



# The influence of paternity on virgin queen success in hybrid colonies of European and African honeybees

STANLEY SCOTT SCHNEIDER\* & GLORIA DEGRANDI-HOFFMAN†

\*Department of Biology, University of North Carolina, Charlotte

†Carl Hayden Bee Research Center

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When African honeybees, *Apis mellifera scutellata*, migrate into an area, substantial hybridization occurs with existing European bee populations. However, over time European traits disappear until the populations become predominantly or entirely African. European patrilineal traits could be lost when hybrid colonies raise virgin queens if African-patriline queens have a survival advantage during reproductive competition. We examined queen competition in observation colonies that contained an African (A) or European (E) matriline, and both patrilines. In colonies of either matriline, the virgin queens that survived the elimination process were those that emerged sooner, piped more, eliminated more rivals and received more vibration signals from workers. Hybrid queens had increased piping and vibration rates and greater fighting success than did purebred queens. However, the effects of hybridization were expressed differently in the two matriline, resulting in significant matriline-by-patriline interactions. In the E-matriline colonies, African-patriline queens emerged sooner, piped more, killed more rivals, were vibrated at higher rates and were more likely to survive the replacement period than were their European-patriline sister queens. In contrast, in the A-matriline colonies, the behaviour of African- and European-patriline queens was highly variable, and the two queen types did not differ for any of the characters examined or the ability to survive the rival elimination period. Thus, African paternity potentially conveys a competitive advantage to queens of European maternity, which would contribute to the spread of African alleles and the loss of European traits in hybrid zones. Furthermore, we observed that vibration signals were produced predominantly by African-paternity workers. The vibration signal may influence the spread of African traits, and this effect may be mediated largely by the behaviour of African-paternity workers.

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Introduced species often have dramatic effects on the genetic structure and behaviour of resident populations (Shigesada & Kawasaki 1997; Holway et al. 1998; Rissler et al. 2000). A recent and spectacular example of an invasion involves the African honeybee, *Apis mellifera scutellata*. Since its introduction into Brazil in 1956, this race of bees has spread throughout Latin America and the southwestern U.S.A. and has largely displaced resident populations of European honeybees within this range (Winston 1992; Rinderer et al. 1993; Hall 1999). African and European bees will readily hybridize (Hall 1990; Sheppard et al. 1991). Indeed, from the time of introduction, hybridization was assumed to result in the intro-

gression of European traits into the African population, giving rise to the 'Africanized' bee of Latin America. However, honeybee populations in invaded areas contain primarily African mitochondrial DNA (mtDNA) (Hall & Smith 1991; Lobo 1995), indicating a loss of European matriline. Resident colonies in invaded regions also lose their European nuclear DNA markers and morphometric characteristics, suggesting a loss of European patrilines through mating and continued backcrossing with African drones (Hall 1990; Rinderer et al. 1991). Thus, gene flow is highly asymmetrical and results in populations becoming increasingly African (Hall & Smith 1991; Hall 1999). Several factors may contribute to this phenomenon (Winston 1992; Harrison & Hall 1993; Lobo 1995), although the ability of African bees to displace European colonies is poorly understood. Nevertheless, the African bee provides an excellent system for investigating the behavioural and genetic mechanisms that mediate biological invasions.

Correspondence: S. S. Schneider, Department of Biology, University of North Carolina, Charlotte, NC 28223 U.S.A. (email: sschmedr@email.uncc.edu). G. DeGrandi-Hoffman is at the Carl Hayden Bee Research Center, 2000 E. Allen Road, Tucson, AZ 85719, U.S.A.

European honeybee alleles may be lost when colonies raise new queens in association with reproductive swarming and emergency queen replacement. During these events, colonies rear multiple virgin queens (VQs) in specially constructed queen cells. Once emerged, VQs typically attempt to destroy unemerged 'rivals' still within queen cells and battle other emerged queens to the death (Winston 1987). The end result is a single surviving VQ that takes mating flights and becomes the new laying queen of the colony. When African honeybees migrate into an area, queens will mate with African and European drones, thereby creating colonies with both African and European patriline workers. When these hybrid colonies swarm or replace their queens, they will rear VQs of both patrilines. If African-paternity VQs have a competitive advantage, then this would contribute to the spread of African alleles and over time the loss of European honeybee characteristics.

Hybridization and paternity could influence queen replacement in two ways. First, it could affect VQ fighting success. The factors affecting queen fighting ability are poorly understood, but may include: (1) body size and weight (Hatch et al. 1999), (2) emergence order, because early emerging VQs may have more opportunities to eliminate rivals still within queen cells (DeGrandi-Hoffman et al. 1998a), (3) 'spraying' behaviour, which consists of a queen ejecting a gut fluid during combat that may disrupt fights and promote VQ survival (Bernasconi et al. 2000), and (4) 'piping' behaviour, which consists of a VQ producing a series of pulsed sounds that may reflect her fighting ability and inhibit the emergence of rivals (Visscher 1993; Schneider et al. 2001). Any influence of paternity on these different VQ characteristics could potentially convey a competitive advantage.

Second, hybridization and paternity could influence worker-queen interactions, which may be the primary determinants for the outcome of VQ rivalry (Visscher 1993; Schneider et al. 2001). A mechanism by which workers may affect VQ behaviour is the 'vibration signal', which consists of a worker rapidly vibrating her body dorsoventrally for 1–2 s while in contact with a VQ (Allen 1959). Emerged queens can receive hundreds of vibration signals an hour, but there is often tremendous variability in the vibration rates experienced by VQs within the same colony (Fletcher 1978b; Schneider 1991). Workers may therefore preferentially direct vibration activity towards certain queens. Schneider et al. (2001) recently demonstrated that in 'pure' African and European colonies, VQs that were vibrated at higher rates relative to their rivals survived longer, piped more, experienced greater fighting success and were more likely to become the new laying queens of their colonies. The vibration signal may therefore give workers a degree of control over which VQ inherits the nest (Schneider et al. 2001). If paternity influences the likelihood of queens receiving the signal, the tendency of workers to perform the signal, or both, then the vibration signal may contribute to the spread of African alleles. However, the effect of paternity on vibration signal behaviour has never been explored.

The purpose of this study was to examine queen success and worker-queen interactions in hybrid colonies con-

taining both African and European patrilines. We had four objectives. First, we examined the effect of hybridization and paternity on VQ emergence order and survival. Second, we assessed the influence of hybridization and paternity on VQ fighting ability and success. Third, we explored the influence of hybridization and paternity on the likelihood of receiving vibration signals and how the signal was associated with queen survival. Fourth, we determined whether worker paternity affected vibration signal performance.

## METHODS

### Study Site, Colony Set-up and Maintenance

The study was conducted during 1999–2000 at the Carl Hayden Bee Research Center, Tucson, Arizona. The queen-replacement process was examined using two types of hybrid colonies created through instrumental insemination, following the procedures of DeGrandi-Hoffman et al. (1998a). European-matriline (E-matriline) colonies arose from European-matriline queens artificially inseminated with the semen from one African and one European drone. These colonies contained two genotypes: pure European and European-African hybrids. African-matriline (A-matriline) colonies arose from African-matriline queens inseminated with the same drone combinations used in the E-matriline colonies, and contained pure African and African-European hybrid genotypes. Our insemination protocol standardized the number of matings between queens and allowed us to examine the effects of hybridization and patriline, as well as possible patriline-by-matriline interactions. The protocol also affected kinship. All workers and queens reared from the same patriline within a colony were 'super-sisters' (relatedness coefficient:  $G=0.75$ ), and those from different patrilines were 'half-sisters' ( $G=0.25$ ).

All queen and drones used for the inseminations were reared from three or more unrelated colonies of each matriline, to ensure that our protocol maintained genetic variability within matrilines and prevented inbreeding. African queens and drones used for the inseminations had black cuticular coloration and were reared from colonies established from swarms captured in southern Arizona that were identified as African using morphometric (Rinderer et al. 1993) and mtDNA analyses (Crozier et al. 1991; Hall & Smith 1991). European queens and drones used in the inseminations were reared from colonies carrying the cordovan (*cd*) gene for body colour. The *cd* trait is a naturally occurring colour variant (Tucker 1986) that produces a distinctive 'light blond' colour when homozygous and an intermediate brown coloration when heterozygous.

Our insemination protocol resulted in patrilines that could be visually distinguished within each matriline based on cuticular coloration. In the E-matriline colonies, worker and queens of European paternity were homozygous for the *cd* allele and showed a uniform blond coloration with indistinct, light-brown abdominal banding. In contrast, African-paternity workers and queens in the E-matriline colonies were darker and had

distinct dark banding patterns. In the A-matriline colonies, workers and queens of African paternity were either completely black or had a black thorax and a solid black band across each abdominal tergite. Workers and queens of European paternity in the A-matriline colonies were lighter and had no black banding on the upper one to three abdominal segments. The coloration patterns were verified by inseminating African and European queens with only one African or European drone (voucher specimens maintained at the Carl Hayden Bee Research Center). This method of identification has been used previously to distinguish between African- and European-paternity queens and workers reared in hybrid colonies (DeGrandi-Hoffman et al. 1998a, b; Schneider & DeGrandi-Hoffman 2002).

We used colour markers to visually discriminate between patriline, rather than genetic markers that had to be identified using molecular techniques, because the outcome of queen replacement is likely to depend upon the cumulative effects of multiple queen–queen interactions and hundreds of worker–queen interactions that occur throughout the rivalry period. Opening the study hives repeatedly to collect these large numbers of bees for genetic analysis would have caused severe disturbances that could have interrupted the queen replacement process.

The inseminated queens were established in five-frame nucleus hives following standard procedures (DeGrandi-Hoffman et al. 1998a) and expanded to 45-litre hive boxes as colony growth warranted. Once a colony contained a large population of workers that were all progeny of the inseminated queen, we transferred four frames with the queen and 6000–8000 workers into observation hives. The Plexiglas walls of the observation hives contained hinged access ports through which emerged VQs were marked with distinguishing colours of paint. We established a total of 13 observation colonies, partitioned between four trials involving three to four colonies each. During each trial, both A- and E-matriline colonies were monitored simultaneously.

### Monitoring Virgin Queen Development and Behaviour

Once transferred to the observation hives, the inseminated queens were allowed to lay for at least 5 days to ensure an ample supply of eggs and young larvae for queen rearing. The laying queens were then removed, which initiated emergency queen replacement. We checked the colonies six to eight times each day, and for every developing queen cell observed we marked its location on the Plexiglas walls of the observation hive and assigned it an identification number.

Six days after the first queen cell was sealed, we began monitoring the colonies continuously for 24 h/day. Observations were conducted by three to four observers to ensure that all colonies were monitored simultaneously during each trial. For each VQ that emerged, we recorded the cell that she emerged from, her paternity (African versus European) and the colour of paint mark she received. We then monitored each VQ continuously,

subdivided into 30-min periods, throughout the time that she was present in the colony (mean  $\pm$  SE observation time per queen =  $39.1 \pm 10.12$  h). At the end of the queen replacement period, we classified each VQ according to her 'fate' (whether she was killed or survived to become the new queen of her colony). At the end of every trial, we classified each colony as either a 'single-VQ colony' (if only one queen emerged, which then destroyed all rivals while they were in their queen cells) or a 'multiple-VQ colony' (if two or more queens emerged, which then fought one another until only one remained).

Two main aspects of behaviour were assessed for each emerged VQ. First, we examined fighting behaviour by recording for each VQ: (1) the number of times that she engaged in spraying during combat, (2) her piping rate (bouts of piping performed per 30-min period), (3) the number of emerged queens that she killed in combat, (4) the number of unemerged queen cells that she destroyed, (5) the total number and proportion of rivals eliminated (emerged queens plus queen cells) and (6) her dry weight. As far as was possible, the corpses of all killed VQs were collected and stored at  $-70^{\circ}\text{C}$ . At the end of the queen elimination periods, we also collected most of the surviving VQs and stored them at  $-70^{\circ}\text{C}$ , although some were allowed to remain in their colonies for use in other parts of the study. Each collected VQ was dried in a desiccating oven for 24 h and then weighed to the nearest milligram.

Second, we examined the vibration signal activity experienced by each VQ. We recorded each time a queen received a vibration signal from an African- versus European-paternity worker. Subsequently, we calculated the vibration rate she experienced, by dividing the total number of signals received by the number of 30-min periods in which she was monitored.

### Determining the Relationships Between Hybridization, VQ Paternity, Emergence Order and Fate

To examine the general effects of early emergence and hybridization on queen success, we used chi-square analysis to compare over all colonies (1) the number of surviving versus killed queens that were the first VQ to emerge and (2) the number of hybrid versus purebred queens that survived the rival elimination process. To examine the effects paternity on queen emergence and survival, we used Fisher's exact tests to compare within each matriline (1) the number of colonies in which the first VQ to emerge was of African versus European paternity and (2) the number of African- and European-paternity VQs that survived versus were killed during the elimination process.

### Determining the Influence of VQ Paternity on Fighting Ability and Vibration Rate

We compared VQs with respect to spraying behaviour, piping rate, the proportion of rivals eliminated, dry weight and the vibration rate experienced during the queen elimination process. We conducted these analyses

from three perspectives. First, we compared surviving versus killed VQs in the A- and E-matriline colonies, to assess whether the relationships between fighting success and queen behaviour previously reported for pure African and European colonies (Schneider et al. 2001) also occurred in hybrid colonies. Second, we compared hybrid versus purebred queens over all colonies, to determine whether hybridization per se influenced VQ behaviour. Third, we compared African- versus European-paternity queens in the two colony types, to assess whether patriline influenced VQ behaviour, and whether this influence was affected by colony matriline.

For each variable examined, we conducted an analysis of variance (ANOVA) to test for differences between individual colonies within the A- and E-matriline groups. If these initial analyses revealed no significant differences, we combined the data within colony matriline and examined each variable using two-way ANOVAs (Sokal & Rohlf 1995), which had one between-subjects factor (colony matriline), one within-subjects factor (VQ paternity or hybrid status) and an interaction between matriline and VQ paternity or hybrid status. If we found significant interactions, we then used single degree-of-freedom planned comparisons to make more detailed contrasts between African- versus European-paternity VQs and hybrid versus purebred queens (Sokal & Rohlf 1995).

We observed enormous variation in VQ piping and vibration rates both within and between colonies. As a result, comparisons of the actual rates in the ANOVAs yielded main effects and interaction terms that were difficult to interpret. We therefore used the methods of Schneider et al. (2001) to standardize our data, by calculating for each VQ a 'relative piping rate' and a 'relative vibration rate', defined as a VQ's individual rate divided by the mean rate for all queens within her colony. We then performed the ANOVAs using the relative values to allow for more meaningful comparisons.

The dynamics of queen replacement may differ between single-VQ and multiple-VQ colonies, because it is only in the latter that direct physical fighting occurs between sister queens. Therefore, we conducted our comparisons, first using the data for all emerged VQs, and next using the data for only the multiple-VQ colonies. Both approaches gave identical statistical results. However, with the exception of dry weight (which is reported for all queens), we report the comparisons for the multiple-VQ colonies, because these allowed for a more direct assessment of physical combat. Also, the use of relative piping and vibration rates was more pertinent for the multiple-VQ colonies. Our data were not normally distributed and were transformed prior to analysis. A square-root transformation was used to normalize the data for relative vibration and piping rates. Data for the proportion of rivals eliminated were arcsine transformed (Sokal & Rohlf 1995).

### Determining the Influence of Worker Paternity on Vibration Signal Performance

Replicated goodness-of-fit tests (Sokal & Rohlf 1995) were used to compare for each VQ the observed number

**Table 1.** The number of African-paternity virgin queens (A-VQs) and European-paternity virgin queens (E-VQs) that were the first to emerge and that survived the queen elimination process in the A- and E-matriline colonies

	A-matriline colonies		E-matriline colonies	
	A-VQs (N=11)	E-VQs (N=5)	A-VQs (N=5)	E-VQs (N=5)
First VQ to emerge	4	3	5	1
Survived	5	2	5	1

of vibration signals received from African- versus European-paternity workers to that expected if workers performed signals at random with respect to their patriline. Expected values were calculated based on the proportion of African- and European-paternity 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, then counting the number of workers of each patriline.

Because of the kinship structure created in our colonies, any observed differences between African- and European-patriline workers in the tendency to vibrate VQs could have reflected kin discrimination, rather than patriline effects per se (Breed et al. 1994; Visscher 1998). To examine the possible influence of relatedness, we used replicated goodness-of-fit tests to compare for each worker patriline the observed number of vibration signals performed on supersister versus half-sister VQs to those expected, based on the proportion of queens belonging to each patriline. Analyses were conducted separately for the A- and E-matriline colonies. Both single-VQ and multiple-VQ colonies were included in the analysis.

We analysed our data using replicated *G* tests and chi-square tests (Sokal & Rohlf 1995), and both approaches gave identical results. However, *G* tests cannot be used for subjects that have observed values of zero, and we had several VQs that received no vibration signals from either African- or European-paternity workers. As a result, we report our chi-square analyses, which allowed us to use the data for all VQs.

All statistical tests were two tailed and the sequential Bonferroni adjustment (Rice 1989) was used to determine significance levels for the multiple comparisons made between colony matriline and VQ patriline. Mean values are reported  $\pm 1$  SE.

## RESULTS

We monitored seven A-matriline colonies, which produced 75 sealed queen cells, 16 of which emerged; the remaining 59 were destroyed during the rival elimination process. Of the 16 emerged VQs in the A-matriline colonies, 11 were of African paternity and five of European paternity (Table 1). Four of the A-matriline colonies were

single-VQ colonies, and the remaining three were multiple-VQ colonies ( $4.0 \pm 1.2$  queens/colony).

We monitored six E-matriline colonies, which produced 57 sealed queen cells, 10 of which emerged and 47 of which were destroyed during the rivalry period. Of the 10 emerged VQs, five were of African and five of European paternity (Table 1). Three of the E-matriline colonies were single-VQ colonies and three were multiple-VQ colonies ( $2.3 \pm 0.3$  queens/colony).

### VQ Emergence Order, Fate and Paternity

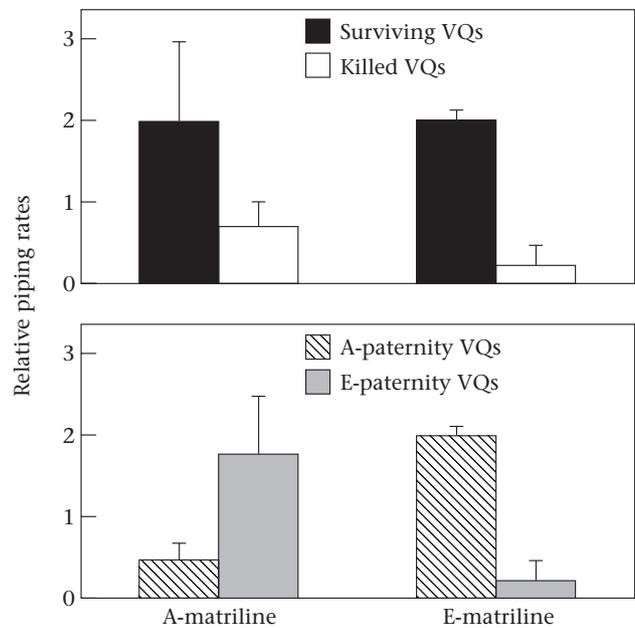
The first VQ to emerge had a strong survival advantage during queen competition. The first-emerging VQ became the new laying queen in 11 of the 13 observation colonies (chi-square test:  $\chi^2_1=6.24$ ,  $P<0.015$ ). When viewed over all queens, hybridization per se did not influence queen survival. Seven of the 10 hybrid queens survived the elimination process, and six of the 16 purebred queens did so ( $\chi^2_1=2.60$ ; NS). More first-emerging queens were of African than European paternity (Table 1), and this pattern was similar for the A- and E-matriline colonies (Fisher's exact test: NS). However, the degree to which paternity and hybridization were associated with VQ survival differed between the two matrilines. In the A-matriline colonies, more than twice as many African-versus European-paternity VQs survived (Table 1), but this difference was not significant (Fisher's exact test: NS). In contrast, in the E-matriline colonies the queens with African-paternity (hybrid VQs) were five times more likely to survive than the purebred queens with European paternity (Fisher's exact test:  $P<0.05$ ; Table 1). Thus, African paternity was associated with earlier emergence and increased survival in VQs, and these relationships were more pronounced in European-matriline colonies.

### VQ Fighting Ability, Fate and Paternity

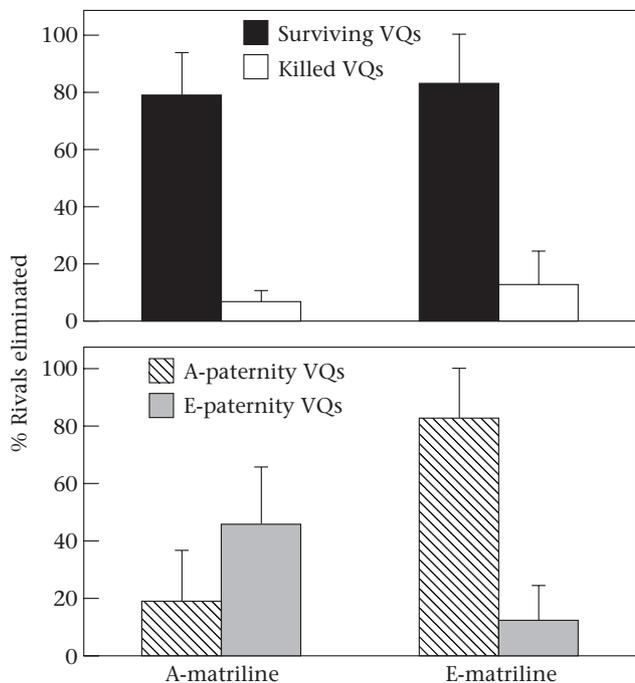
For each aspect of fighting behaviour examined, there were no differences among the multiple-VQ colonies within the A- and E-matriline groups (ANOVA:  $F_{4,18}<0.60$  for all comparisons, NS). We therefore combined data within matriline. We observed only four instances of spraying and this behaviour was excluded from the analyses.

Compared to the queens that were killed in the multiple-VQ colonies, surviving queens had greater relative piping rates ( $F_{1,15}=11.96$ ,  $P=0.0035$ ; Fig. 1) and eliminated a greater proportion of rivals ( $F_{1,15}=49.81$ ,  $P<0.0001$ ; Fig. 2). These trends in VQ fate were similar in the two colony matrilines ( $F_{1,15}<0.62$  for both comparisons, NS).

Hybridization influenced queen fighting behaviour. Compared to purebred queens, hybrid VQs had greater relative piping rates ( $F_{1,15}=10.80$ ;  $P=0.005$ ; Fig. 1) and eliminated more rivals ( $F_{1,15}=12.52$ ;  $P=0.003$ ; Fig. 2). However, the effects of hybridization were expressed differently in the A- and E-matriline colonies, resulting in highly significant matriline-by-patriline interactions for both piping activity ( $F_{1,15}=11.79$ ,  $P=0.0037$ ) and



**Figure 1.** Mean  $\pm$  SE relative piping rates for the surviving versus killed virgin queens (VQs) and the African- versus European-paternity VQs in the A- and E-matriline colonies. The analyses were restricted to three multiple-VQ A-matriline colonies (which produced seven African- and five European-paternity VQs) and three multiple-VQ E-matriline colonies (which produced four African- and three European-paternity VQs).



**Figure 2.** Mean  $\pm$  SE proportion of rivals eliminated by the surviving versus killed virgin queens (VQs) and the African- versus European-paternity VQs in the A- and E-matriline colonies. Details as in Fig. 1.

the proportion of rivals eliminated ( $F_{1,15}=13.72$ ,  $P=0.0021$ ).

In the A-matriline colonies, the relative piping rates and proportions of rivals eliminated by the hybrid

European-paternity VQs were two to three times greater than those of the purebred African-paternity queens (Figs 1, 2). However, these differences were not significant (ANOVA:  $F_{1,15} < 4.39$ ; NS for both comparisons), because of the pronounced variability observed for the behaviour of the European-paternity queens (Figs 1, 2). In contrast, in the E-matriline colonies, the relative piping rates and proportions of rivals eliminated by the hybrid African-paternity VQs were six to seven times greater than those of the purebred European-paternity queens (Figs 1, 2), and these differences were highly significant ( $F_{1,15} > 7.81$ ,  $P < 0.0136$  for both comparisons).

Queen weight was not a factor in VQ success, nor did it differ based upon hybrid status or paternity. Based upon the dry weights of 21 VQs, surviving queens averaged  $0.044 \pm 0.001$  g, and those that were killed averaged  $0.042 \pm 0.002$  g ( $F_{1,17} = 0.26$ , NS). The weights of hybrid versus purebred VQs and African- versus European-paternity queens did not differ in either colony matriline ( $F_{1,17} < 0.48$ , NS for both comparisons).

In summary, regardless of colony matriline, the queens that survived the rival elimination process were those that showed greater piping activity and fighting success. These relationships were influenced by hybridization and paternity, especially in the European-matriline colonies, in which African paternity was associated with an advantage in fighting behaviour, although it did not influence queen size.

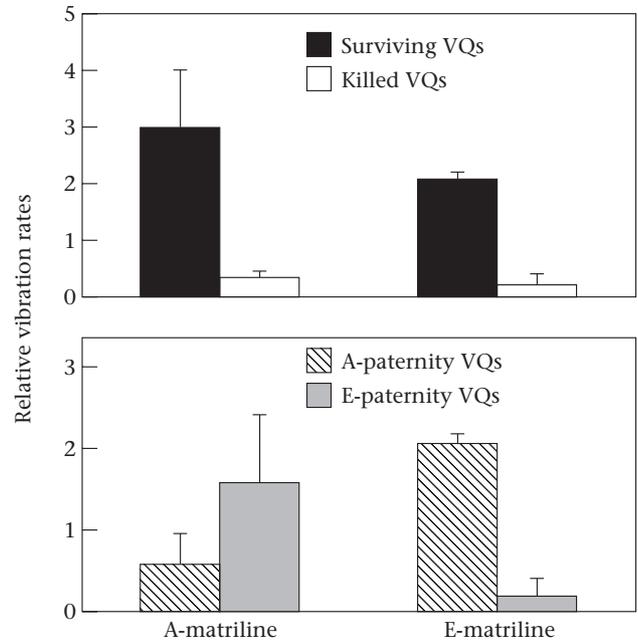
### Vibration Rate Experienced, VQ Fate and Paternity

Relative vibration rates did not differ between the multiple-VQ colonies within the A- and E-matriline groups ( $F_{4,18} < 0.40$  for both comparisons, NS). The data were therefore combined within matriline.

Surviving VQs experienced relative vibration rates that were highly significantly greater than those of the queens that were killed ( $F_{1,15} = 41.65$ ,  $P < 0.0001$ ; Fig. 3), and this trend was similar in both colony matriline ( $F_{1,15} = 0.01$ , NS). Hybridization influenced vibration rates, and, compared to the purebred queens, hybrid queens were vibrated more ( $F_{1,15} = 10.72$ ;  $P = 0.005$ ). However, the A- and E-matriline colonies differed markedly in the extent to which hybridization affected vibration rates, resulting in a highly significant matriline-by-patriline interaction ( $F_{1,15} = 12.20$ ,  $P = 0.0033$ ).

In the A-matriline colonies, the relative vibration rates experienced by European-paternity VQs were more than double those of their African-paternity rivals (Fig. 3). However, this difference was not significant ( $F_{1,15} = 3.32$ , NS), because of the pronounced variability in the vibration rates observed for the European-paternity queens (Fig. 3). In contrast, in the E-matriline colonies, the hybrid African-paternity VQs were vibrated at almost 10 times the rate experienced by their purebred European-paternity rivals and this difference was highly significant ( $F_{1,15} = 9.03$ ,  $P = 0.0089$ ; Fig. 3).

Thus, in both matriline, higher vibration rates were associated with increased VQ success. This relationship was influenced by hybridization and paternity, particularly in European-matriline colonies. In these colonies,



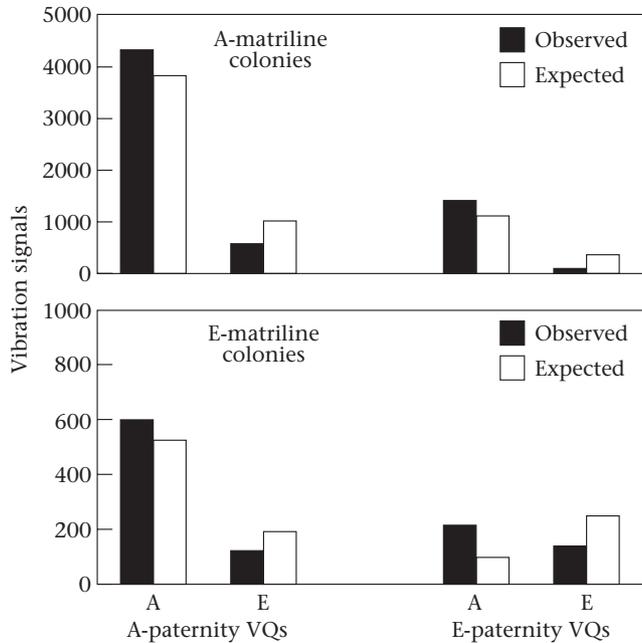
**Figure 3.** Mean  $\pm$  SE relative vibration rate experienced by the surviving versus killed virgin queens (VQs) and the African- versus European-paternity VQs in the A- and E-matriline colonies. Details as in Fig. 1.

the VQs that were vibrated at higher rates were hybrid queens of African paternity, and these were also the queens that showed greater fighting success and piping activity.

### Worker Paternity and Vibration Signal Production

African-paternity workers were more likely than European-paternity workers to perform vibration signals on VQs, regardless of queen patriline (Fig. 4). In the A-matriline colonies, African-paternity workers performed significantly more vibration signals than expected on both African-paternity VQs ( $\chi^2_{11} = 316.7$ ,  $P < 0.0001$ ) and European-paternity queens (chi-square test:  $\chi^2_5 = 352.2$ ,  $P < 0.0001$ ). Likewise, in the E-matriline colonies, African-paternity workers performed more signals than expected on African- ( $\chi^2_5 = 81.3$ ,  $P < 0.0001$ ) and European-paternity VQs ( $\chi^2_5 = 237.9$ ,  $P < 0.0001$ ). Heterogeneity values were significant for all comparisons (A-matriline colonies:  $\chi^2_{10} > 28.0$ ,  $P < 0.01$ ; E-matriline colonies:  $\chi^2_4 > 44.0$ ,  $P < 0.0001$ ), indicating that the degree to which African paternity affected worker signalling behaviour varied between VQs.

Relatedness showed no consistent effect on vibration signal performance. African-paternity workers showed a strong supersister preference and performed more signals than expected on African-paternity VQs in both matriline (for each comparison,  $\chi^2_1 > 183.5$ ,  $P < 0.0001$ ). In contrast, European-paternity workers showed a half-sister preference in the A-matriline colonies ( $\chi^2_1 = 143.2$ ,  $P < 0.0001$ ), but showed no kin preference during signal production in the E-matriline colonies ( $\chi^2_1 = 0.74$ , NS).



**Figure 4.** The number of vibration signals performed by African-paternity (A) versus European-paternity (E) workers and the number expected on African- and European-paternity virgin queens (VQs) in all A- and E-matriline colonies.

In summary, African paternity in workers was associated with a greater tendency to perform vibration signals on queens. If a preference for certain queens was shown, it was always shown for African-paternity VQs, whether they were supersisters or half-sisters of the workers producing the signals. Paternity, therefore, had a stronger influence on vibration signal activity than did relatedness.

## DISCUSSION

In both African- and European-matriline hybrid colonies, the VQs that survived the queen replacement process were those that emerged sooner, piped more, eliminated a greater proportion of rivals and were vibrated at higher rates relative to the other queens in their colonies. Our results are consistent with the findings of [Schneider et al. \(2001\)](#) for pure African and European colonies, although these authors reported a less consistent association between early emergence and queen success. We observed no effects of VQ weight on survival. Similarly, [Hatch et al. \(1999\)](#) and [Tarpy et al. \(2000\)](#) found no clear influences of weight and size on queen quality or survival during the elimination process. Taken together, these studies suggest that the factors most closely associated with VQ survival and fighting ability are emergence order, piping behaviour and the vibration signal. Furthermore, [Schneider et al. \(2001\)](#) found the same associations between these behaviours and VQ success during both emergency queen replacement and reproductive swarming. The observed relationships between VQ behaviour and survival may therefore occur in all contexts in which new queens are produced.

Hybridization affected the aspects of VQ behaviour that we examined. When viewed over all queens combined, hybrid VQs piped at higher rates, eliminated more rivals and received more vibration signals than did purebred queens. However, the extent to which hybridization influenced queen success was strongly dependent upon colony matriline, as was evidenced by the pronounced matriline-by-patriline interactions observed for most of the factors investigated. In the European-matriline colonies, hybrid VQs with African paternity emerged sooner, piped more, destroyed more rivals, were vibrated at higher rates and were more likely to survive the elimination process than were their purebred, European-paternity sister queens. The earlier emergence of African-patriline VQs has been previously reported ([DeGrandi-Hoffman et al. 1998a](#)) and may reflect a shorter developmental time for African queens ([Fletcher 1978a](#)). Early emergence may provide African-paternity VQs more opportunities to destroy unemerged rivals still in queen cells ([DeGrandi-Hoffman et al. 1993](#)). The greater fighting ability of the African-patriline VQs did not result from increased size, because queens from the two patrilines were similar in weight. The increased piping activity and fighting success of the African-paternity VQs may therefore have reflected inherent behavioural differences between African- and European-paternity queens reared from the same European matriline. Alternatively (or additionally), the increased piping and fighting success of the African-patriline VQs may have been related to the greater vibration rates they experienced, because the vibration signal can elicit piping and may influence queen aggressive interactions ([Fletcher 1978b](#); [Schneider 1991](#); [Schneider et al. 2001](#)). Regardless of the specific mechanisms involved, our results suggest that for queens of European maternity, African paternity may convey a competitive advantage in hybrid colonies.

In contrast, in the African matriline colonies, paternity had no consistent influence on queen behaviour and survival. In the A-matriline colonies, purebred African-patriline VQs tended to emerge sooner, but hybrid European-paternity VQs showed more piping activity, had slightly greater fighting success and experienced greater relative vibration rates. However, none of these differences was significant. Regardless, queens from the two patrilines did not differ in the likelihood of surviving the elimination process. These data must be interpreted cautiously because of the relatively few queens examined and the marked variability in the behaviour of VQs in the A-matriline colonies. Nevertheless, our results suggest that the advantages associated with hybridization and African paternity observed for the European-maternity VQs did not occur, or occurred at much lower levels, in the A-matriline colonies.

There are at least three possible explanations for these matrilineal differences. First, the absence of a consistent African-patriline advantage in the A-matriline colonies could have resulted if African traits for queen characteristics were dominant. Dominance of African traits has been demonstrated for worker defensive behaviour ([Guzman-Novoa & Page 1994](#); [DeGrandi-Hoffman et al. 1998b](#)). If African traits for the examined queen

characteristics are also dominant, then in the A-matriline colonies all queens may have been African-like, because the genome of each was at least 50% African. If so, then the variability in fighting success, piping activity and vibration rates observed in the A-matriline colonies may have reflected behavioural differences between essentially African queens, rather than differences between patriline. In contrast, in the E-matriline colonies, VQs were either pure European or half African. A dominance of African traits would therefore have conveyed an advantage to only the African patriline queens.

Second, rather than a dominance of African traits, hybrid queens may have displayed overdominance for the behaviours examined. This hypothesis is consistent with the greater behavioural rates and increased fighting success observed when comparing all hybrid versus purebred queens. We may not have detected significant overdominance effects in the A-matriline colonies because of the relatively few VQs monitored. However, it seems unlikely that hybridization effects and limited sample sizes alone can fully account for our results. Overdominance was not exhibited for queen weight. Furthermore, we found consistent, highly significant differences between hybrid and purebred queens in the E-matriline colonies, despite the fact that we monitored even fewer VQs with European maternity (Table 1). Thus, even if overdominance occurred, advantages associated with African paternity were more pronounced than any general effects that may have arisen from hybridization per se, especially in the E-matriline colonies.

Third, the observed matrilineal differences could have been influenced by our methodology. In particular, our use of the *cd* colour marker could potentially have affected the outcome of queen replacement in two main ways. It has been suggested that the *cd* trait may convey slightly lower viability in drones under some circumstances (Tucker 1986; Berg et al. 1997). If the cordovan trait reduces queen viability, then the associations that we observed between European paternity, lower fighting ability and reduced survival in our E-matriline colonies may simply have been artefacts arising from the inferior quality of queens homozygous for the *cd* allele. No differences would have been observed in the A-matriline colonies, because the cordovan trait was not expressed in these queens. However, there is no known association between the cordovan trait and the viability of queens or workers (Taber & Wendel 1958; Tucker 1986; Schneider & DeGrandi-Hoffman 2002), and the subviability of *cd* drones has been questioned (DeGrandi-Hoffman et al., in press). The *cd* trait is a natural colour variant that occurs throughout a large geographical range (Tucker 1986; Page et al. 1991). Expression is not associated with inbreeding and there is no known linkage between the *cd* allele and any aspect of queen quality and behaviour (Tucker 1986; R. E. Page, Jr, personal communication). Bee breeders regularly produce cordovan queens for sale (Koehnen 1999; Glenn & Glenn 2001) and a recognized commercial value argues against decreased viability in *cd* bees. Our E-matriline colonies were outbred, and several were among the strongest that we maintained during our study. Indeed, our African- and European-paternity

queens did not differ in dry weight in either matriline, and in one E-matriline colony the surviving VQ was a pure *cd* queen. The cordovan trait was therefore not consistently associated with reduced queen size or success. Thus, there is no evidence that the lower fighting ability and survival of the European-patriline queens in our E-matriline colonies resulted from subviability associated with the cordovan coloration.

The *cd* marker may also have affected our results by influencing kin discrimination. Our colonies had only two patrilines, one of which contained the *cd* allele. Workers may preferentially interact with supersister queens (Page & Robinson 1990; Visscher 1998; Osborne & Oldroyd 1999), and the use of a limited number of patrilines may allow for artificially high levels of nepotism (Carlin & Frumhoff 1990; Breed et al. 1994). Furthermore, under such simplified colony genetic structure, the *cd* trait may actually promote patriline discrimination and contribute to the appearance of supersister preferences (Frumhoff 1991; Breed et al. 1994). Therefore, the differences that we observed between the A- and E-matriline colonies may not have reflected a patriline advantage per se, but rather an enhanced subfamily discrimination resulting from the greater expression of the *cd* trait in the E-matriline colonies. However, studies using the *cd* marker and two patrilines frequently give results on nepotism very similar to those that use other genetic markers and multiple patrilines (reviewed in Visscher 1998). The influence of the *cd* trait on kin discrimination and worker–queen interactions is therefore unclear. We found no consistent associations between the *cd* marker, relatedness and the tendency of workers to perform vibration signals on VQs. Indeed, the apparent kin preferences that we observed in our colonies may have arisen coincidentally from a preference by all workers to perform vibration signals on African-paternity VQs, regardless of relatedness. However, we cannot rule out possible viability and nepotism effects resulting from the *cd* marker at this time. Definitive conclusions will require the examination of queen rivalry in colonies containing multiple African and European patrilines that can be distinguished using molecular markers, although such an approach may make it difficult to conduct the detailed behavioural observations needed to fully assess the mechanisms of queen replacement (see Methods). The available evidence suggests that an influence of paternity provides the most parsimonious explanation for the observed competitive advantage for our African-patriline VQs.

The associations that we observed between the vibration signal and queen success are consistent with the hypothesis by Schneider et al. (2001) that workers use the signal to influence VQ aggressive interactions and the outcome of queen replacement. In the present study, VQs were often vibrated at high rates when approaching rivals, which elicited bursts of running that removed them from potential battles (see also Schneider 1991). Workers could therefore potentially use the signal to promote the survival of certain VQs by delaying their aggressive interactions until they have greater maturity and fighting ability. Our results suggest that the vibration

signal may have played an important role in the spread of the African honeybee, because in European-hybrid colonies the highest relative vibration rates were experienced by African-paternity queens. Furthermore, African-paternity workers were more likely to perform vibration signals on all VQs. Thus, any effect of the vibration signal on an African advantage in hybrid areas may be largely mediated by the behaviour of African-paternity workers.

Taken together, our results may help account for the loss of European traits that typically occurs when African bees invade areas containing resident European honeybee populations (Hall 1999; Hall & McMichael 2001). Initial matings between European queens and African drones may promote the inheritance of the European colonies by African-paternity VQs in subsequent queen replacement events. These hybrid VQs will then mate disproportionately with African drones, because (1) African colonies tend to reach high densities (Boreham & Roubik 1987; McNally & Schneider 1996), (2) drone production may be greater in African versus European nests (Winston 1992), and (3) offset mating periods between African and European drones may result in an African drone advantage (Rowell & Taylor 1988; Taylor & Rowell 1988). Each successive generation of the European-hybrid colonies will therefore experience an increasing introgression of African-paternity alleles from both queens and drones. Our colonies reared relatively few queens, so definite conclusions cannot be drawn at this time. Nevertheless, the available evidence suggests that an African-patriline advantage during queen rivalry may inevitably initiate a process that results in the loss of European-paternity alleles. In a related study (Schneider & DeGrandi-Hoffman 2002), we found that workers in hybrid colonies did not show patriline preferences when feeding and incubating developing queen larvae. Thus, an African-patriline advantage may not occur during queen rearing, but rather may be confined primarily to postemergence queen behaviour and worker-queen interactions.

Although our results suggest a mechanism for the disappearance of European patriline, they cannot explain the loss of European matriline that typically occurs in areas where African bees have migrated. Although European colonies in invaded areas may experience increasing introgression of African-paternity alleles, they should continue to express European mtDNA in successive generations of queens and workers. However, the loss of European matriline has been documented in numerous mtDNA studies (Hall & Muralidharan 1989; Smith 1991; Schneider & Hall 1997; Hall 1999). A variety of reasons have been suggested for this phenomenon, including a numerical advantage for African matriline (Page 1989; Smith 1991), European colony take-overs by African swarms (Danka et al. 1992; Vergara et al. 1993), and negative heterosis in European-African hybrid worker bees that might create a selective disadvantage in some environments (Harrison & Hall 1993; Schneider & Hall 1997; Hall & McMichael 2001). However, the relative importance of these different factors is poorly understood, and future research should focus on the specific

mechanisms that underlie the differential success of matriline in invaded regions.

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