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Research article

The influence of worker behavior and paternity on the development and emergence of honey bee queens

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Summary. The interactions of worker honey bees with queens cells could influence the outcome of the queen replacement process, and could potentially contribute to the spread of the African honey bee in the New World if workers exhibit racial preferences during queen rearing. We examined worker-queen cell interactions in hybrid colonies that contained African and European patrilines. Worker interactions were associated with a queen's emergence success (e. g. the probability that she would develop to emergence). Compared to queen cells that were destroyed, those that emerged were initiated sooner during the rearing process, were started from younger brood, were visited and incubated at higher rates, and received more vibration signals from workers. In contrast, the worker interactions examined did not influence emergence order (e. g. the sequence that queens emerged relative to one another). African- and European-paternity queens experienced similar emergence success, and did not differ in the rates at which workers visited, incubated or vibrated their cells. African-patriline workers were more likely to engage in queen rearing than their European-paternity nestmates. Workers of both patrilines exhibited super-sister preferences when visiting queen larvae, but showed variable or no kin discrimination during incubation and vibration signal activity. Thus, honey bee workers may use a variety of mechanisms to influence queen development. However, they do not exhibit marked racial or kinship preferences when interacting with queen cells, suggesting that the queen rearing process has not contributed strongly to the spread of the African bee in the Western Hemisphere.

Key words: Queen rearing, worker-queen interactions, vibration signal, Africanized honey bee.

Introduction

Honey bee colonies produce virgin queens (VQs) during reproductive swarming, supercedure and emergency queen replacement (Winston, 1987). Queen production involves two distinct phases: (1) queen rearing, in which multiple VQs are reared in specially constructed queen cells, and (2) polygyny reduction, in which emerged VQs destroy unemerged rivals in their queen cells and battle other emerged queens to the death (Hatch et al., 1999; Tarpy et al., 2000). The end result is a sole surviving VQ who becomes the new laying queen of the colony. The outcome of queen replacement has a pronounced impact on colony survival and worker inclusiveness fitness. The ability of workers to influence the replacement process may therefore be under strong selection (Visscher, 1993). Indeed, workers use a variety of mechanisms to regulate aggressive interactions between emerged VQs, which may influence the outcome of polygyny reduction (Fletcher, 1978a; Schneider, 1991; Gilley, 2001; Schneider et al., 2001). Furthermore, these worker-queen interactions may have influenced the spread of the African honey bee in the New World, by contributing to a competitive advantage for African-patriline queens (Schneider and DeGrandi-Hoffman, 2002; Schneider et al., 2002).

Workers could influence the queen rearing process by affecting "emergence success" (the likelihood that a queen cell will develop to emergence) and "emergence order" (the sequence in which VQs emerge relative to one another within a colony). Large proportions of queen cells are often destroyed before development is completed (Hatch et al., 1999), suggesting that workers strongly influence emergence success. Likewise, workers may sometimes regulate emergence order by inhibiting or delaying the emergence of certain VQs (Fletcher, 1978b; Gilley, 2001). This in turn could affect the ultimate outcome of queen replacement, because early emerging queens (especially those that emerge first) may have increased survival (Schneider et al., 2001; Schneider and

DeGrandi-Hoffman, submitted). However, we have a limited understanding of the role that workers play in the queen rearing process. In particular, we know relatively little about (1) the mechanisms workers use to affect queen development and emergence, (2) the characteristics of developing queens that influence these worker interactions, and (3) the worker characteristics that influence the tendency to engage in queen rearing.

There are at least five main mechanisms that workers could use to influence the emergence success and emergence order of VQs. The timing of cell initiation (how quickly a queen's rearing is begun during the replacement process) and the age of brood selected for rearing could affect queen development. During emergency queen replacement, most queen cells are initiated within 48 h of queen loss (Pettis et al., 1995; Hatch et al., 1999) and most successfully developing VQs are started from eggs versus larvae (Hatch et al. 1999). Cell initiation time and brood age may, in turn, influence subsequent worker interactions with queen cells, such as cell visitation rates, incubation behavior and the performance of vibration signals. These interactions could affect emergence success and order, because nutrition and temperature strongly influence development (DeGrandi-Hoffman et al., 1993; Hatch et al., 1999) and the vibration signal may delay emergence (Fletcher, 1978b; Schneider et al., 2001).

One of the main characteristics that may influence how workers interact with queen cells is paternity. Honey bee queens typically mate with 10–17 drones (Oldroyd et al., 1998), which results in multiple patrilines (subfamilies) within a colony. If queens reared from different subfamilies vary in quality or attractiveness to workers, then VQ paternity could influence the interactions experienced during development and emergence (Tilley and Oldroyd, 1997; Tarpy et al., 2000). Also, worker paternity could influence the tendency to engage in queen rearing (Robinson et al., 1994). For example, in hybrid colonies that contain African and European patrilines, workers preferentially interact with emerged VQs from African patrilines and most of these interactions are performed by African-paternity workers (Schneider and DeGrandi-Hoffman, 2002). These paternal effects may, in turn, contribute to the spread of African bees in the Western Hemisphere (Schneider et al., 2002). Paternity also affects the relatedness between queens and workers. The tendency of workers to preferentially interact with more closely related queens has been suggested repeatedly (reviewed in Breed et al., 1994; Visscher, 1998).

The purpose of this study was to investigate queen rearing in honey bee colonies. We had three main objectives. First, we examined the influence of cell initiation time, brood age and worker visitation, incubation and vibration signal activity on queen cell emergence success and emergence order. Second, we investigated the effect of VQ paternity on development and emergence. Third, we assessed the influence of worker paternity and relatedness on the tendency to engage in queen cell interactions. We examined queen rearing in colonies containing one African and one European patriline, which allowed us to assess the influence of paternity on VQ success and worker behavior, as well as investigate the possible contributions of queen rearing to the spread of African bees in the New World. Also, the simplified genetic structure created in our colonies facilitated the assessment of kinship effects. If kin preferences are not strongly exhibited in colonies with only two subfamilies, then it is unlikely that relatedness plays a major role during queen rearing in colonies with a normal genetic composition.

Methods and materials

Colony set up and maintenance

The study was conducted from June-August, 1999 and 2000 at the Carl Hayden Bee Research Center, Tucson, AZ. We created two types of hybrid colonies through instrumental insemination, following the procedures of DeGrandi-Hoffman et al. (1998a). African-matriline (A-matriline) colonies were established by inseminating African-matriline queens with the semen from one African and one European drone. European-matriline (E-matriline) colonies arose from European-matriline queens inseminated with the same drone combinations used for the A-matriline colonies. Our insemination protocol established two patrilines within each colony and also affected kinship. Workers and queens reared from the same patriline within a colony were super-sisters (relatedness coefficient, G=0.75), while those from different patrilines were half-sisters (G=0.25).

All queens and drones used for the inseminations were obtained from separate sources to prevent inbreeding. The African queens and drones had black cuticular coloration and were reared from colonies established from swarms captured in southern Arizona that were identified as African using mophometric and mitochondrial DNA analyses (Rinderer et al., 1993; Crozier et al., 1991). European queens and drones were reared from colonies carrying the Cordovan (*cd*) gene for body color. The *cd* trait is a naturally occurring color variant (Tucker, 1986) that produces a distinctive "light blond" color when homozygous and an intermediate brown coloration when heterozygous in dark bees.

Our insemination protocol resulted in patrilines that could be visually distinguished within each matriline based on cuticular coloration. In the A-matriline colonies, workers and queens of African paternity were solid black or had a black thorax with a distinct black band across each abdominal tergite. In contrast, European-patriline workers and queens in the A-matriline colonies had lighter coloration and no banding on the upper 1–3 abdominal segments. In the E-matriline colonies, workers and queens of European paternity were homozygous for the *cd* allele and exhibited a uniform blond coloration with indistinct, light-brown abdominal banding. African-paternity workers and queens in the E-matriline colonies were darker in coloration and had distinct abdominal banding patterns. The coloration patterns were verified by inseminating African and European queens with only one African or European drone (DeGrandi-Hoffman et al., 1998a; voucher specimens maintained at the Carl Hayden Bee Research Center). This method of patriline identification has been used previously to examine the effects of African versus European paternity on queen development time (DeGrandi-Hoffman et al., 1998a), worker defensive behavior (DeGrandi-Hoffman et al., 1998b), and the fighting ability of emerged queens (Schneider and DeGrandi-Hoffman, 2002).

We used color markers to visually discriminate between patrilines, rather than genetic markers that had to be identified using molecular techniques, because queen rearing and emergence involve hundreds or thousands of worker-queen cell interactions that occur throughout a period of 14–16 days (Winston, 1987). Opening the study hives repeatedly to collect these large numbers of bees for molecular analysis would have seriously disrupted worker behavior and altered the queen rearing process.

The inseminated queens were established in 5-frame nucleus hives and expanded to 45-L hive boxes as colony growth warranted. Colonies

were allowed to develop until all workers were progeny of the inseminated queens. Each colony was then moved into an observation hive by transferring the queen, one frame of honey comb, and three frames of brood comb with their associated worker bees. We shook additional bees from the brood areas of the parental nests into the observation colonies until each contained 6000 – 7000 workers, to establish similar numbers of younger bees in each colony for queen rearing. The inseminated queens were allowed to lay for at least five days in the observation hives to ensure an ample supply of eggs and young larvae for queen rearing. The laying queens were then removed, which initiated queen replacement. Each colony was checked 4–6 times daily and every forming queen cell was given an identifying number and its location was marked on the Plexiglass walls of the observation hives. We established a total of 15 observation colonies (seven A-matriline and eight E-matriline), subdivided into four trials involving 3–4 colonies each. During each trial, both A- and E-matriline colonies were monitored simultaneously.

Determining queen cell initiation time, brood age, emergence and paternity

Initiation times were estimated by categorizing each cell as begun within 24 h, 25–48 h, 49–72 h, 73–96 h, and more than 96 h following the removal of the laying queen. We approximated the age of the egg/larva from which each queen cell was initiated by calculating backwards from the day the cell was sealed. Queen cells initiated from newly laid eggs require five days of development until sealing (Winston, 1987); lesser amounts of time until sealing indicate that older brood were selected for rearing. Each queen cell was assigned to one of five brood-age categories, following the methodology of Hatch et al. (1999): (1) egg 0–24 h, (2) egg 24–48 h, (3) egg 48–72 h, (4) larva 0–24 h, (5) larva 24–48 h. These age categories have an inherent 24–hour measurement error (Hatch et al., 1999) and development time may vary with genotype (DeGrandi-Hoffman et al., 1993). Nevertheless, the age classification scheme allowed us to make relative comparisons among queen cells reared from the same matriline within a common hive environment.

At the end of a trial, every cell was classified according to its emergence success (whether it emerged or was destroyed before emergence). For each cell that developed to emergence, we noted the emergence order (whether it was the first, second, etc. to emerge within its colony) and recorded the paternity of the emerged VQ. Whenever possible, we also determined the paternity of the VQs that were destroyed before emergence. However, such queens typically were killed before they had sufficient cuticular coloration to allow for paternity identification. Furthermore, the corpses were quickly dismembered by workers and morphometric and molecular determinations of paternity were not possible.

Determining worker visitation, incubation and vibration signal behavior

Once queen cells had been initiated, we monitored each observation colony for 30-min periods 6–8 times daily throughout the queen rearing process. During each 30-min period, we recorded every time a developing queen was visited by an African- or European-paternity worker. A worker was considered to be visiting a queen if it had its head inside a queen cell for at least 5 s. We did not record the time that each worker spent in a queen cell. Once a worker enters a cell, it is impossible to determine what activity (if any) it is performing. Thus, even though workers can remain in queen cells for up to several minutes, there is no way to accurately determine if feeding or queen care is occurring throughout that period. However, trophallactic food exchange can occur in less than 3 s of contact (Wainselboim and Farina, 2000), and thus workers that remained in a cell for 5 s or more probably engaged in at least some queen care. We therefore focused on the number of visits a cell received, rather than the amount of time per visit, as a rough estimate of queen care. Subsequently, we calculated for each queen cell a "visitation rate", defined as the total number of visits divided by the total number of 30-min observation periods.

We began monitoring incubation behavior after queen cells were sealed. During each 30-min observation period, we conducted three counts (each separated by at least 5 min) of the number of African- and European-paternity workers sitting on each cell. We then calculated for each cell an incubation rate, defined as the total number of incubating workers per 30-min period. The amount of time that each worker spent on a cell was not measured and some workers may have been included in more than one consecutive count. However, our purpose was to assess the total incubation activity a cell received and not the total number of different workers performing incubation.

Vibration activity was monitored on sealed queen cells, because few or no signals are performed on cells prior to sealing (Fletcher, 1978b). During each 30 min observation period, we recorded every time a queen cell received a vibration signal from an African- or European-paternity worker. We then calculated for each cell a vibration rate (total signals received/30 min).

Determining the relationships between worker interactions, VQ emergence success and emergence order

We used three approaches to assess the influence of the different factors monitored. First, we used logistic regression analysis (Sokal and Rohlf, 1995) to determine the importance of each of seven variables (cell initiation time, brood age, visitation rate, incubation rate, vibration rate, matriline and colony) to emergence success and emergence order. This approach identified the relative contribution of each variable after partitioning the effects of the other variables. However, some of our variables may have been inter-dependent and the logistic regression analysis may not have reflected completely these relationships. For example, cell initiation time and brood age could potentially influence the rate at which workers visit, incubate and vibrate queen cells. Initiation time and brood age could therefore show significant effects on emergence success, while the different worker interactions may not reach significance once these effects are removed. This would not necessarily indicate that visitation, incubation and vibration activity play no role in queen development, but rather that the contribution of these variables cannot be fully assessed outside the context of initiation time and brood age. Thus, for our second approach, we determined the correlation of each variable that was significant in the logistic regression with the other variables, using Spearman correlation analysis. Then, for each variable that exhibited a significant correlation, we compared that variable between emerged versus destroyed cells using a 2-way ANOVA, which had one between-subjects factor (colony matriline) and an interaction between colony matriline and emergence success (Sokal and Rohlf, 1995). In this manner, we were able to examine the combined effects of all variables, as well as the contributions of the specific worker interactions that may have varied with a queen's rearing history.

Visitation rates were not normally distributed and were log-transformed prior to analysis. Vibration rates often varied tremendously among colonies and among queen cells within the same colony. As a result, comparisons of the actual rates yielded results that were difficult to interpret. We therefore calculated for each queen cell a "relative vibration rate", by dividing each cell's individual rate by the mean rate for all cells within a colony (see also Schneider et al., 2001; Schneider and DeGrandi-Hoffman, 2002). We then conducted our analyses using the relative values, to allow for more meaningful comparisons. A square root transformation of relative vibration rates was conducted prior to the analyses to normalize the data.

The sequential Bonferroni adjustment (Rice, 1989) was used to determine significance levels for the correlation matrices and the multiple comparisons made within and between matrilines in the ANOVAs. All mean values are reported as $\pm$ one SE.

Determining the influence of queen paternity on emergence and worker interactions

We examined within each matriline the effect of VQ paternity on emergence success and emergence order using contingency table Chi-square

tests to compare (1) the total number of African- versus European-paternity queens that emerged, and (2) the number that were the first queen to emerge in their colonies. Chi-square tests were also used to assess the influence of queen paternity on the timing of cell initiation and the age of brood selected for rearing (cells within the contingency tables were combined when necessary to ensure expected values of five or greater; Sokal and Rohlf, 1995) . We examined the effects of queen paternity on worker visitation, incubation and vibration rates using 2-way ANOVA that had one between-subjects factor (colony matriline), one within-subject factor (VQ paternity), and a matriline by patriline interaction. These analyses were restricted to those queen cells for which VQ paternity could be determined and thus involved only a subset of the total data. The sequential Bonferroni adjustment was used to determine significance levels for the multiple comparisons made between queen patrilines.

Determining the influence of worker paternity and kinship on queen cell interactions

The influence of worker paternity on the tendency to interact with queen cells was examined using replicated goodness-of-fit tests (Sokal and Rohlf, 1995). We used Chi-square analysis to compare for each queen cell the observed numbers of visits, incubations and vibration signals performed by African- versus European-paternity workers to those expected if these interactions occurred randomly with respect to worker patriline. Expected values were calculated based on the proportion of African- and European-patriline workers in each colony. The proportions were estimated at the end of each trial by randomly collecting 100–200 workers from a frame of brood comb, killing them by freezing, and then counting the number of workers of each patriline. These analyses utilized the data for all queen cells. Each matriline was examined separately.

To examine kinship effects, we used Chi-square analysis to compare for each worker patriline the observed and expected number of visits, incubations and vibration signals directed toward cells containing supersister versus half-sister queen larvae. The analyses were restricted to those cells for which VQ paternity could be determined. A separate analysis was conducted for each matriline.

Results

Factors influencing emergence success and emergence order

Our observation colonies produced a total of 132 sealed queen cells. The seven A-matriline colonies produced 75 cells (10.7±1.28 per colony), of which 16 emerged and 59 were destroyed. Two of the E-matriline colonies failed to raise any queens to emergence and were excluded from the study. The remaining six E-matriline colonies produced 57 cells (9.5±1.67 per colony), 10 of which emerged and 47 were destroyed. Thus, on average, colonies in both matrilines reared 10 queen cells to pupation and these cells experienced an emergence success rate of about 20%. The numbers of cells for which we were able to monitor the different aspects of queen rearing are indicated in Table 1.

The logistic regression analysis revealed that in combination the seven variables examined had a highly significant effect on whether queen cells emerged or were destroyed (likelihood ratio Chi-square = 28.87; df = 7, P < 0.0002). This effect resulted primarily from the very strong relationship between emergence success and cell initiation time (Table 1). Cells initiated earlier in the queen rearing process were more likely to develop to emergence. Of the 24 emerged cells for which initiation time could be determined, 75% were started within 48 h of queen loss (Fig. 1). In contrast, 75% of the cells that were destroyed before emergence were initiated more than 48 hours following removal of the laying queen (Fig. 1).

Table 1. Results of the logistical regression analysis for the effect of the seven variables examined on the emergence success and emergence order of queen cells. Samples sizes refer to the number of queen cells for which each variable could be determined

	N	Chi-square	P
Emergence Success			
Cell Initiation Time	120	11.97	0.001
Brood Age	112	2.52	0.112
Visitation Rate	109	2.56	0.109
Incubation Rate	125	0.78	0.375
Vibration Rate	125	1.56	0.211
Matriline	132	1.66	0.197
Colony	132	1.99	0.159
Emergence Order			
Cell Initiation Time	24	0.69	0.407
Brood Age	23	2.33	0.127
Visitation Rate	22	0.01	0.907
Incubation Rate	25	0.67	0.414
Vibration Rate	25	0.50	0.478
Matriline	26	0.01	0.904
Colony	26	0.14	0.710

When the effect of cell initiation time was removed, none of the other variables contributed significantly to emergence success (Table 1). However, cell initiation time had a significant, positive correlation with brood age (r = 0.56; df = 111; P < 0.001), and significant, negative correlations with visitation rate (r = –0.20; df = 108; P < 0.043), incubation rate (r = –0.49; df = 115; P < 0.001) and relative vibration rate (r = –0.21; df = 115; P < 0.023). These correlations suggested that earlier initiated cells tended to be started from younger brood, were visited, incubated and vibrated at higher rates, and as a result were more likely to develop to emergence than

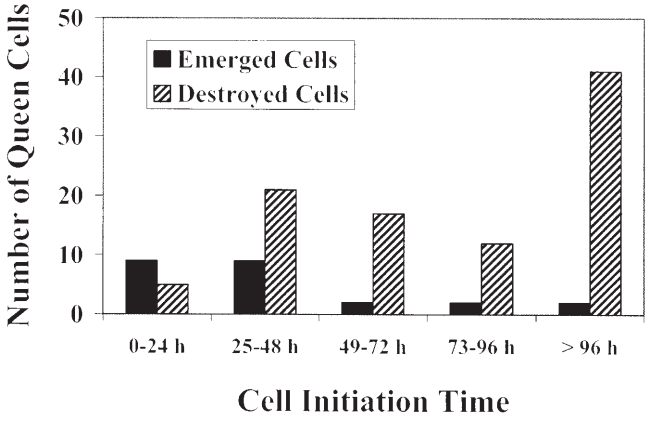

Figure 1. The number of emerged and destroyed queen cells that were initiated during each of the five periods following the removal of the laying queen

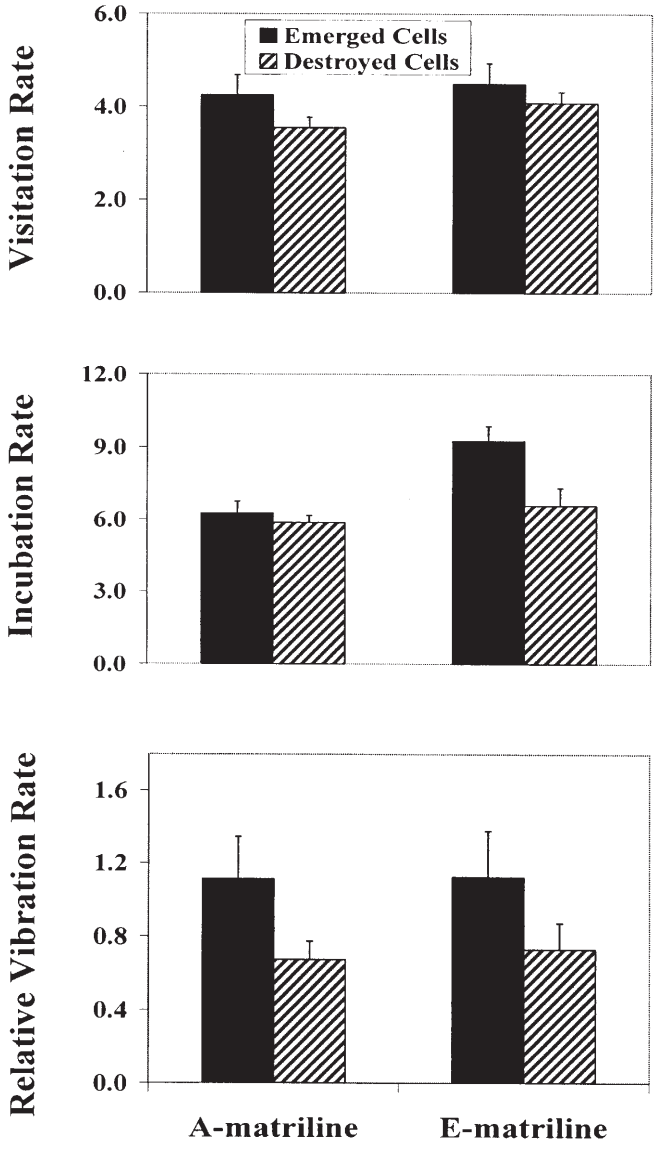

Figure 2. Mean ± SE visitation rates (upper figure), incubation rates (middle figure) and relative vibration rates (lower figure) experienced by emerged versus destroyed queen cells in the African(A)- and European(E)-matriline colonies

were cells initiated later after queen loss. Indeed, compared to cells that were destroyed, those that emerged experienced higher relative vibration rates ($F=6.64$; $df=1, 124$; $P<0.011$), greater incubation rates ($F=9.23$; $df=1, 124$; $P<0.003$), and slightly, but not significantly, higher visitation rates ($F=2.82$; $df=1, 108$; $P=0.096$; Fig. 1). The relationships between emergence success, visitation rates and vibration rates were exhibited similarly in both matrilines (for both interaction terms, $F<0.02$; $P>0.65$), while that for incubation rate was more pronounced in the E-matriline colonies ($F=5.09$; $df=1, 124$; $P<0.026$; Fig. 2). In summary, cell initiation time, and its effect on subsequent worker interactions, appeared to be the major determinant for the successful development and emergence of virgin queens.

Table 2. The mean ± SE visitation rate, incubation rate and relative vibration rate experienced by the queen cells that gave rise to African (A)- versus European (E)-paternity VQs. Rates refer to the number of worker interactions occurring per 30-min period. Samples sizes indicate the number of cells of each patriline for which the different variables could be determined

	N	African-paternity VQs	European-paternity VQs
Visitation Rate	14A; 11E	4.52 ± 0.41	4.31 ± 0.51
Incubation Rate	16A; 12E	7.24 ± 0.60	7.81 ± 0.69
Vibration Rate	16A; 12E	0.98 ± 0.21	1.16 ± 0.23

In contrast, emergence order was uninfluenced by the different aspects of queen rearing examined. The logistic regression analysis revealed that in combination the seven variables monitored had no significant effect on the sequence in which VQs emerged in their colonies (likelihood ratio Chi-square = 9.85; $df=7$; $P>0.19$). Likewise, none of the variables individually contributed significantly to emergence order (Table 1).

Influence of VQ paternity on emergence success, emergence order and worker interactions

VQ paternity was determined for a total of 30 sealed queen cells. Paternity could be assigned to 18 of the sealed cells in the A-matriline colonies, 12 of which contained an African-patriline and six a European-patriline VQ. Paternity was determined for 12 sealed cells in the E-matriline colonies, five of which contained an African- and seven a European-paternity queen.

VQ paternity did not influence emergence success. Similar numbers of African- and European-paternity VQs were reared to emergence (Chi-square = 0.53; $df=1$; $P>0.05$), and this trend did not differ between the two matrilines (Chi-square = 1.84; $df=1$; $P>0.05$). Queen paternity did not influence cell initiation time or the age of brood selected for rearing. There was no difference in the number of African- versus European-paternity VQs initiated during the different time periods following queen removal (Chi-square = 1.89; $df=2$; $P>0.05$), nor was there a difference in the number of cells of each patriline started from the different brood-age categories (Chi-square = 0.81; $df=2$; $P>0.05$). Also, African- and European-paternity VQs did not differ in the rate at which their cells were visited ($F=0.34$; $df=1, 24$; $P>0.53$), incubated ($F=0.02$; $df=1, 27$; $P>0.88$) or vibrated ($F=0.33$; $df=1, 24$; $P>0.56$) by workers (Table 2). These trends were exhibited to similar extents in both matrilines (for all interaction terms, $F<2.42$; $P>0.13$).

VQ paternity also had no clear effect on emergence order. More than twice as many first-emerging queens in our 13 study colonies were of African- versus European-paternity (9 versus 4 VQs, respectively). However, this difference was not significant (Chi-square = 1.92; $df=1$; $P>0.05$).

Table 3. The number of visits, incubations and vibration signals performed by African(A)- versus European(E)-paternity workers and the number expected (in parentheses) summed over all queen cells in the A- and E-matriline colonies. Samples sizes refer to the number of cells for which data were collected

	A-matriline Colonies			*E-matriline Colonies*		
	A-workers	E-workers	N	A-workers	E-workers	N
Visits	2671(2561)	522(632)	62	1956(1718)	1194(1432)	47
Incubations	20,507(18,877)	2314(3944)	71	12,811(12,580)	8821(9052)	54
Vibration Signals	4652(4877)	1391(1166)	71	306(281)	214(239)	54

Table 4. The number of visits, incubations and vibration signals performed by African(A)- versus European(E)-paternity workers and the number expected (in parentheses) summed over all cells containing African- and European-paternity queens in the A- and E-matriline colonies. Samples sizes refer to the number of cells of each patriline for which data were collected

	African-paternity VQs			*European-paternity VQs*		
	A-workers	E-workers	N	A-workers	E-workers	N
A-matriline Colonies						
Visits	600(536)	127(191)	10	331(380)	159(110)	5
Incubations	3973(3800)	746(919)	11	1572(1498)	359(433)	6
Vibration Signals	1601(1488)	228(341)	11	965(1164)	524(325)	6
E-matriline Colonies						
Visits	211(183)	145(173)	4	215(235)	204(184)	6
Incubations	2216(2106)	1365(1475)	5	3101(2681)	1500(1910)	6
Vibration Signals	38(46)	50(42)	5	134(109)	52(77)	6

Influence of worker paternity and kinship on queen cell interactions

Worker paternity had a strong effect on the tendency to interact with queen cells. African-paternity workers performed many more visits and incubations than expected in both matrilines, and more vibration signals than expected in the E-matriline colonies (for all comparisons, Chi-square >144.0; $P<0.001$; Table 3). In the A-matriline colonies, more vibration signals were performed by European-paternity workers (Chi-square $=1127.2$; $P<0.0001$; Table 3). There was, however, pronounced variability among the different queen cells in the extent to which worker paternity influenced the different interactions (for all comparisons, heterogeneity Chi square >139.0; $P<0.001$).

Kinship did not consistently influence the different worker-queen cell interactions monitored. Relatedness affected the tendency to visit queen cells. In both colony matrilines, significantly more visits than expected were made to super-sister queens by both African- and European-paternity workers (for all comparisons, Chi square >47.0; $P<0.001$; Table 4). In contrast, kin preferences were not exhibited during incubation behavior. In both matrilines, most incubations were performed by African-paternity workers (for all comparisons, Chi-square >178.0; $P<0.0001$; Table 4), regardless of their relatedness to the developing queens. The variability in kinship effects was most clearly seen in the tendency of workers to perform vibration signals on queen cells. In the A-matriline colonies, both African- and European-patriline workers showed super-sister preference during signal performance (for both comparisons, Chi-square >122.0; $P<0.001$), while in the E-matriline colonies, the patrilines exhibited half-sister preferences when vibrating queen cells (for both comparisons, Chi-square >15.4; $P<0.01$; Table 4). However, for each interaction monitored there was tremendous variability among queen cells in the extent to which kin preferences were exhibited (for all comparisons, heterogeneity Chi square >37.0; $P<0.001$).

In summary, African paternity in workers was associated with a greater tendency to engage in queen rearing for all interactions monitored, with the exception of vibration signals performed on queen cells in the A-matriline colonies. Kinship influenced the tendency to visit queen cells, but had variable or no effect on incubation and vibration signal activity.

Discussion

The patterns of queen cell production that we observed were consistent with those reported from previous studies of queen rearing. The numbers of queen cells/colony in our study (10.7 and 9.5 respectively for the A- and E-matrilines) fell within the range of 6.0 – 27.1 cells/colony reported for European colonies (Punnett and Winston, 1983; Fell and Morse, 1984; Winston et al., 1989; 1990; Engels et al., 1993; Pettis et al., 1995), but were slightly higher than the 4.8 – 8.7 cells/colony reported for African honeybees (Fletcher and Tribe, 1977;

Winston, 1979; Pettis et al., 1995; Schneider et al., 2001). We observed the initiation of queen cells throughout five or more days following queen removal. Similarly, Pettis et al. (1995) reported continued queen cell production after three or more days of queenlessness, although Hatch et al. (1999) observed no further cell initiation after 48 h following queen loss. The proportion of sealed cells that were destroyed in our colonies (approx. 80%) was consistent with the 53% destruction rate reported by Hatch et al. (1999).

Queen emergence success

The factor most strongly associated with emergence success in our study colonies was the timing of queen cell initiation. Cells initiated earlier in the queen replacement process were started from younger brood, received higher visitation, incubation and vibration rates and were more likely to develop to emergence than were later-initiated cells. Thus, during emergency queen replacement, workers could convey a developmental advantage to certain queens by rearing them from young brood soon after queen loss, and then interacting with the resulting larvae at heightened rates. Hatch et al. (1999) also reported a tendency for workers to select young brood for queen rearing during the early period following queenlessness. Schneider et al. (2001) also observed higher vibration rates on emerged versus destroyed cells in pure European and African colonies, although these differences did not reach significance.

We do not fully understand the cause-and-effect relationships between greater worker interactions with queen cells and emergence success. Some queen larvae may receive higher levels of visitation, incubation and vibration activity because of an inherently greater ability to develop to emergence. Alternatively (or additionally), increased worker interactions might contribute directly to greater developmental success. If increased visitation rates contribute to increased feedings, then the resulting nutritional benefits could enhance development. Greater incubation activity may result in a higher brood nest temperature, which is a major factor regulating developmental success (DeGrandi-Hoffman et al., 1993; Hatch et al., 1999). The effects of the vibration signal on virgin queen development are unknown. However, the significantly higher vibration rates observed for emerged versus destroyed cells suggest that workers may use the signal to influence whether queens develop to emergence.

Queen emergence order

We observed no associations between any of the factors examined and VQ emergence order. In particular, we found no relationship between the vibration signal and emergence order. In contrast, several authors have suggested that workers use the signal to delay queen emergence (Fletcher, 1978b; Bruinsma et al., 1981). However, Grooters (1987) found no association between the signal and emergence order, while Schneider et al. (2001) observed delayed emergence by vibrated cells in some colonies, but not others. Indeed, our observations suggest that the vibration signal may be more associated with queen development than the order of emergence. Several studies have revealed that workers can regulate queen emergence by resealing emerging VQs and physically confining them within their cells (Fletcher, 1978b; Grooters, 1987). Thus, the behaviors examined in our study may be more associated with influencing whether queens develop to emergence, whereas emergence order may be regulated by other worker-queen cell interactions.

VQ paternity and emergence

There was no difference in the number of African- and European-paternity queens that developed to emergence, and cells from the two patrilines did not differ in the timing of initiation, the age of brood selected for rearing, or in visitation, incubation and vibration rates. VQ paternity, therefore, had no observable effect on emergence success or the worker-queen cell interactions that we monitored.

Our results also suggest that the Cordovan color marker did not influence the queen rearing process. Several authors have suggested that the Cordovan marker may contribute to reduced viability, because *cd* drones may have lower survival and mating success under some circumstances (Tucker, 1986; Berg et al., 1997). If the *cd* marker resulted in sub-viable queens, then we would have expected less care to be given by workers and higher destruction rates for European-paternity VQs. These trends should have been especially pronounced in the E-matriline colonies, in which European-patriline queens were homozygous for the Cordovan trait. However, we observed no differences in emergence success or worker interactions for African- versus European-paternity cells in either matriline. Furthermore, there are no known associations between the *cd* marker and any aspect of queen quality or behavior (Taber and Wendel, 1958; Tucker, 1986; Schneider and DeGrandi-Hoffman, 2002). It is therefore unlikely that our results reflected artifacts arising from the use of the Cordovan color variant.

While we found no effect of VQ paternity on emergence success, we did find a slight, but non-significant tendency for African-paternity VQs to be the first queens to emerge in their colonies. Similarly, DeGrandi-Hoffman et al. (1998a) and Schneider and DeGrandi-Hoffman (2002) reported earlier emergence of African-patriline queens in hybrid colonies. Our results suggest that this earlier emergence may reflect an inherent racial difference in developmental time, rather than differences in the extent to which worker interact with African- versus European-paternity cells (see also DeGrandi-Hoffman et al., 1993; Pettis et al., 1995).

Worker paternity, relatedness and interactions with queen cells

Worker paternity influenced the tendency to engage in most of the behaviors that we examined. African-paternity workers

performed more visitation and incubation behavior in both colony matrilines, and also performed more vibration signals on queen cells in the European-matriline colonies. Likewise, African-paternity workers are more likely to perform vibration signals on emerged VQs in hybrid colonies (Schneider and DeGrandi-Hoffman, 2002). Thus, in general African-paternity workers may be more involved in queen rearing and polygyny reduction in hybrid colonies than are their European-paternity nestmates.

The role of kinship in the queen rearing process was ambiguous. Workers of both patrilines exhibited super-sister preferences during visitation behavior, but inconsistent or no kin preferences during incubation and vibration signal activity. Furthermore, there was pronounced variability among the different queen cells in the extent to which worker nepotism was exhibited during the different interactions. Previous studies have also revealed slight, but inconsistent and highly variable kin preferences during queen rearing (reviewed in Breed et al., 1994; Visscher, 1998). The use of a limited number of subfamilies in kinship studies may allow for artificially high levels of nepotism (Breed et al., 1994). Also, under such simplified colony genetic structure, the Cordovan color marker may enhance patriline discrimination and contribute to the appearance of super-sister preferences (Frumhoff, 1991; Visscher, 1998). We used colonies containing only two patrilines and the *cd* marker, yet found consistent super-sister preferences for only the rate at which workers visited queen cells. It therefore seems unlikely that relatedness is a major factor influencing queen rearing in colonies with a typical number of subfamilies (see also Visscher, 1998).

Our results, in concert with those of previous studies, suggest that workers use different suites of behavior to influence the various components of the queen replacement process. The timing of cell initiation, the age of brood selected for rearing, and visitation, incubation and vibration signal activity may be used to create a pool of rival queens that develop to emergence. The order in which queens emerge may be regulated through mechanisms that physically confine VQs in their cells (Fletcher, 1978b; Grooters, 1987). Once queens emerge, they are often bitten, chased and vibrated by workers, which may inhibit or interrupt their aggressive interactions and help to determine the ultimate victor in polygyny reduction (Gilley, 2001; Schneider et al., 2001; 2002; Schneider and DeGrandi-Hoffman, 2002). Worker interactions may therefore be primary determinants for the outcome of many aspects of queen replacement. Furthermore, some of these interactions may have contributed to the spread of the African honey bee in the Western Hemisphere. Emerged African-patriline VQs receive more vibration signals from workers, which may contribute to a competitive advantage during queen competition (Schneider et al., 2001; Schneider and DeGrandi-Hoffman, 2002). In contrast, we found that VQ paternity had little influence on worker interactions with queen cells. Thus, African paternity may influence the queen replacement process primarily by affecting worker-queen interactions during the polygyny reduction period, and may play less of a role during queen rearing.

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