Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis

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Abstract

Honey bee males and queens mate in mid air and can fly many kilometres on their nuptial flights. The conservation of native honey bees, such as the European black bee (*Apis mellifera mellifera*), therefore, requires large isolated areas to prevent hybridisation with other subspecies, such as *A. m. ligustica* or *A. m. carnica*, which may have been introduced by beekeepers. This study used DNA microsatellite markers to determine the mating range of *A. m. mellifera* in two adjacent semi-isolated valleys (Edale and Hope Valley) in the Peak District National Park, England, in order to assess their suitability for native honey bee conservation and as isolated mating locations. Three apiaries were set up in each valley, each containing 12 colonies headed by a virgin queen and 2 queenright drone producing hives. The virgin queens were allowed to mate naturally with drones from the hives we had set up and with drones from hives owned by local beekeepers. After mating, samples of worker larvae were taken from the 41 queens that mated successfully and genotyped at 11 DNA microsatellite loci. Paternity analyses were then carried out to determine mating distances and isolation. An average of 10.2 fathers were detected among the 16 worker progeny. After correction for non-detection and non-sampling errors, the mean effective mating frequency of the test queens was estimated to be 17.2, which is a normal figure for honey bees. Ninety percent of the matings occurred within a distance of 7.5 km, and fifty percent within 2.5 km. The maximal mating distance recorded was 15 km. Queens and drones did occasionally mate across the borders between the two valleys, showing that the dividing mountain ridge Losehill does not provide complete isolation. Nevertheless, in the most isolated part of Edale sixty percent of all matings were to drones from Edale hives. The large majority of observed mating distances fell within the range of Hope Valley, making this site a suitable location for the long term conservation of a breeding population of black bees.

Introduction

During the past few 100 years the distribution of honey bees, *Apis mellifera*, in Europe has been severely affected by man, particularly by the introduction and propagation of non-native subspecies (Ruttner 1988a). Honey bee mating is, contrary to other domesticated animals, very difficult to control, so that gene flow between honeybee subspecies is common (Franck et al. 1998; Garnery et al. 1998a, 1998b). This has resulted in hybridisation and introgression, or even the replacement of one subspecies by another. In Germany, massive importation of *A. m. carnica* has led to the almost complete replacement of native *A. m. mellifera* (Kauhausen-Keller and Keller 1994; Maul and Hähnle, 1994). We are only recently beginning to understand the consequences of this interference, which will increase genetic homogenisation and decrease natural diversity (Rhymer and Simberloff 1996; Olden et al. 2004).

Apis mellifera mellifera is the native subspecies in Britain and NW Europe, whereas other European subspecies have been introduced. The Italian subspecies *A. m. ligustica* was occasionally introduced to Britain as early as 1859 (Dews and Milner 1991; Cooper 1986). Mass importation into Britain started around 1915 following the loss of many colonies due to the "Isle of Wight" disease, which eliminated a large proportion of the native honey bee population. Several other subspecies, such as *A. m. carnica*, and *A. m. cecropia* have been and continue to be introduced. Greater honey production, quicker spring build up, lower swarming tendency, and lower defensiveness were some of the motivations for introduction (Ruttner 1988a). In addition, queens are often imported simply because they are cheaper or more available. However, beekeepers in Britain and NW Europe are becoming increasingly interested in conserving the native subspecies. Bee Improvement and Bee Breeders' Association (BIBBA) actively promotes the conservation of *A. m. mellifera*, especially in Britain and Ireland, while Societas Internationalus pro Conservatione *Apis mellifera mellifera* (SI-CAMM) has much of its membership in Scandinavia.

Conservation of native honey bee subspecies is desirable for conserving European biodiversity and this conservation is not incompatible with the desire of beekeepers to have better bees. Honey bee populations are variable and by selecting breeding stock beekeepers can favour desirable characteristics. For example, black bee colonies in Derbyshire, England, are highly variable in their defensivity (tendency to sting) (F.L.W. Ratnieks, personal observation). This trait is highly heritable making it practical for beekeepers to breed and keep native bees that are not highly defensive, and hence are easier to keep, especially in a country such as England with a high density of people. In addition, black honey bees almost certainly have characteristics that make them more suitable than other subspecies to the climate of NW Europe. Many beekeepers in northern England value the ability of black bees to survive in a climate with low summer temperatures and unsettled weather that frequently prevents foraging even in spring and summer. Another issue of the conservation is that in order to produce hybrid bees such as Buckfast bees, which some beekeepers claim to be more productive in respect to honey yield, it is necessary to keep some stocks or populations pure.

In 1997 the BIBBA began requeening hives owned by beekeepers in the Hope Valley in the Peak District, Derbyshire, England using *A. m. mellifera* queens that they had bred from stock obtained within the British Isles. The aim was to establish a geographically confined population of native honey bees, *A. m. mellifera* that would be semi-isolated from gene flow from neighbouring areas. The Hope Valley was chosen because it is of a suitable size (c. 12×6 km) and because it is surrounded by low mountains (up to 600 m) and moorlands. The mountains should provide some isolation, both by their height and windy tops and because they are unsuitable for keeping bees. Edale is a smaller valley (c. 6×3 km) leading into the Hope Valley (see Figure 1). In Edale one of us (FR) noticed that no honey bees could be seen on flowers, and local people confirmed that there were no beekeepers in the last decade. This strongly suggested that Edale had no honey bee colonies, either in hives or natural nests. Edale, therefore, had potential as an isolated mating site for selective breeding.

In honey bees the mating system is characterized by "drone congregation areas" that are visited by males from many colonies (Baudry et al. 1998). Virgin queens visit these on one to several nuptial flights, which typically take place in the second week of the queen's adult life (Woyke 1964), and
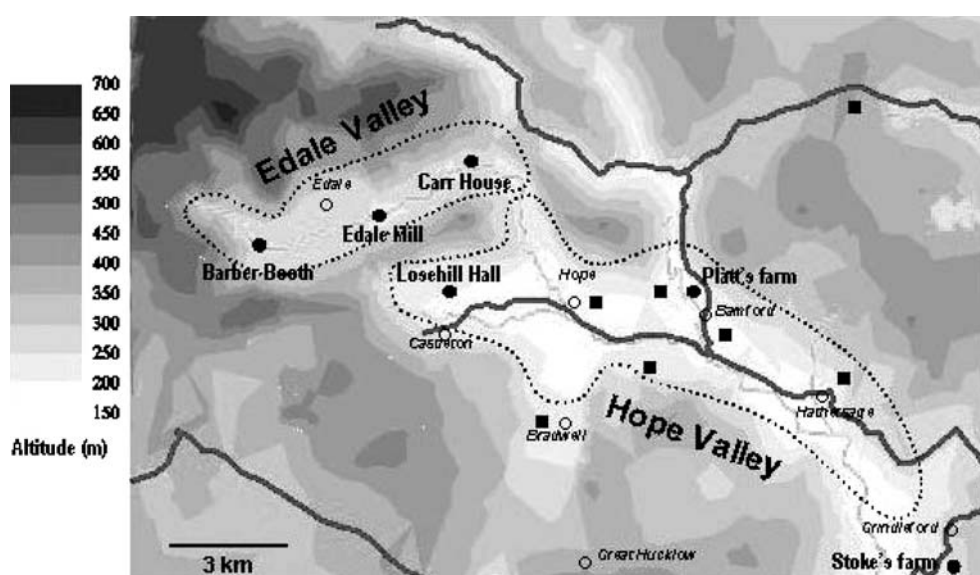
Figure 1. Map of Edale and Hope Valley, with the approximate areas indicated by dotted-line envelopes. Virgin queens flew from mating nucleus hives in six apiaries (•) and mated with drones from the same six apiaries and from hives belonging to local beekeepers (■). Villages are indicated by open circles and names.

mate with numerous drones (Ruttner 1988b). The degree of multiple mating (polyandry) is always high but varies between the different subspecies of *A. mellifera*, with *A. m. larmarckii* having the lowest mean observed numbers of mating at 5.0 and *A. m. capensis* the highest at 34.0 (Franck et al. 2000). The black honey bee *A. m. mellifera* has intermediate mating frequencies averaging 16.5 (Estoup et al. 1994; Kryger and Moritz 1997). Virgin queens normally fly 2–3 km, and drones further (Ruttner and Ruttner 1966; Böttcher 1975). The maximum recorded mating distance between a queen's hive and those of her mates is 17 km (Winston 1987). No studies have investigated the distribution of mating distances, so that it is unknown how exceptional this distance is.

Several new studies of honey bees in Europe have shown that most *A. m. mellifera* populations are threatened by hybridisation and introgression with other introduced honey bee subspecies (Garnery et al. 1998a, 1998b; Jensen et al. 2005). For the conservation of remaining *A. m. mellifera* populations it is thus important to gain information on mating distances and isolation at a local scale. The aim of the present study was to determine mating distances and isolation in Hope Valley and Edale in order to plan future conservation and controlled breeding activities. To do this we

used DNA microsatellite markers to determine the fathers of the worker progeny of queens that mated naturally in these valleys.

Materials and methods

Experimental design

The basic design was for virgin queens and drones to make mating flights from hives at different locations in the two semi-isolated valleys, so that a distribution of mating distances could be obtained by paternity analysis of worker progeny using DNA microsatellites. The paternity analysis used data on the genotypes of the worker progeny, the genotypes of their mothers (the mated test queens) and the genotypes of their possible fathers (the drone producing queens) (Figure 2). Male Hymenoptera are haploid and are produced by arrhenotokous parthenogenesis. As a result, the summation of offspring drone genotypes can be used to determine their mother queen's diploid genotype (Figure 2).

Experimental apiaries and their positioning

Six experimental apiaries were established; three in Hope Valley and three in Edale. In Hope Valley
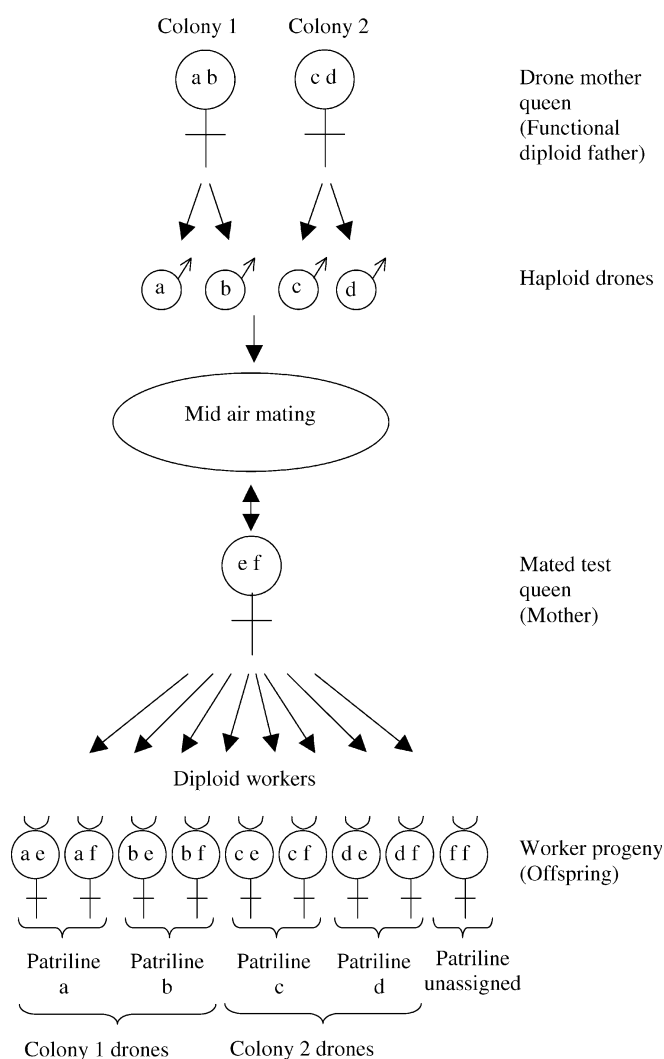
Figure 2. Transmission of genes in the experimental setup. The drone mother queens contributed their gametes to the worker progeny of the test queen via matings of their haploid sons. The genotype of each drone mother queen was deduced from the genotypes of pooled tissue from c. 10–20 drones per colony. The genotype of each mated test queen was deduced from her worker progeny. Subsequently the workers were assigned to a drone mother queen through paternity analysis.

the apiaries were approximately 4–6 km apart and placed in the west (Losehill Hall), centre (Platt's farm) and east (Stoke's farm) of the valley, respectively (Figure 1). In Edale the apiaries were approximately 2.5 km apart with the centrally located apiary at Edale Mill being about 2.5 km from the other two (Barber Booth to the west and Carr House to the east) and from the Losehill Hall apiary in Hope Valley. Within Edale matings could be achieved by level flying along the valley, whereas Losehill, a mountain ridge of c. 500 m, separates Losehill Hall from Edale. Since Edale is connected to Hope Valley at the village Hope (see

Figure 1), bees from Edale could still meet and mate with bees from Losehill Hall by level flying along that route, but would have to fly further. Each apiary contained 12 queen mating colonies and two drone producing colonies. In addition, two more apiaries were set up in Hope Valley each with just two drone-producing hives.

Drone producing and queen mating hive set up

Drone production was stimulated in the experimental apiaries by giving colonies frames of drone comb and sugar syrup. The numbers of drones

reared in each drone producing colony were estimated by photographing the drone comb approximately 4 weeks before mating took place. Samples of 10–20 drone pupae were taken from each colony for genetic analysis to determine the genotype of the drone mother queen.

Queen mating hives were set up with 4–6 frames of bees and brood but without any males or a queen. A marked virgin queen was then introduced into each mating hive using a mailing cage. The virgins were reared using standard queen rearing procedures (Laidlaw and Page 1997). All queens were sisters, reared from the same breeder colony, except for a few that developed as emergency queens from worker cells. The virgin queens were released from their cages at approximately 1 week of age and allowed to mate. Approximately 6 weeks later, worker pupae or larvae were sampled from each colony with a successfully mated queen and used for genetic analyses.

Local colonies

Beekeepers in the Hope Valley allowed us to inspect their colonies for drone production approximately 4 weeks before the experimental matings took place. Drone production was observed in 25 of the colonies inspected. Samples of 4–20 drone pupae were taken from all these colonies for genotyping and paternity assignment. The local beekeepers are organised in a club and cooperated fully with us, so that we believe to have a fairly complete sample of colonies in Hope Valley. We also obtained DNA of a single worker bee from all the colonies to estimate the background allele frequencies in the Valley. These estimates were applied for statistical inferences of the likelihood of deduced queen genotypes and for paternity analysis.

Genetic Analysis

Drone and worker samples

Equal amounts of tissue were taken from each drone from a given colony (a single leg from pupae or an equal amount of larval tissue) and DNA was extracted from the pooled tissue sample with the DNeasy tissue kit (QIAGEN, Inc., Santa Clara, California). Seventeen microsatellite loci (A7, A8, A24, A28, A43, A88, A113, Ap33, Ap36, Ap43, B124, A14, A76, A79, Ap218, Ap85, Ac11) were amplified and analysed for each of the 37 drone mother colonies according to standard procedures (Baudry et al. 1998; Solignac et al. 2003). The combination of the eleven most variable loci (Table 1) produced multilocus genotypes that could uniquely identify drones from each drone mother colony. DNA was also extracted from 16 immature workers from each mated test queen using the Chelex® extraction technique (Walsh et al. 1991). The same 11 microsatellite loci were amplified and analysed for each of these workers.

Paternity analysis

Genotypes of each of the mated test queens were deduced from the genotypes of their 16 worker offspring using MateSoft Version 1.0 b (Moilanen et al. 2004), which analyse male-haplodiploid mating systems based on the expression of co-dominant genetic markers, such as DNA microsatellites. Because honeybee queens mate multiply, MateSoft's "broad deduction" method was chosen. MateSoft calculates the weighted probabilities of all possible queen genotypes, based on the observed allele frequencies in the population. At any locus the queen genotype may be determined unambiguously or there may be several alternative possibilities. When the analysis indicated several possible queen genotypes, we only used the loci where the weighted probabilities of the most likely genotype were above 0.80.

Worker offspring were assigned to drone mother queens using the likelihood-based method in Cervus 2.0 (Marshall et al. 1998), a software package which performs large-scale parentage analysis in diplo-diploid mating systems using co-dominant genetic markers. To overcome complications due to haplodiploidy, worker progeny were assigned to drone mother queens rather than to the father drones themselves, because drones can be regarded as the flying gametes of a queen, each drone producing clonal sperm. We assumed a genotyping error rate across all loci and individuals of 1% and a sampling coverage of 95% of the candidate parents (drone mother queens), thus allowing for the possibility that there were additional colonies we did not know about. Statistical confidence limits of the most likely parental assignments were obtained from the difference in

Table 1. The 11 DNA microsatellite markers used for paternity analysis and the locus-specific $MgCl_2$ concentration, annealing temperature (T_a), number and size range of the alleles, expected heterozygosity (H_e), average exclusion probabilities for a single parent (Exclusion 1) and a second parent when the first parent is known (Exclusion 2)

Locus	[$MgCl_2$]	T_a (C)	No. alleles	Size range(bp)	H_e	Exclusion 1	Exclusion 2
A7[a]	1.2 mM	55	8	105–126	0.601	0.211	0.386
A113[b]	1.2 mM	55	11	200–236	0.429	0.105	0.266
Ap43[c]	1.2 mM	55	6	134–149	0.638	0.220	0.368
A85[d]	1.5 mM	55	7	190–202	0.740	0.348	0.530
B124[a]	1.5 mM	55	13	216–250	0.887	0.627	0.772
A76[a]	1.2 mM	60	24	209–315	0.896	0.660	0.795
A79[c]	1.2 mM	60	9	91–118	0.515	0.146	0.308
Ap36[c]	1.2 mM	55	11	141–169	0.860	0.601	0.752
Ap33[c]	1.2 mM	55	15	223–257	0.876	0.554	0.716
Ac11[d]	1.5 mM	55	10	111–129	0.781	0.404	0.582
A14[d]	1.5 mM	55	15	216–256	0.816	0.479	0.651
			Mean = 11.7			Combined = 0.99785	Combined = 0.99996

Average exclusion probabilities were obtained by summing individual exclusion probabilities across all combinations of genotypes, weighted by genotype frequencies. The combined exclusion probabilities across all loci represent the average probability of excluding a single randomly chosen unrelated individual from parentage.
Data are based on 734 individuals. The markers used were obtained from: [a] Estoup et al. 1994; [b] Estoup et al. (1995); [c] Baudry et al. (1998) and [d] Solignac et al. (2003).

the log likelihood of the most likely and the second most likely parent compared to a test statistic produced in a simulation model. Paternity assignment of particular worker offspring to a particular drone mother queen was, however, only accepted if it involved at most two mismatches between the putative drone mother queen (father), the mated test queen (mother) and the offspring genotypes.

Worker progeny from each mated test queen that were assigned to the same drone mother queen might originate from either the same drone or from brother drones. By subtracting the mated test queen's own genotype we were able to group sibling offspring into patrilines (Figure 2). We thus estimated the observed mating frequency of each mated queen by the number of patrilines detected in her offspring sample. The effective mating frequency of each mated test queen was calculated with a correction for finite sample size and unequal paternal contributions (paternity skew) (Pamilo 1993; Boomsma and Ratnieks 1996).

$$m_e = (n - 1)/\left(n \sum_{i=1}^{k} y_i^2 - 1\right)$$

where k is the number of patrilines observed, y_i is the observed proportion of the ith patriline, and n is the number of workers genotyped.

Results

Accuracy of paternity analysis

Our genetic analyses had great power. The probability of justifiably excluding a single randomly chosen unrelated drone mother queen from parentage at one or more loci was 99.75 when only data on the genotypes of the offspring worker and candidate parent (drone mother queen) were used. This rose to 99.99 when the deduced genotypes of the mated test queen were also used (Table 1). 595 (90.7%) of the genotyped offspring were assigned to the experimental or beekeeper-owned drone mother queens from Edale and Hope Valley with a confidence of $P > 0.80$, (548 with $P > 0.95$). 61 worker offspring (9.3%) could not be assigned to any of the known drone mother queens, and are thus most likely to have fathers from non-sampled hives belonging to beekeepers in the Hope Valley or from drones that had flown in from outside.

Non-assignable offspring were observed in 28 of the 41 mated test queens.

Mating frequency and mating distances

The 16 worker progeny from each of the 41 mated queens gave an average of 10.2 (SE ± 2.02) fathers detected per queen. The lowest recorded number of detected fathers was 5 and the highest 14 (Figure 3). As a result, the entire analysis is based on 418 confirmed matings in total. There was no significant difference between the mean number of observed matings per apiary in an unbalanced analysis of variance ($F_{5,35} = 0.12$, $P = 0.987$). The mean estimated effective paternity per queen was 17.2 (SE ± 10.9). We defined mating distance as the distance between the position of the drone producing hive and the position of the mating hive

hosting the mated queen. The maximum mating distance was approximately 15 km, which was observed in one queen. Most of the offspring resulted from mating distances of 7.5 km or less (Figure 4). Approximate one fifth of the matings occurred between queens and drones that originated from the same apiary.

Mating locations

More than half of the matings (53.8%) in the three apiaries in Edale took place with drones produced in Edale and two thirds (66.4%) of the matings in the three apiaries in Hope Valley took place with drones produced in Hope Valley (Table 2). Overall, approximately 80% of all matings of Edale queens took place with drones produced in Edale and the two immediately
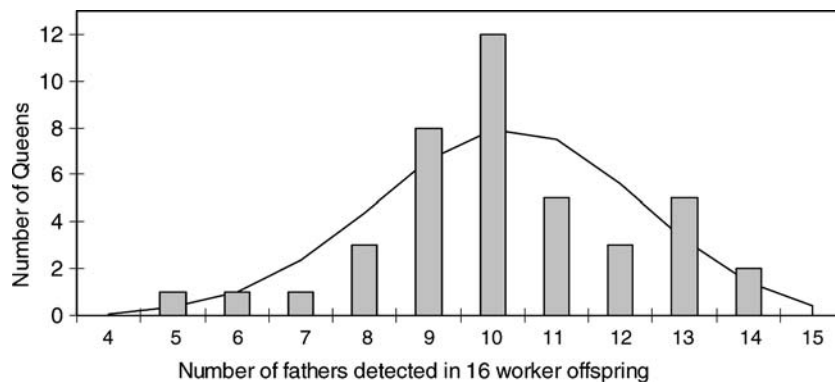

Figure 3. Distribution of observed number of matings of test queens based on genetic analysis of 16 worker offspring per queen. The curve is a fitted binominal distribution.
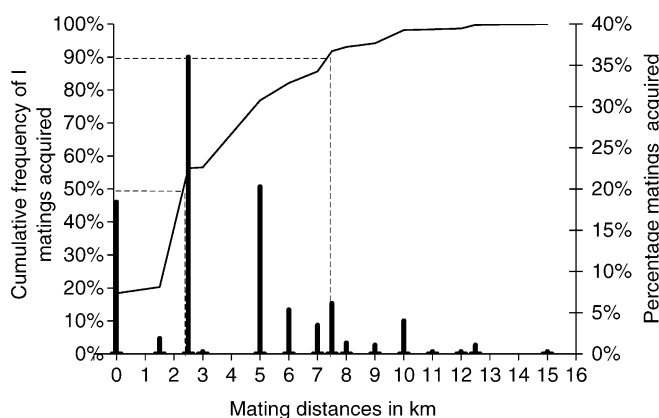

Figure 4. Distributions of mating distances. The cumulative distribution of mating distances is plotted as the curve, whereas the bars indicate the separate percentages of matings obtained at specific mating distances. Ninety percent of the offspring resulted from mating distances of 7.5 km or less and half of the offspring from mating distances of 2.5 km or less (see dotted lines).

Table 2. Proportion of matings according to location (see Figure 1) of the drone mother colonies

Drone mother colony locations	Queen mating apiaries in Edale			All Edale
	Barber Booth	Edale Mill	Carr House	
Edale	60.00%	54.29%	49.14%	53.87%
Hope Valley	40.00%	40.00%	37.93%	39.11%
Edale, Hope, Losehill Hall	85.88%	81.43%	71.55%	78.60%
Unknown	0.00%	5.71%	12.93%	7.01%
Drone mother colony locations	Queen mating apiaries in Hope Valley			All Hope Valley
	Losehil Hall	Platt's farm	Stoke's farm	
Edale	19.75%	7.14%	10.34%	14.50%
Hope Valley	61.73%	78.57%	62.07%	66.40%
Unknown	18.52%	14.29%	27.59%	19.10%

adjacent Hope Valley locations, Losehill Hall (9%) and Hope (16%). This shows that Edale is relatively but not fully isolated. In addition, the proportion of matings in Edale that could not be assigned to a known drone mother queens increased from zero in the most westerly apiary, Barber Booth, to 13% when moving east in the direction of Hope.

A higher proportion of matings from Hope Valley (19%) could not be assigned to known drone mother queens compared to the matings in Edale (7%) (Table 2). The two Edale apiaries in the middle (Edale Mill) and west (Barber Booth) both had queens that only mated with drones from Edale hives, whereas all queens from the eastern Edale apiary, Carr House, had mated with at least one drone originating outside Edale. In the three Hope Valley apiaries some of the queens had mated with drones originating from Edale, especially at the western most apiary Losehill Hall where two thirds of the queens had mated with at least one drone from Edale. This suggests that gene flow occurs both ways between the two valleys and that drones are able to fly over Losehill, the mountain directly between the Losehill Hall apiary and the Edale Mill apiary.

Discussion

The high resolution of the genetic markers and the large sample size of matings imply that our results present a clear picture of mating distances in two adjacent valleys. We were able to show that queens and drones from the two valleys do mate. The 11 microsatellite loci provided the necessary power to determine the origin of most of the drone fathers and to distinguish father drones that were brothers. The results were in good agreement with previous studies of mating frequency and mating distance in honey bees.

Polyandry

Polyandry is the rule in honey bees and the number of matings per queen is high. Several papers have discussed the evolutionary aspects of polyandry in honey bees and other social insects (e.g. Boomsma and Ratnieks 1996; Palmer and Oldroyd 2000). The current view is that polyandry increases the fitness of a queen through increased genetic variability among her worker offspring. Advantages of increased intra-colonial genetic variability may be improvements in social organisation and tolerance to environmental changes including pathogens.

Division of labour and reproduction greatly reduces the effective population sizes of social insects like honey bees, because a very small number of individuals produce all the offspring, while the large majority are non-reproducing workers that help their mother to raise siblings and maintain the colony (Crozier 1979). The number of colonies in a given honeybee population is therefore much closer to the effective population size than the actual numbers of bees. However, when queens are mated to multiple males, the effective population size increases considerably (Crozier and Page 1985), so

that high queen-mating frequencies are desirable in honey bee conservation.

Our estimate of queens mating with 17.2 drones is in close agreement with previous estimates for *A. m. mellifera* (on average 16.5) (Estoup et al. 1994; Kryger and Moritz 1997) and indicates that mating was normal. The mating frequency in our study might be slight overestimations due to the rather small number of offspring sampled, especially for colonies with high numbers of observed patrilines (Tarpy and Nielsen 2002). The location of apiaries can also have a significant effect on mating frequencies. In *A. m. carnica* the effective mating frequency was higher in a mainland apiary compared to an island apiary, where high wind speeds and relative low temperatures (15–20 °C) prevail during the mating flights (Neumann et al. 1999). It remains to be explored, however, whether native *A. m. mellifera* that have adapted to such harsh environments for thousands of years might perform better than *A. m. carnica*, which evolved in a continental environment.

Reproductive isolation

A considerable recent research effort has focused on locating and characterising the remaining populations of native black bees in Europe (Franck et al. 1998; Garnery et al. 1998a, 1998b; Jensen et al. 2005). Now that these efforts are becoming successful, maintaining stocks of native black bees becomes relevant. In some countries controlled breeding for certified bee breeders takes place on small islands. In Denmark, for example, such locations have to be approved every year by the Danish Plant Directorate (Anonymus 1993). However, island-based isolated mating stations are not practical in all countries, and controlled breeding is often achieved by creating mating stations isolated by distance or topography such as mountain valleys (Ruttner 1988b), so that detailed information on mating distances becomes important. In the present study the mating distances were mostly below 8 km. Peer and Farrrar (1956) observed mating distances of 9–10 km by using cordovan queens and cordovan drones even though wild type drones were abundantly available at shorter distances. (Cordovan is a single-locus recessive body-colour marker). The maximal mating distance recorded in our study was approximately 15 km, which is in accord with other biological observations (Klatt 1929, 1932; Peer 1957).

Edale, which was free of honey bee colonies prior to our experiment, is semi-isolated in terms of honey bee mating. As expected, the geographically most isolated apiary, Barber Booth in western Edale, which is surrounded on three sides by inhospitable mountains and moorlands, was the most isolated location in terms of honey bee mating. An increasing proportion of matings to drones originating from outside Edale was observed further down the valley in the direction of Hope, where several local beekeepers live. Edale queens also mated with drones from Losehill Hall, and vice versa, showing that Losehill Mountain does not provide complete reproductive isolation. Queens, drones or both were apparently able to fly over or around this 500 m high mountain (but rising only c. 300 m from the valley bottoms), since quite many colonies contained offspring of fathers from both sides of Losehill. This corroborates a study by Ruttner (1976) that used a colour mutant and marked drones to show that they were able to overcome differences in altitude of 500 m or more and return to their hives. This indicates that even considerable differences in altitude are not sufficient to provide complete mating isolation, and that other factors such as the overall topography of the terrain and the local climate also play a role.

About one fifth of the queen matings in Hope Valley were to drones from unknown drone mother queens suggesting that significant gene flow from the surrounding areas is unavoidable even in fairly well isolated valleys. However, the actual proportion of outside matings is almost certainly lower as the limited number of drones in some of the samples from beekeeper-owned colonies may have resulted in undetected alleles in their mothers. This implies that some of the unassigned offspring might in fact have been offspring of these drone mothers. A recent method of genotyping live queens from small pieces of wing tip (c. 2 mm^2) (Châline et al. 2004) would eliminate problems with undetected queen alleles and could be used to determine the genotypes of drone mother queens and mated test queens directly instead of having to infer them from samples of progeny. In addition, there may have been some beekeeper-owned colonies that we were not aware during our sampling.

Conservation implications

Over the last century the number of beekeepers in NW Europe and thus the number of honey bee colonies, in particular *A. m. mellifera*, has decreased significantly. Wild colonies are rare because very few old hollow trees have remained standing in modern landscapes and because Varroa mite infections tend to be fatal for untreated colonies. The maintenance of populations of native black honey bees thus relies on active cooperation with beekeepers, as all beekeepers in a certain area need to be comply with keeping native honey bees only to maximize the success of conservation efforts. A case in point illustrating such an active cooperative conservation effort is the requeening program of the honey bee population in Hope Valley that was initiated by BIBBA.

Our present results show that the effective mating distance is similar to the size of the Hope Valley, confirming that this area is a reasonable location for maintaining a panmictic and relatively pure population of black honey bees. It will be necessary, however, to continue the conservation programme in the entire area of the valley and to further reduce hybridisation with imported bees, such as the yellow Italian bee *A. m. ligustica*, if necessary by requeening.

Several studies have investigated the effect of commercial honeybees on the native fauna of other, annual and often solitary bees. Recently Forup and Memmott (2005) showed a negative association between bumblebee and honeybee abundance, but no apparent effect of honeybee density on bumblebee diversity. So, although there might be competition between honeybees and other bees, the ultimate effect of these interactions are as yet unclear. However, it is prudent to also take the presence of other endangered bees and non-bee pollinators into consideration when designing new potential *A. m. mellifera* reserves.

Conservation and improvement of native honey bee populations is a challenge due to the spectacular open-mating system of honey bees. The information obtained in the present study is, therefore, important for evaluating the status and improvement of both new and existing reserves for native honey bees. More specifically it appears that Hope Valley and Edale can play complementary roles in the conservation of *A. m. mellifera* in Britain through, respectively, their suitability for maintaining a large breeding population, and the possibility for controlled matings.

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