cohorts of bees introduced at 12-day intervals into colonies left with the original queen (control), requeened with a mated queen, requeened with a virgin queen, or made queenless and allowed to rear a new queen

Table 1. The mean size of wintering population produced based on calculations in Fig. 3, in number of bees ± S.E., in colonies with one of four queen treatments

Treatment	Mean size of wintering population (± S.E.)
Group A: n = 4 Control, original queen	16995 ± 1856
Group B: n = 6 Requeened, mated	20339 ± 1045
Group C: n = 6 Requeened, virgin	17984 ± 3314
Group D: n = 6 Requeened, reared	14379 ± 1395

C and D, respectively. The distributions in Figure 3 were all significantly different from each other (Kolmogorov-Smirnov tests, P < 0.01).

Although a one-way ANOVA (SAS Institute, 1990) found no significant difference in winter population size between treatments (Table 1, P > 0.25), the estimates may indicate real trends. Colonies requeened with mated queens had larger estimated mean wintering populations, followed by colonies requeened with virgin queens, then the control colonies. Although colonies which reared a replacement queen produced winter bees over a longer period, small brood areas at the start and finish of this period contributed to their small winter bee populations.

Discussion

There is a gradual transition within colonies from a population of short-lived summer bees to long-lived winter bees. The survivorship curves for subsequent cohorts within a treat-

ment showed that over time, the proportion of each cohort that was comprised of overwintering bees increased (indicated by inflection points at which the rate of mortality was dramatically reduced). An examination of the 31 August cohort (bold, dashed line in Fig. 1) illustrates the difference in the development of the wintering population between requeened and control colonies: in control colonies, this was the first cohort of the season containing winter bees, whereas no bees from the equivalent cohorts in requeened treatments survived the winter. For the first time, temporal shifts in the development of the winter bee population resulting from requeening have been estimated. Requeening treatments delay the appearance of the first winter bees, as well as the bulk of their production.

Estimates of worker survival could not be made during cold weather due to dense clustering. Because actual counts of marked bees could not be made after 30 October, the survivorship curves assume a constant loss of bees from then until counts could be made again on 11 March. Due to this limitation, the slope of the survivorship curves for some overwintering cohorts depicted in Fig. 1 appear to have changed dramatically on 30 October. It is possible that the real shift in bee survival occurred after 30 October, and because we used the proportion of each cohort alive at the inflection points to determine the number of winter bees in the cohort, our estimates of the winter bee populations may be artificially inflated. This was not a concern for control colonies because most of the cohorts contributing to the winter bee population had an inflection point prior to 30 October (especially the ones that contributed heavily to the wintering population). For these cohorts in the control colonies, an average of 29% of the total cohort was lost between the fall inflection point to the first spring sampling on 11 March (slope: $-2.2 \times 10^{-3} \pm 0.2 \times 10^{-3}$). If this proportional loss is applied to requeened colonies between 30 October and 11 March, the requeened colonies overestimated this loss, at most, by 6% over the same period, indicating that if inflection points occurred after 30 October, the error introduced was minimal. It is also a strong indication that the point at which the mortality rate changed in the survivorship curve was at or very close to 30 October. We feel that this margin of error is small enough to justify the presentation of the results in Fig. 3.

The results of this study suggest that the development of a winter bee population is not stimulated solely by environmental cues associated with winter in a temperate climate, but is also influenced by factors within the colony. Our survivorship curves suggest that worker mortality decreases (therefore worker longevity increases) as brood production declines in the fall; colonies in which brood rearing ceased earlier showed earlier shifts in worker survival. Requeening a colony influences brood production, extending it perhaps because younger queens tend to lay longer into the fall (Free and Racey, 1968) or because of the effects of the break in brood production which result from requeening.

It is known that worker mortality is related to the amount of brood reared. Lifespan is strongly influenced by the intensity with which workers perform their two principal tasks,
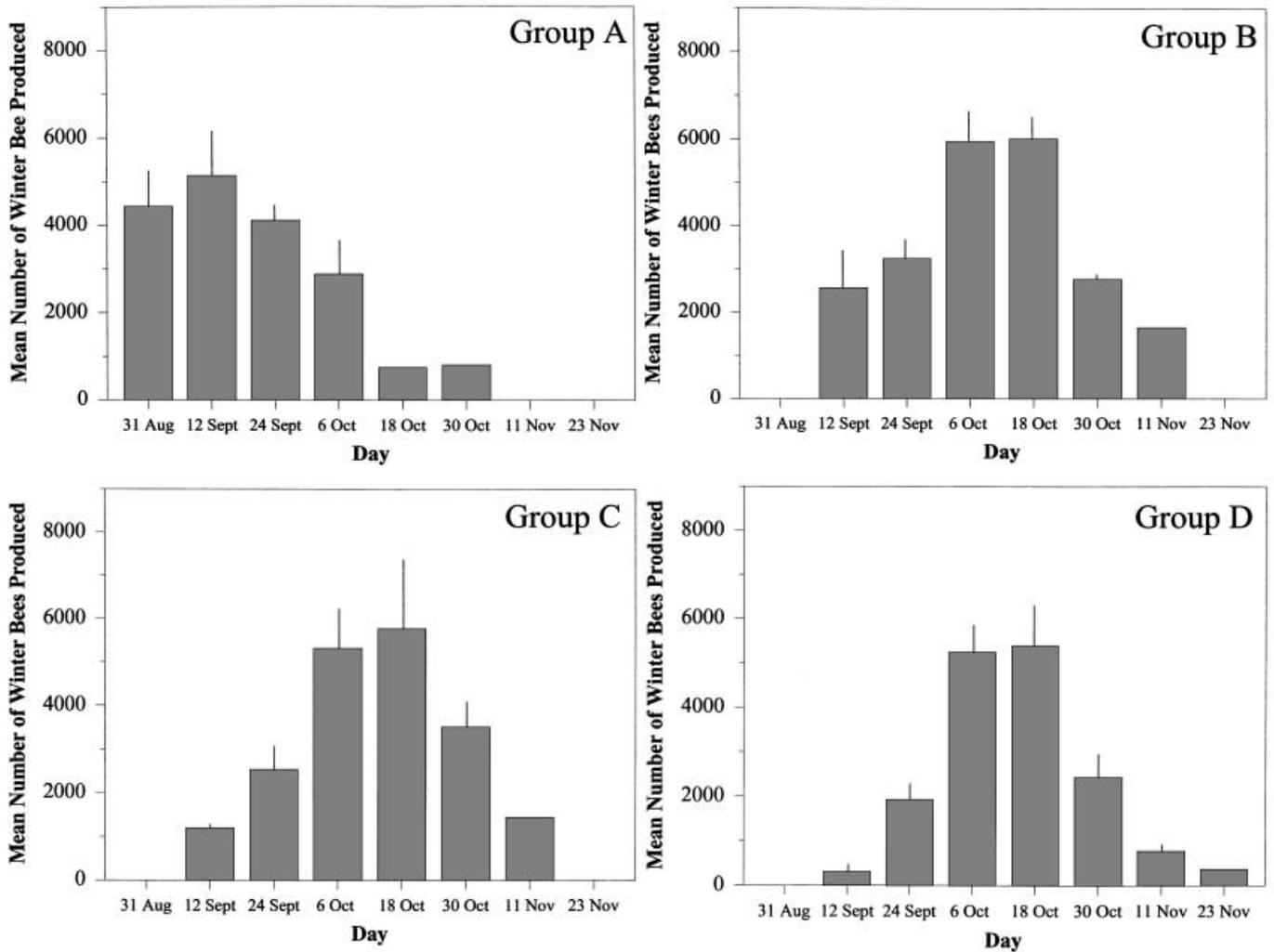
Figure 3. Mean number of winter bees produced (± S. E.) over 12-day intervals until fall brood rearing ceased in colonies receiving one of the following queen treatments: A, original package queen (control); B, requeened with mated queen; C, requeened with virgin queen; or D, made queenless and allowed to rear queen

nursing and foraging. In-hive duties such as nursing are typically performed by young bees before they switch to foraging and because the time and energy devoted to foraging is relatively fixed (Neukirch, 1982), it is the intensity with which brood rearing is performed during the early in-hive period that largely determines length of life (Maurizio, 1950). Theoretical modelling of worker longevity and seasonal nurse load show changes in population structure similar to those observed in colonies as fall brood rearing ceases (Omholt, 1988).

JH levels in workers correspond to seasonal activity: JH titres increase in summer bees from the time they begin to feed larvae until they initiate foraging, but remain low in winter bees until brood rearing resumes in the spring (Huang and Robinson, 1995). It is not known if all winter bees, regardless of absolute age, have the same probabilities of task performance in spring, or if the first winter bees produced in fall initiate brood care and/or foraging earlier. Our observation, which agrees with Free and Spencer-Booth (1959) and Fukuda and Sekiguchi (1966), that all winter bees die at

around the same time, regardless of time of emergence or requeening treatment, would suggest the former explanation, but a closer examination of spring behaviour with respect to age is required. Knowledge of differences in JH titre and probability of task performance between overwintered bees of the same age would also address this question.

Although changes in JH titre were not examined in this study, our results agree with Huang and Robinson (1995), who suggested that fall changes in colony demography could explain the seasonal reduction in JH biosynthesis and titres. The presence of older bees can suppress hormone-mediated behavioural development in younger bees (Huang and Robinson, 1992). Since birth rates decline as fall brood rearing comes to a halt, older bees become a larger proportion of the colony population. It is this greater presence which could initiate the appearance of winter bees. If brood rearing continues relatively longer in a requeened colony, birth rates would not slow until later in the season, thus it would take longer to reach a critical proportion of older bees to induce a change to winter bee physiology.

The microclimate associated with large amounts of brood, i.e., temperature of 35 °C and increased CO_2 levels, has been shown to cause a steady rise in JH titres, as is seen in young summer bees (Bühler et al., 1983). If workers are not exposed to this microclimate, titres do not rise. As the area of brood shrinks in colonies in the fall, emerging bees are more likely to find themselves displaced from the brood area. The likelihood of being in the brood area is largely dependent on the egg-laying activity of the queen, and in our requeened colonies where brood was present longer into the fall, the appearance of winter bees would be expected to be delayed to some degree according to this hypothesis.

During the development of the winter bee population, there is a period during which emerging bees can become short- or long-lived individuals in all colonies. At present, we know little about how this is determined. Differences in larval care may affect whether a bee is long- or short-lived; newly emerged bees that overwinter have significantly greater dry weight, protein, fat, triglycerides, glycogen and glucose content than bees that do not survive to winter (Kunert and Crailsheim, 1988). If winter bee status is not determined at the larval stage, it is possible that emerging bees may experience conditions that increase the probability of becoming a winter bee. Experiments combining quantification of JH titres and the observation of the behaviour of same-aged bees that do and do not overwinter are necessary.

Acknowledgements

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