Genetic Characterization of Commercial Honey Bee (Hymenoptera: Apidae) Populations in the United States by Using Mitochondrial and Microsatellite Markers

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Ann. Entomol. Soc. Am. 102(4): 666–673 (2009)

ABSTRACT Genetic diversity levels within and between the two commercial breeding areas in the United States were analyzed using the DraI restriction fragment length polymorphism of the COI-COII mitochondrial region and 10 polymorphic microsatellite loci. The western commercial breeding population (WCBP) and the southeastern commercial breeding population (SCBP) were sampled in 1993–1994 and again in 2004–2005. The goal of this study was to characterize the genetic composition of these populations and to measure potential changes in genetic diversity and composition across the sampling period. The mitochondrial DNA haplotypes C1 and C2, characteristic of the most popular bee strains (Italians and Carniolans, respectively) sold in the United States, were the dominant haplotypes at both sample dates. The frequency of *Apis mellifera mellifera* M haplotypes, M4, M7, and M7′, decreased during the 10-yr span. An A1 haplotype characteristic of Africanized bees was found in the SCBP from 2005. Microsatellite analysis showed there was a loss of alleles in both the WCBP and SCBP, but the losses were not significant due to simultaneous gains of new alleles into these populations between 1993 and 2005. Genetic differences that occurred between the 1993–1994 WCBP and SCBP were still detectable in these populations sampled a decade later, suggesting that these populations could be useful sources of diversity for each other in the future.

KEY WORDS genetic diversity, *Apis mellifera*, commercial populations, mitochondrial DNA, microsatellites

Honey bees are the most economically valuable pollinators of agricultural crops worldwide. One third of the total human diet is dependent on plants pollinated by insects, predominately honey bees (McGregor 1976). In North America, honey bees pollinate >90 crops, worth ≈14.6 billion dollars annually (Morse and Calderone 2000). Production of almonds in California is a $2 billion dollar enterprise (Council 2007) and is almost entirely dependent upon honey bees. However, the honey bee, *Apis mellifera* L., is not native to the Americas. Across an endemic range of Europe, Africa and western and central Asia, the honey bee evolved and adapted to a large variety of climatic and ecological conditions. Currently, 26 different subspecies are recognized within the endemic range, with classification based on morphology (Ruttner 1992, Sheppard et al. 1997, Sheppard and Meixner 2003). From this large pool of honey bee subspecies, a modest subset was exported to North America (Sheppard 1989a, 1989b).

The first recorded importation occurred in 1622, when the Virginia Company sent ships full of seeds, fruit trees and various animals, including bees (Horn 2005). The majority of honey bee importations occurred between 1859 and 1922. In 1922, in response to the discovery of a parasitic honey bee mite in Europe, a law was passed that restricted the importation of adult honey bees into the United States (Sheppard 1989a, 1989b). Between 1859 and 1891 seven additional subspecies were brought into the United States. However, only two subspecies were favored by the beekeeping community (*A. m. carnica*, and *A. m. ligustica*) and remain available as selected strains today in the United States.

The majority of the honey bee queens produced and sold in the United States come from two geographically distinct commercial queen-producing regions, each contributing roughly equal numbers of queens for sale. The western queen-producing region is primarily located in central California, with some operations in southern California (Fig. 1). The second major queen-producing area is in the southeastern United States, with the majority of operations located from Florida through to Texas (Fig. 1).

Commercial honey bee breeding populations are maintained by queen producers who typically select for traits desirable to apiculture, including honey produc-

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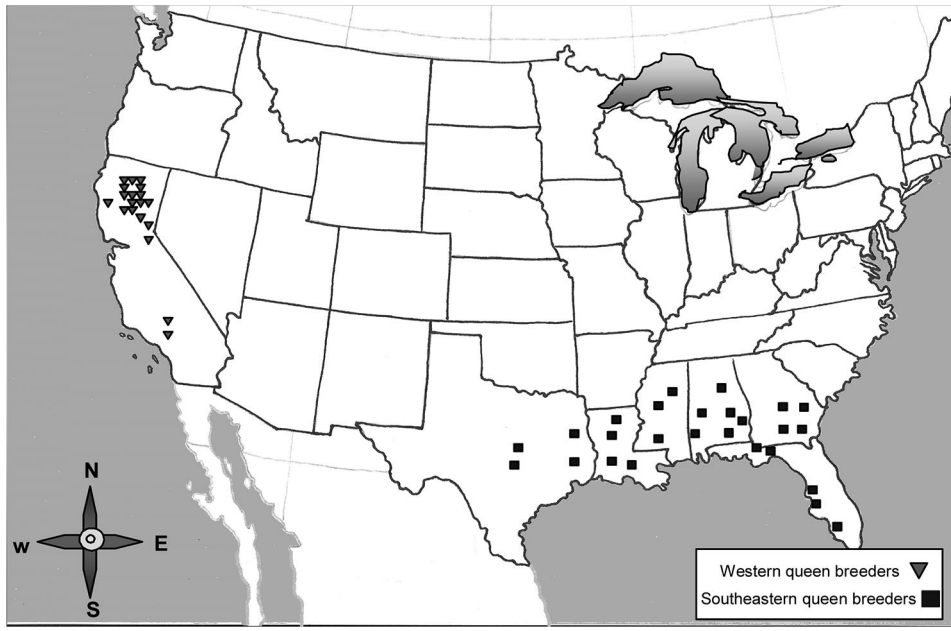


Fig. 1. A map of the two distinct commercial queen breeding regions of the United States showing collection sites from 2004–2005.

tion, colony growth, colony survivorship and temperament. Coloration is also a major criterion used for the selection of different strains (D.A.D., unpublished data).

As a whole, the honey bee-breeding industry uses a small number of queen mothers (<600) to produce nearly 1 million replacement queens for beekeepers in the United States (Schiff and Sheppard 1995, 1996). The genetic basis for these replacement queens is determined by the number of queen mothers used by queen producers and the total genetic diversity of males to which they mate.

Queen producers from the western portion of the U.S. market two main strains of queens, the Italian honey bee originally derived from *A. m. ligustica*, and the Carniolan honey bee, derived from *A. m. carnica.* Due to the open aerial mating behavior of honey bees, the queens produced are some admixture of the different gene pools that are present at these central California sites.

Queen producers in the southeast also market *A. m. ligustica* and *A. m. carnica.* However, the presence of feral populations may have influenced the genetic make up of this southeastern population (Schiff and Sheppard 1995). Genetic remnants of *A. m. mellifera*, the "dark bee" from northern Europe, were still present in the southeastern managed population in 1993 in low frequency, reflecting contributions from the feral population. Schiff et al. (1994) found that >30% of the mitochondrial DNA (mtDNA) in the feral population from this region could be attributed to *A. m. mellifera.* Thus, in the early 1990s, the feral population represented a "gene pool" somewhat separate from the commercial population, reflecting the historical introduction and spread of *A. m. mellifera.*

In 1987, a mite, *Varroa destructor* Anderson & Trueman, parasitic on honey bees, became established in the United States and rapidly spread (Wenner and Bushing 1996). Untreated colonies often died within 2 yr and feral populations were, for the most part, eliminated (Ritter 1988, Sanford 2001, Seeley 2007). Large annual winter losses of managed colonies also occurred due to this mite, although beekeepers could usually recover numbers by making colony splits in the spring (Korpela et al. 1992). The apparent mite-induced loss of the feral honey bee population and mite-associated overwintering losses of managed colonies may have reduced the genetic diversity of U.S. honey bee populations, through bottleneck effects and genetic drift.

Here, we address three main questions: 1) Was there a change in genetic diversity in the western commercial breeding population (WCBP) and southeastern commercial breeding population (SCBP) during the past decade? 2) Do genetic differences that were detected >10 yr ago between the WCBP and SCBP still remain? 3) Did the disappearance of feral populations disproportionately affect the SCBP? Queen producer populations were chosen for this study, because these operations annually produce 1 million daughter queens, an amount sufficient to requeen approximately one third of the estimated 3.2 million managed colonies in the United States (Council 2007).

Materials and Methods

Collection Protocol. *WCBP.* In 1994 samples of adult honey bee workers were collected from 178 colonies from 21 commercial apiaries in the western United States (Fig. 1) (Schiff and Sheppard 1996). A sample

consisted of 200–300 workers from each queen-mother colony. Of these 178 queen-mother colonies, 176 were reanalyzed in the current study. All samples were stored at −80°C until analysis. In May 2004, the western queen producing area was resampled, with 212 colonies sampled from fifteen queen-producing operations. Of these fifteen queen-producing operations, 11 had been sampled in 1993–1994. Several hundred adult workers were collected from each of the 212 queen-mother colonies and stored in 95% ethanol.

SCBP. In 1993, honey bee workers were collected from 185 colonies from 22 different queen producers in the southeastern United States (Fig. 1) (Schiff and Sheppard 1995). All samples were stored at −80C. Of these 185 colonies, 155 queen-mother colonies were reanalyzed in the current study. In June 2005, operations within the southeastern queen-producing region were resampled. Adult honey bee workers were collected from 132 queen-mother colonies from 20 queen-producing operations. Of these 20 operations, eleven had been sampled in 1993–1994. Two samples were also collected from a commercial beekeeper concerned with the temperament of a particular stock.

Sampled adult workers were daughters of the queen-mothers used by queen producers during the sampling season and represented the genetic stock currently being sold to beekeepers. After collection, the 2005 samples were stored in 95% ethanol. Survey data on the numbers of queens produced, the selection criteria and source of stock were gathered from each queen producer.

Molecular Analysis. Total DNA was extracted from half the thorax or the middle leg of a single adult honey bee worker from each queen-mother colony and placed in 150 μl of 10% chelex and 5 μl of proteinase K solution (Walsh et al. 1991). These samples were placed in a thermocycler for 1 h at 55°C, 15 min at 99°C, 1 min at 37°C, and 15 min at 99°C. The extracted DNA was stored at −80°C until used for polymerase chain reaction (PCR) amplification.

mtDNA analysis was performed on the intergenic region between the COI and COII genes. This region was amplified from one worker bee from each queen-mother colony by using PCR and primers E2 (5′-GGCAGAATAAGTGCATTG-3′) and H2 (5′-CAATATCATTGATGACC-3′) (Cornuet et al. 1991, Garnery et al. 1992). Each sample reaction was performed in 25 μl containing 2.7 μl of *Taq* buffer (Promega, Madison, WI), 1.5 mM $MgCl_2$, 25 nmol of each dNTP, 25 pmol of E2 and H2 primers, 0.001 mg of bovine serum albumin (BSA), 11.8 μl of distilled water, 0.6 U of *Taq* polymerase (Promega), and 1.25 μl of DNA extract. The conditions for PCR amplification were 30 cycles at 92°C for 30 s, 1.5 min at 47°C, and 2 min at 63°C.

To determine the size of the amplified region, a 6-μl aliquot of PCR product was electrophoresed on a 1.4% agarose gel. Amplified fragments were visualized for size scoring using ethidium bromide. The remaining 19 μl from each sample were digested for 6 h at 37°C with 4 U of restriction enzyme DraI. The restricted DNA fragments were separated on a 10% acrylamide gel and stained with ethidium bromide. Fragment sizes were estimated, and mtDNA haplotype names within honey bee "mitochondrial lineages" were assigned to each sample (Garnery et al. 1993). The DraI test differentiates up to 50 distinct mtDNA haplotypes within the three mitochondrial lineages of *A. mellifera* (Franck et al. 2000). One-way analysis of molecular variance (AMOVA) was performed among and within populations and pairwise Fst values for each population and the overall Fst value were calculated using the software program ARLEQUIN 3.1 (Excoffier et al. 2005). Populations were grouped as four regions: SCBP93, SCBP05, WCBP94, and WCBP04. Chi-square tests were performed to ascertain changes in haplotype frequencies between sampling dates in WCBP of 1994 and 2004 and the SCBP of 1993 and 2005.

Ten variable microsatellite loci were characterized for all samples: A7, A24, A28, A88, A113, and B124 (Estoup et al. 1995); Ap43 (Garnery et al. 1998); and Ap55, Ap66, and Ap81 (Solignac et al. 2003). These loci have been shown to be good proxies for assessing genetic variation in honey bees at the population level. Amplification of the 10 loci was split into two multiplex reactions. Amplification of extracted DNA was performed using 10-μl PCR reactions containing 1× Promega reaction buffer and 1.5 U of *Taq* polymerase (Promega), 3 mM dNTP mixture, 1.0–4.0 mM of florescent dye-labeled primer, 0.001 mg of BSA, 1 μl of DNA, and a variable concentration of $MgCl_2$. The loci in plex 1, A24, A28, A88, Ap66, and B124, had a final concentration of 1.5 mM $MgCl_2$. The loci in plex 2, A7, A113, Ap43, Ap55, and Ap81, had a final concentration of 1.2 mM $MgCl_2$. All reactions were amplified at 95°C for one 7-min cycle, 30 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 30 s, and 72°C for a 60 min cycle. The amplifications were processed using a 3730 automatic sequencer (Applied Biosystems, Foster City, CA). Microsatellite fragment sizes were scored using GeneMapper software, version 3.5 (Applied Biosystems).

Allele frequencies and single locus (and multiple locus genotype) frequencies for each of the four populations (WCBP 1994, WCBP 2004, SCBP 1993, and SCBP 2005) were summarized in GENETIX (Belkhir et al. 2002). Allelic richness (a_g), which is calculated by adding the number of alleles seen in a population to the expected number, given the number of genes examined in the population and the allele frequencies observed over a set of populations, was calculated using HP-RARE 1.0 (Kalinowski 2005). The number of private alleles or alleles unique to a particular population, in each of the populations also was calculated in HP-RARE 1.0 (Kalinowski 2005). ARLEQUIN 3.1 (Excoffier et al. 2005) was used to calculate pairwise Fst values by locus and over all loci.

The number of alleles at each locus and the loss and gain of alleles at each locus across the two sampling periods were calculated from GENETIX (Belkhir et al. 2002) allele frequency data. Paired *t*-tests (SPSS Inc. Chicago, IL) on the number of alleles at each locus ($n = 10$) for each population were used to test for significant losses or gains in diversity between 1993–1994 and 2004–2005. Exact tests for Hardy–Weinberg (HW) equilibrium for each locus, genotypic linkage

Table 1. Mitochondrial mtDNA haplotype frequencies for the WCBP in 1994 and 2004

mtDNA haplotype for WCBP	WCBP 1994 (n = 176)	WCBP 2004 (n = 212)
C1	0.386	0.425
C2	0.517	0.574
C3	0.063	0
M3	0.028	0.011

n is the number of colonies sampled.

disequilibrium among loci, and genetic structure over all loci by using F statistics, were computed in GENEPOP, web version 3.4 (Raymond and Rousset 1995b). Population structure also was analyzed using a Bayesian model-based clustering method provided in STRUCTURE (Pritchard et al. 2000). This program infers population structure of populations by using allele frequencies of unlinked markers (microsatellites). The parameter set was programmed for independent alleles and individuals to have a mixed ancestry. The program was asked to place individuals from all four commercial populations into one, two, three, and four groups and the analyses consisted of 10^5 burn-in replicates and a run length of 10^5 replicates. The best estimate of K or number of populations was determined by looking at the values of log Pr(X/K) and the value of α.

Results

Collection Survey. In 1994, the 21 western queen breeders used 295 breeder queens to produce 406,800 queens. The mean production from each breeder queen was 1,379 daughters per queen (Schiff and Sheppard 1996). In 2004, the western queen breeders used fewer queen mothers (218) to produce 430,000 daughter queens. Thus, each breeder queen produced an average of 1,972 daughter queens.

The southeastern queen production numbers were similar. In 1993, the 22 southeastern queen breeders used 308 breeder queens to produce 483,900 queens for sale. On average, each breeder queen produced 1,571 daughter queens (Schiff and Sheppard 1996). In 2005, the southeastern queen breeders used 255 breeder queens to produce 439,500 marketable queens, for a mean production of 1,724 daughters/queen.

mtDNA. Three mtDNA haplotypes were detected in the WCBP sampled in 2004 (Table 1), and four mtDNA haplotypes were seen in samples collected a decade earlier. The most common mtDNA haplotype for both years was C2 (52% in 1994 and 57% in 2004). The haplotype frequencies were significantly different between 1994 and 2004 (χ^2 = 21.7, df = 4, P = 0.0002). The frequency of the C1 mtDNA haplotype increased during the 10-yr span from 38% in 1994 to 43% in 2004. The C3 mtDNA haplotype occurred in 6% of the sampled colonies in the 1994 WCBP but was not detected in 2004. The frequency of the M3 mtDNA haplotype was 3% in 1994 and 1% in 2004.

Six different mtDNA haplotypes (C1, C2, M3, M7, M7′, and A1) occurred in samples collected from the SCBP in 2005 (Table 2). Haplotype frequencies were significantly different between 1993 and 2005 in the SCBP (χ^2 = 17.4, df = 8, P = 0.03). Frequencies of mtDNA haplotypes from the two sampling periods (1993 and 2005) again show the dominant haplotype increasing: C1 increased from 45 to 56% from 1993 to 2005. The M3 and A1 mtDNA haplotypes were absent in 1993 but were seen at 1 and 2%, respectively, in the 2005 SCBP. The frequency of mtDNA haplotype C2 decreased from 47% in 1993 to 40% in 2005. In 1993, M7 and M7 mtDNA haplotypes made up 2 and 3% of the SCBP, respectively, whereas they both occurred in ≈1% frequency in the 2005 population. Two samples analyzed from a commercial beekeeping operation in the south had the A1 mtDNA haplotype.

Table 2. Mitochondrial mtDNA haplotype frequencies for the SCBP in 1993 and 2005

mtDNA haplotype	SCBP 1993 (n = 155)	SCBP 2005 (n = 132)
C1	0.445	0.568
C2	0.471	0.393
C3	0.026	0.00
M3	0.00	0.009
M4'	0.013	0.00
M7	0.019	0.009
M7'	0.026	0.009
A	0.00	0.015

n is the number of colonies sampled.

Based on mtDNA, Fst values were not significantly different from zero within the two regions WCBP (1993 vs. 2004, Fst = 0.003) and SCBP (1994 vs. 2005, Fst = 0.012). A higher level of genetic divergence was found between the SCBP of 2005 and the WCBP from 2004 (pairwise Fst values = 0.044). The Fst value is significantly different from zero (P = 0.001). The overall Fst value was 0.04 (P = 0.001). The analysis of molecular variance showed that most of the variation was from within populations (sum of squares = 334) rather than among populations (sum of squares = 11). The percentage of variation within populations was 96.2 in contrast to 3.8 among populations.

Microsatellites. *WCBP.* The number of alleles, mean number of alleles and private alleles are all estimates after rarefaction. Rarefaction is an approach used to produce comparable estimates of the number of alleles and number of private alleles in populations with varying sample sizes. In 1994, the number of alleles per locus ranged between 4.9 (Ap66) and 25.4 (A7) in the WCBP, and in 2004 the numbers of alleles per locus in the WCBP ranged from 5.5 (A88) to 18.5 (A7) (Table 3). The mean number of alleles per locus for the WCBP in 1994 was 12.8 and 11.5 in 2004. The mean expected heterozygosity values for the WCBP from 1994 and 2004 were 0.667 and 0.633, respectively. The mean observed heterozygosity values for the WCBP from 1994 and 2004 were 0.575 and 0.531, respectively. The number of private alleles per locus for 1994 and 2004 in the WCBP is given in Table 3. A single locus, Ap66, showed an increase in the number of private alleles in the west in 1994 from 0.00 to 6.01 in 2005.

Table 3. Number of alleles per locus (allelic richness) and number of private alleles per locus for the WCBP and SCBP (estimates based on rarefaction)

	WCBP 1994	WCBP 2004	SCBP 1993	SCBP 2005
Locus A24				
Allelic richness	7.53	7.40	5.36	6.41
Private alleles	1.21	1.09	0.86	1.91
Locus A28				
Allelic richness	6.67	5.48	3.42	4.72
Private alleles	3.29	2.10	0.34	1.65
Locus A88				
Allelic richness	7.30	5.55	5.94	8.04
Private alleles	1.91	0.16	0.41	2.52
Locus AP66				
Allelic richness	4.99	11.00	5.67	8.14
Private alleles	0.00	6.01	1.00	3.47
Locus B124				
Allelic richness	12.73	12.68	11.14	11.45
Private alleles	1.51	1.47	1.17	1.48
Locus A7				
Allelic richness	25.44	18.50	15.19	13.57
Private alleles	8.70	1.77	4.45	2.87
Locus AP113				
Allelic richness	11.32	10.30	10.70	10.38
Private alleles	2.74	1.71	1.49	1.18
Locus AP43				
Allelic richness	17.62	13.15	11.64	9.00
Private alleles	8.87	4.39	5.70	3.06
Locus AP55				
Allelic richness	13.94	12.18	10.08	11.09
Private alleles	1.79	0.04	0.95	1.97
Locus A81				
Allelic richness	15.06	9.04	5.42	5.98
Private alleles	7.82	1.79	2.11	2.66

However, reductions in the number of private alleles were found for nine out of 10 loci. Loci A7 and A81 experienced large losses of private alleles over the 10-yr span in the WCBP (8.70–1.77 for locus A7 and 7.82–1.79 for locus A81).

In the 1994 WCBP, 128 alleles in total were found in the 10 screened loci. Of the 128 alleles detected in 1994, only 92 were found in the samples taken in 2004 for the same population, a loss of 36 alleles. All of the 36 alleles lost during this sampling interval occurred in very low frequency (below 0.03) in 1994. Twenty-two "new" alleles were detected in the 2004 WCBP. In total, 114 alleles were detected in the 2004 WCBP. The decline from 128 to 114 total alleles was not significant based on a paired *t*-test (df = 9, $t = 0.3$, $P = 0.9$). Eight of the 22 alleles that were detected in the 2004 WCBP are known to occur in U.S. populations (feral 1980–1990, SCBP 1993). However, 14 alleles had not been previously detected in U.S. honey bee populations.

The allele frequencies in the WCBP differed between 1994 and 2004 and were highly significant in seven (A28, Ap66, B124, A7, Ap113, Ap43, and A81) of 10 loci, based on probability or Fisher exact tests (1995a). All pairs of populations across six loci (A28, Ap66, B124, Ap113, Ap43, and A81) had significantly different genotypic distributions between the two sampling dates, WCBP 1994 and 2004.

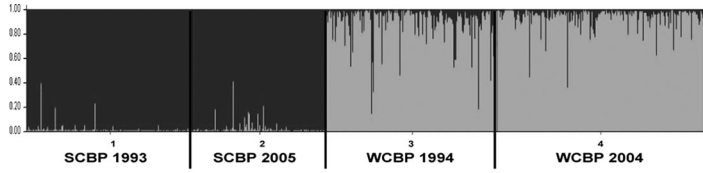
SCBP. In 1993, after rarefaction, the number of alleles per locus for the SCBP ranged from 3.4 (locus A28) to 15.2 (locus A7) (Table 3). In 2005, the number of alleles per locus for the SCBP varied from 4.7 (locus A28) to 13.6 (locus A7) (Table 3). The mean number of alleles per locus for 1993 and 2005 samples was 10.2 and 9.9, respectively. The mean expected and observed heterozygosity for all 1993 samples and loci was 0.64 and 0.57, respectively, and for the 2005 samples and loci 0.62 and 0.53, respectively.

In 1993, the SCBP had a total of 102 alleles. Of the 102 alleles detected in 1993, only 77 were found in the samples taken in 2005 for the same population. Several of the 25 lost alleles were found in very low frequencies (below 0.045) in 1993. Twenty-two new alleles were detected in the 2005 SCBP. In combination with the 77 previously known alleles, 99 alleles in total were detected in 2005 SCBP. Of those 22 alleles, 19 were present in other established U.S. honey bee populations (feral 1980–1990, WCBP 1994). Three alleles were unique to the 2005 SCBP, being undetected in previous populations. The SCBP experienced a significant gain in the number of alleles per locus from 1993 to 2005 (df = 9, $t = 0.02$, $P = 0.009$).

Allelic richness (corrected for population sizes) and the number of private alleles per locus for the SCBP are shown in Table 3. Seven of 10 loci gained more private alleles during the 10-yr sample period. Three loci showed a reduction in the number of private alleles, notably A7 and Ap43, which dropped from 4.5 to 2.9 and 5.7–3.1, respectively. The population differentiation test (Raymond and Rousset 1995a) indicated that genic differentiation occurred at six loci (A24, B124, Ap113, Ap43, Ap55 and A81) between the SCBP 1993 and 2005 populations. Genotype distributions also were found to be significantly different between the SCBP of 1993 and 2005 for the same six loci.

WCBP versus SCBP. The genic and genotypic differentiation tests (below) across all loci were significant when comparing WCBP and SCBP in both 1993–1994 and 2004–2005. The analysis for linkage disequilibrium found no significant deviation from equilibrium among the 45 locus pairs at the 5% level for any of the commercial populations. In both commercial populations (west and southeast) at both sampling times (1993–1994 and 2004–2005), significant departures from Hardy–Weinberg equilibrium were detected using the "exact HW test." The *U* test for heterozygote deficiency showed these deviations were caused by a deficiency of heterozygotes.

The output of the Bayesian-Markov assignment algorithm implemented in STRUCTURE supported the differences found in the allelic composition of the WCBP and SCBP. The probability of K = 2 was most strongly supported (LnP(D) = −21552, Var [LnP(D)] = 446.4 and a1 = 0.05) (Fig. 2a and b). Population pairwise Fst values ranged from 0.002 to 0.017 for the six unique pairwise comparisons. The within-region Fst values of 0.002 and 0.005 indicate little genetic divergence occurred within SCBP and WCBP, respectively, over time. The Fst values between the WCBP and SCBP had a 10% difference (0.012 in 1993–1994 and 0.017 in 2004–2005), indicating genetic differences between the two queen-pro-



Given population	Inferred cluster 1	Inferred cluster 2	Known population
1	0.983	0.017	SCBP1993
2	0.976	0.024	SCBP2005
3	0.107	0.893	WCBP1994
4	0.058	0.942	WCBP2004

Fig. 2. a). Bar plot produced by Bayesian based cluster analysis software STRUCTURE showing best fit, K = 2 populations. The SCBP and WCBP samples clustering into different populations. b). Proportion of membership of each pre-defined population in 2 clusters.

ducing areas still remained. Allele frequency data can be accessed by emailing D.A.D.

Discussion

For most plants and animals in agriculture, it is important to maintain adequate levels of genetic diversity, while selecting for desired traits. This equilibrium can be challenging to sustain. Genetic variation is crucial not only in a commercial breeding environment but also for survival in a dynamic natural environment. Increased levels of genetic diversity are correlated with increased longevity and overall genetic health of a species. The importation of only a few subspecies, reduction in commercial and feral populations due to *V. destructor*, and current queen breeding practices, are all events that may have reduced the genetic diversity of U.S. honey bee population during their history in North America. The neutral markers used in this study serve as proxies reflecting the overall diversity present in these populations.

mtDNA. In the early 1990s the production queens advertised for sale in the United States were mainly Italian, Carniolan, or Caucasian, with the exception of several commercial strains of variable genetic origin, such as Buckfast, Starline, and Yugo (all typified by C lineage mtDNA). This was supported by the mtDNA results for the 1994 WCBP and the SCBP in this study. Based on the 2004–2005 beekeeper interviews, 16 of 36 queen producers obtained their breeder stock from universities or government programs that have developed specific genetic lines. However, the majority of the queen mothers used by the industry were still from producer-maintained Italian and Carniolan stocks (D.A.D., unpublished data from beekeeper interviews). The reliance the beekeeping industry has put on the production of these two strains was mirrored by changes in the mitochondrial haplotypes frequencies, at least for the WCBP breeding population, in which both C1 and C2 haplotypes increased. However, the pooled mitochondrial variation in the two commercial breeding populations did not significantly change over the course of 10 yr. High frequencies of C haplotypes characterized the commercial populations produced in both regions. Differences within the two regions were characterized by losses of various M haplotypes that existed in low frequencies in WCBP and SCBP in 1993 and 1994 and the addition of an A haplotype found in SCBP in 2005.

In the WCBP, increases in frequency of the C1 (*A. m. ligustica*) and C2 (*A. m. carnica*) mtDNA haplotypes (Kraus et al. 2007) were evident over the 10-yr period between sampling. The loss of the C3 mtDNA haplotype was probably due to drift and the selective pressure to maintain these other two strains of honey bees. The presence of the M3 mtDNA haplotype typically found in western Europe and common in *A. m. mellifera* shows that genetic remnants from early introductions still remained in the WCBP in 1994 and the SCBP of 2005. However, the M3 haplotype frequency continued to decline as fewer queen breeders selected queen mothers from local stocks in 2004–2005, compared with 1993–1994.

The mtDNA results for the SCBP were similar to the WCBP. More M haplotypes were detected in the SCBP than in the WCBP, probably due to contributions from the once prevalent feral population that existed in that region. Several of these M haplotypes, M3, M7, and M7′, are known from western Europe, specifically the Iberian Peninsula (Franck et al. 1998, 2001). However, as in the WCBP, there was also a reduction of M haplotype patterns. The M haplotype made up 6% of the SCBP in 1993 and decreased to 1% in 2005 (Tables 1 and 2), presumably caused by reduced use of local stocks, greater reliance on university stocks or simply genetic drift due to using fewer queen mothers leading to reductions of uncommon haplotypes by chance.

The A1 haplotype (a haplotype associated with Africanized bees) pattern found in the SCBP signifies the presence of the African mitochondrial lineage in U.S. honey bee populations. Although the geographic origin of the A1 haplotype is still uncertain, it was reported that the frequency of A1 increased from south to north in Africa, forming a clinal gradient (Moritz et al. 1994, Garnery et al. 1995, Franck et al.

2001). This latitudinal gradient was supported by Collet et al. (2006)), who reported that the frequency of A1 increased toward northern and northeastern Brazil. Sheppard et al. (1999) reported that colonies from Argentina characterized by the A1 haplotype may have originated from the North African subspecies *Apis mellifera intermissa*, which was introduced into the United States ≈65 yr before the introduction of *Apis mellifera scutellata* to Brazil.

The presence of African mtDNA and nuclear DNA markers in the southern United States suggests that there has been introgression of Africanized alleles into U.S. honey bee populations (Whitfield et al. 2006; D.A.D. et al., unpublished data). When queen breeders use queen mothers with African mtDNA the dissemination of African haplotypes across the United States is likely. The queen-breeder operation that contained the A1 haplotype used eight breeder queens that year to make 6,000 daughter queens. Assuming that they all made equal numbers of daughters, the A1 breeder queen would have produced 750 A1 daughters for sale to beekeepers throughout the United States

Microsatellite DNA. There was no significant loss in overall genetic diversity in the WCBP or the SCBP over the 10-yr period of the survey. However, there was a change in the allelic composition of these two populations. WCBP and SCBP both lost alleles over the 10-yr span, which could be due to drift, sampling error or queen-breeding practices. However, the gain of new alleles into both of these populations due to sampling error or new sources, such as Africanized populations, Russian stocks, or other unknown importations, helped to compensate for the loss of alleles within these populations. Due to only a 50% sampling overlap of queen breeders between the two sample times (1993–1994 and 2004–2005), allelic losses and gains were tested for significance in the 11 overlapping operations by using paired *t*-tests. When only the data from the 11 queen-breeding operations that were sampled in both time periods were analyzed, there was no significant loss or gain in alleles in the WCBP between 1994 and 2004 or between both the WCBP and SCBP between 1993–1994 and 2004–2005. However, there was a significant gain ($P = 0.009$) in alleles in SCBP between 1993 and 2005.

Microsatellite data revealed no significant differences in the variance of allele frequencies between the two populations (WCBP and SCBP) based on pairwise Fst values (0.041 for 1993–1994 and 0.044 for 2004–2005), but pairwise Fst values were 10% higher in comparison to within population divergence (0.005 and 0.012) between 1993–1994 and 2004–2005. The software program STRUCTURE could best separate sampled individuals into two populations (Fig. 2). Significant allelic and genotypic differentiation between the WCBP and SCBP revealed that different amounts and combinations of alleles made up these two populations. Therefore, there were still differences in the genetic composition between the WCBP and SCBP after 10 yr. These differences may reflect the unique historical conditions under which the west and southeast incorporated their mix of original source populations and ongoing importations of Australian and Russian bee stocks. The lack of extensive E-W interchange between queen producers and the geographical barriers of the desert southwest may have played a role in retaining genetic distinctiveness between these two queen-breeding regions.

The WCBP experienced a greater loss of original alleles than the SCBP. Reductions in the amount of private alleles occurred in nine of 10 loci in the WCBP samples. Only three loci showed a reduction in the number of private alleles in the SCBP samples. The SCBP resilience to allelic loss might be attributed to exposure to the feral gene pool. The SCBP shared a higher proportion of alleles, (89%) with the once established feral populations compared with the WCBP (77%) (D.A.D. et al., unpublished data). Therefore, the historical presence and proximity of feral honey bee populations to the SCBP may have helped reduce the loss of alleles in this population. The arrival and presence of Africanized bees and other new honey bee stock in the southeast could also be responsible for the gain of alleles into the SCBP.

The overall genetic diversity of the WCBP and SCBP did not change over the 10-yr span. However, the allelic makeup of these populations did change. The WCBP and SCBP were genetically different from one another, and the SCBP actually gained alleles during the 10-yr span. These findings suggest that the WCBP and the SCBP could benefit from sharing germplasm to help maintain diversity. This research also suggests that the current queen production practices of producing 1,000,000 daughter queens from <500 queen mothers, is having little effect on genetic diversity due to drift and the inflow of new alleles. Without the arrival of alleles from Africanized honey bees, Australian packages, Russian stock and other imports there was a decline in the genetic diversity of WCBP. Therefore, the maintenance of adequate genetic diversity in U.S. commercial honey bee populations will probably depend on the future inflow of new alleles.

Acknowledgments

We thank the numerous queen breeders and beekeepers throughout the United States who participated in this project and opened their hives and doors to honey bee research. We are also grateful to the Washington State Beekeepers Association for grants to fund field and laboratory work. Deborah Delaney was supported in part by a fellowship from the Washington State University Center for Reproductive Biology.

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Received 25 November 2008; accepted 11 March 2009.