Environmental physiology of the invasion of the Americas by Africanized honeybees

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Synopsis The expansion of Africanized honeybees (AHB) through the Americas has been one of the most spectacular and best-studied invasions by a biotype. African and European honeybees (EHB) hybridize, but with time, tropical and subtropical American environments have become dominated by AHB that exhibit only 20–35% genetic contribution from western European bees, and a predominance of African behavioral and physiological traits. EHB persist in temperate environments. Clines between AHB and EHB exist in ecotones of South and Central America, and are forming in North America. What individual-level genetic, behavioral and physiological traits determine the relative success of the AHB as an invader in the neotropics, and of the EHB in temperate areas? Preference for pollen versus nectar may be an important trait mediating these ecological trade-offs, as preference for pollen enhances nutrient intake and brood production for the AHB in the tropics, while a relative preference for nectar enhances honey stores and winter survival for EHB. AHB exhibit morphological (higher thorax-to-body mass ratios) and physiological (higher thorax-specific metabolic rates) traits that may improve flight capacity, dispersal, mating success and foraging intake. Enhanced winter longevity, linked with higher hemolymph vitellogenin levels, may be a key factor improving winter survival of EHB. Data from South America and distributions of AHB in the southwestern United States suggest that AHB–EHB hybrids will extend 200 km north of regions with a January maximal temperatures of 15–16°C. The formation of biotypic clines between AHB and EHB represents a unique opportunity to examine mechanisms responsible for the range limit of invaders.

Introduction

Spread of Africanized honeybees (AHB) throughout the Americas represents one of the best-documented, spectacular and lethal examples of an invasion by a biotype. However, the AHB do not survive or displace European honeybees (EHB) at high latitudes or elevations. The mechanisms responsible for the competitive success of the AHB in the neotropics, and for EHB in temperate areas remain poorly understood. In this contribution, we review and provide new data on 3 topics related to the environmental physiology of the invasion by African bees. Firstly, we discuss the role of hybridization between AHB and EHB in the invasive process, and provide new data on this topic for a feral population in Arizona. Secondly, we review data regarding the eventual northern range limits of AHB in the United States. Finally, we consider specific behavioral and physiological traits that may contribute to ecological differences between AHB and EHB: foraging preference, longevity and flight capacity.

A brief history of the invasion

Honeybees are not native to the Americas. Over the past few centuries, beekeepers have introduced a variety of EHB strains (*Apis mellifera ligustica*, *A. m. carnica*, *A. m. mellifera*, *A. m. lamarckii*) and transported them widely throughout the Americas to use for honey production and pollination (Winston 1987). Escaping swarms from these domesticated hives established feral populations of honeybees beginning in the 1700s and 1800s (Winston 1987). These EHB, primarily derived from feral European populations adapted to temperate conditions, were successful in North American temperate and even desert habitats (Winston 1987; Loper and others 1999). However, EHB introduced into tropical areas for management exhibited poor survival and growth, impeding beekeeping and agriculture throughout much of Latin America.

In the 1940s, there were reports of very large honey harvests by beekeepers in Africa, prompting the Brazilian scientist Warwick Kerr to bring 57

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A. m. scutellata queens from Africa to Brazil in the 1950s. Virgin queens were distributed and swarms established local feral populations (Winston 1992b; Schneider and others 2004). Feral AHB reproduced and spread rapidly. Within 30 years, AHB had spread throughout the lowlands and lower latitudes of South America. In the next 11 years, they had spread across Central America and Mexico, entering Texas in 1990 and Arizona in 1994. In the 15 years AHB have been in the United States, expansion has slowed, but the bees have spread extensively throughout much of southwestern United States. (Winston 1992a; Winston 1992b; Schneider and others 2004).

Population genetic patterns in the African honeybee invasion

One of the primary ongoing questions regarding the AHB invasion is how the African and European genomes interact. There are 3 classic predicted outcomes when a genetically distinct population invades areas where there is an already established population. First, the invader (AHB) may completely replace the native (EHB). If the populations are sufficiently genetically divergent that hybrids are of low fitness, they are predicted to form geographically stable regions of hybridization, or tension zones. A "tension zone" shows a steep and smooth character transition (cline) from one parental type to the other, and is maintained by endogenous selection against hybrid genotypes and the continued dispersal of parental alleles into the zone (Barton and Hewitt 1984). As the dispersal distances of honeybees are large (100 km), a tension zone could span a broad distance. A third possibility is that the genomes of the parental species are highly compatible, and resulting hybrids may suffer little to no endogenous selection. Hybrid swarms occur when 2 populations interbreed freely, and hybridization produces admixtures with higher or equal fitness than either parental type. Hybrid superiority may emerge when a particular admixture results with a higher fitness than either parental type. At an invasion front a hybrid type may replace both parental populations, and/ or may disperse successfully into novel habitats not inhabited by parental types (Anderson and Hubricht 1938; Arnold 1997; Pinto and others 2005).

These 3 classic outcomes of an invasion become more complex when parental and hybrid fitness varies with environment, as clearly occurs for AHB and EHB. A cline may occur at an ecotone in which both parental and hybrid distributions are determined by environmental factors (Rand and Harrison 1989; Ross and Harrison 2002).

In tropical regions where the AHB has been present for decades, there has been strong directional introgression of the Africanized genome into previously European populations, so that the AHB mitotype has essentially replaced EHB mitotypes (Hall and Muralidharan 1989; Hall and Smith 1991). These data would support the assertion that AHB populations in this region have spread as continuous maternal lineages, and have essentially replaced the EHB (Hall and Muralidharan 1989; Smith and others 1989; Schneider and others 2004). However, compiled studies using nuclear markers indicate that these populations contain a significant (20–35%) genetic contribution from EHB, primarily *A. m. mellifera*, with a minimal (<5%) contribution from *A. m. ligustica* (Lobo and others 1989; Del Lama and others 1990; Suazo and others 1998; Schneider and others 2004; Clarke and others 2005). Possibly a large part of this hybridization occurred in the initial reproductive seasons of the invasion, when AHB queens had few AHB drones to mate with.

The data available support the formation of a stable hybrid between the AHB and EHB in the Americas, with a circa 70% African AHB spreading throughout tropical and subtropical environments, and environmental selection determining the distribution of parental and hybrid forms at ecotones. The persistent cline between AHB and EHB has been studied in South America, across transects in southern Brazil and Uruguay, using the allozymes malate dehydrogenase-1 (Mdh) and hexokinase as markers (Lobo and others 1989; Diniz and others 2003). Over ~200 km, the proportion of the fastest Mdh allele varies from a high of 75% in one of the Brazilian populations, to a low of 33% in southern Uruguay. Across the same transect, relative proportions of the AHB mitotypes varied from 100% in southern Brazil and northern Uruguay to 31 and 50% in 2 populations in southern Uruquay. Beyond 35°S, only EHB mitotypes are reported. There was no evidence for gametic disequilibrium between mitochondrial and nuclear genotypes (Diniz and others 2003), suggesting a panmictic population with fitness of hybrid and parental genotypes governed by environmental factors across an ecotone.

The fact that mtDNA of neotropical honeybees is near 100% African, while nuclear markers are 20–35% European, does suggest direct interactions that advance the African maternal lineage. One such mechanism is that AHB colonies occasionally usurp EHB colonies, directly invading and displacing them (reviewed by Schneider and others 2004). There is also evidence that AHB virgin queens are more likely to become the functional queen of a hybrid colony,

due to earlier emergence and worker interactions (DeGrandi-Hoffman and others 1998; Schneider and DeGrandi-Hoffman 2002).

Invasion of the Africanized genome into North American populations of EHB

In contrast to the tropical areas where Africanized bees were first introduced, the EHB has been extensively managed in the southern United States and in the Yucatan region of Mexico, and feral populations of EHB are more abundant. Reports of northward moving AHB populations in the United States and Mexico indicate that they remain primarily African, but with a stronger EHB genetic component than reported in South America and Central America. In southern Texas, mtDNA and microsatellite nuclear markers showed replacement of local EHB populations over a 5 year period with a hybrid population containing a 25–37% EHB genetic contribution (Pinto and others 2005). Feral colonies studied in the Yucatan 11 years after Africanization exhibit ~60% African mitochondrial and nuclear markers (Clarke and others 2005). Possibly the degree of Africanization in these regions will increase further with time.

We found a similar pattern for Africanized bees as they moved into Arizona. We monitored a feral population in southern Arizona (Oracle Junction) from 1992 through 1999. This period spans the first documentation of Africanized bees in the area in 1995 (Loper 1995, 1996; Loper and others 1999). This Sonoran desert habitat was well populated by EHB before Africanization, with a high of over 220 colonies in 1991. We sampled the population twice per year, by locating all observable colonies and checking all known sites where colonies had been located previously. Colonies in this habitat tend to nest in the same rock cavities, allowing us to reliably locate bees from year to year. To collect samples for genetic analyses, workers were captured at the colony entrance, placed on dry ice and quickly transferred to a −60°C freezer.

Two workers per colony were analyzed for mitotype. Total DNA was extracted using a 5% Chelex solution (Walsh and others 1991). We then amplified a 485 bp fragment of the cytochrome B locus by PCR and the resulting DNA fragment was digested with the BglII restriction endonuclease for 3 h at 37°C (Crozier and others 1991). Resulting fragments were electrophoresed in 1% agarose, and visualized with EtBr stain. European colonies (*A. m. mellifera* or *ligustica*) possess the BglII restriction site and result in 2 distinct fragments while African (*A. m. scutellata*) colonies lack the restriction site, resulting in a single band (Smith and others 1989).

Prior to 1995, we also routinely amplified and cut the COI region with XbaI, which cuts at a restriction site for *A. m. ligustica* but not *A. m. mellifera*. Our data indicated a high percentage of *A. m. mellifera* mitotype (over 70%) (Loper and others 1999). A small percentage of colonies were also identified as *A. m. lamarckii*, the Egyptian honeybee, which was likely brought in over a century ago and has not been used in any appreciable level for beekeeping. The presence of the *A. m. mellifera* and *A. m. lamarckii* mitotypes, and corresponding morphological evidence indicate that the population has been feral for an extensive period, with limited input from managed colonies (Loper and others 1999).

We additionally used allozyme electrophoresis to analyze Mdh allelic frequencies and to compare genotypic frequencies with Hardy–Weinberg expectations of genetic stability (Richardson and others 1982). We measured Mdh genotypes for ~10 workers per colony. As in previous studies, we differentiated 3 allelic types, designated Slow (Mdh^{65}), Medium (Mdh^{83}) and Fast (Mdh^{100}). In 4 sampling periods, from 1992, 1994 and in spring 1995 before Africanization, allelic frequencies within the population remained stable, at ~21% F, 30% M and 49% S (Loper and others 1999). Genotype distributions indicated the population was in Hardy–Weinberg equilibrium during that time.

African mtDNA first appeared in the feral Arizonan population in 1995, with the percentage of colonies exhibiting African mtDNA rising to ~74% by 1999 (Fig. 1). The data suggest that African mitochondrial frequencies were still rising but approaching an equilibrium level (Fig. 1). Consistent with this interpretation, a study in 2005 at another site in the Sonoran desert of Arizona reported African mtDNA in 86% of colonies (Rabe and others 2005).

The capture of the first AHB swarms within the area allowed us to characterize the genotypes of the Africanized colonies on the invasion front moving into Arizona. Eight of 35 captured swarms in spring 1995 had AHB haplotypes. The Mdh allelic frequencies within these swarms (60% F, 12% M and 28% S) were significantly different from established colonies and from EHB haplotype swarms. In contrast, EHB swarms had allelic frequencies (17% F, 23% M and 60% S) similar to established colonies (Fig. 2). The presence of ~40% medium and slow alleles in the AHB swarms suggests colonies on the swarm front were likely Africanized hybrids, rather than purely African types. This corresponds with data for Texas

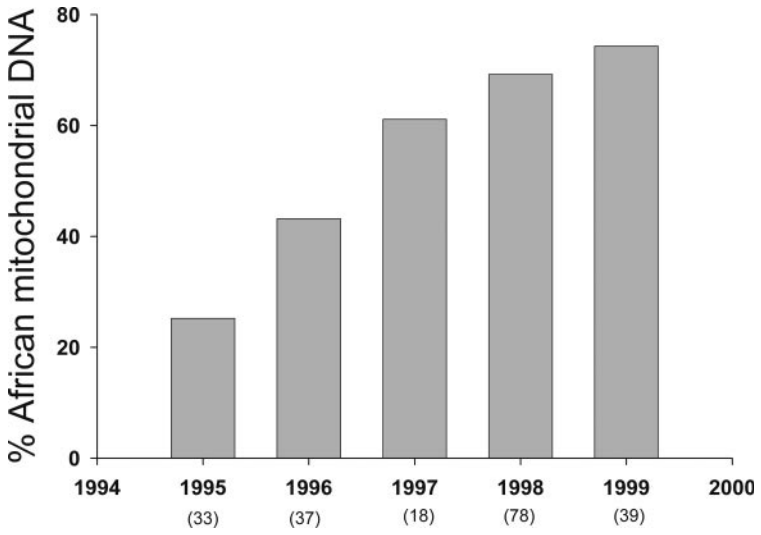

Fig. 1 Percent of the colonies of a feral Arizona honeybee population exhibiting African mtDNA. All colonies sampled from 1991 to 1994 had European mtDNA. From 1995 to 1999, the percentage of African mtDNA increased progressively. The number under each year indicates the number of colonies sampled that year.

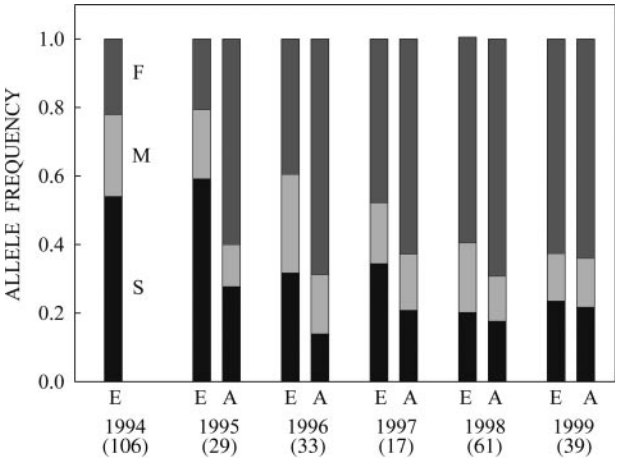

Fig. 2 Proportion of each of the 3 malate dehydrogenase (Mdh) allozymes for each mitotype (E = European, A = African) and year in a feral Arizonan population of honeybees. The number below the year indicates the number of colonies sampled that year; 6 workers were assayed per colony. European and African mitotypes differed strongly in their MDH allele at the beginning of the invasion (1995) but the equivalent MDH allele frequencies in E and A mitotypes by 1999 suggests a panmictic population.

(Pinto and others 2005) and the Yucatan (Clarke and others 2005).

Frequencies of Mdh alleles within the population began to change as the number of established colonies with AHB mitotypes increased (Fig. 2). In 1995 and 1996 colonies with AHB versus EHB mitotypes had very different distributions of Mdh alleles, indicating that established colonies with AHB and EHB were different subpopulations, and suggesting that Africanized colonies initially moved in and replaced European feral colonies (Fig. 2). Correspondingly, genotypic distributions within the population were statistically different from those expected for Hardy–Weinberg equilibrium (1995: $X^2 = 14.2$, $P < 0.02$, $n = 2026$; 1996: $X^2 = 369.2$, $P < 0.0001$, $n = 1145$). By 1997, however, the population was in Hardy–Weinberg equilibrium ($X^2 = 5.7$, $P = 0.34$, $n = 510$), and remained statistically stable through 1999 (1998: $X^2 = 1.88$, $P = 0.87$, $n = 1353$; 1999: $X^2 = 4.58$, $P = 0.47$, $n = 799$). In 1998 and 1999, Mdh markers had stabilized, with no further, statistically distinguishable changes. The population contained 74% African haplotypes and was in Hardy–Weinberg equilibrium for Mdh. At this time, Mdh allelic frequencies within the feral population were essentially indistinguishable from the Africanized bee swarms that first moved into the area in 1995. Africanization was rapid (2–3 years), and may have occurred even more rapidly in the swarm population than the resident colonies.

The transition to similar Mdh frequencies for both AHB and EHB haplotypes indicates maternal and paternal gene flow between the invading Africanized bees and the established European colonies, a similar result to that found in Texas (Pinto and others 2005). If Africanized colonies simply replaced EHB in this area, we would expect retention of differences in Mdh frequencies between the subpopulations, but the loss of EHB mitochondrial haplotypes. The lack of evidence for disequilibrium among nuclear and mtDNA genotypes suggest that a stable, AHB-dominated hybrid form now exists in this region.

Africanized bees were not the only invader into the population during this period. Corresponding with the appearance of Africanized bees, the population was also attacked by *Varroa* mites, causing a population crash in Fall 1995 through 1996 (Loper and others 1999). Likely, the presence of *Varroa* contributed to the genetic shift, but even before the crash the population had begun a rapid shift to Africanization (Loper and others 1999). Similarly, a crash in EHB colonies induced by mite attack is hypothesized to be a mechanism allowing rapid Africanization in Texas (Pinto and others 2005).

Overwintering and range limits of AHB

A question of general interest for any invasive species is where they will be able to exist in their new habitat. For an environmental physiologist, invasive biotypes such as the AHB provide a fascinating test-case for understanding the mechanisms that underlie animal range limits. Based on the population genetic data reviewed above, range limits for AHB and hybrid genotypes are likely set by a complex and variable suite of extrinsic factors, including both biotic (predation and diseases) and abiotic (for example, temperature and precipitation) factors (Kinlan and Hasting 2005). Range limits for invasive species can also be strongly influenced by hybridization and/or competition with local populations (Cox 2004), and this certainly is possible for AHB as they begin to encounter EHB populations adapted to their local environments. Feral EHB are smaller in more southern areas of California (Daley and others 1991), suggesting that honeybees exhibit either plastic or evolutionary responses to environmental variables that may influence range limits.

If abiotic factors alone determine the range limits of the AHB, then the eventual distribution of AHB in the United States should be predictable from the abiotic characteristics of AHB range limits in regions where they have existed for long periods. In Africa, *A. m. scutellata* is distributed throughout eastern and southern Africa, with their southern distribution limited by ocean (~34°), (Hepburn and Radloff 1998). In lowland regions of South America, AHB have been reported to not overwinter south of 34°S (Kerr and others 1982; Taylor and Spivak 1984). Africanized bees do not colonize at elevations above 2500 m in South Africa (Hepburn and Radloff 1998). In South America, *A. m. scutellata* are limited to the west by the Andes, and in South America and Central America do not occur at elevations above 3000 m (Kerr and others 1982; Lobo 1995).

Taylor and Spivak (1984) used the data of Kerr and colleagues (1982) to consider a variety of environmental factors that might determine the range limits of Africanized bees in South America, including minimum mean, and maximum temperatures during winter, and the number of frost-free days. Their analysis demonstrated that the number of frost-free days was, in fact, not a good predictor of Africanized bee distribution in South America, as this parameter would predict that AHB should extend far south of 34°S along the coast. The best predictor of locations where AHB could overwinter was for mean high temperatures to be above 16°C for the coldest month (July) (Taylor and Spivak 1984).

Taylor and Spivak (1984) combined the South American AHB distribution data with 1975 United States climate data to predict regions of the United States within which AHB would eventually saturate and be able to overwinter. Their analysis suggested that Africanized bees should extend throughout much of the southern half of the southeastern states of Louisiana, Alabama and Mississippi, and as far north as central California and North Carolina in coastal areas, but otherwise be limited to southern Texas and Arizona.

Temperatures in many regions of the United States have warmed considerably since 1975, the year used in the Taylor and Spivak (1984) analysis, so we reconsidered their model with new climatic data. If temperature is a major factor in determining AHB range limits, then interannual variation also may be important. We examined how the 16°C isotherm for maximal temperature in January, suggested as a predictor by Taylor and Spivak (1984), varied from 1995 to 2005 (the years since the AHB invasion), using climatic data from the National Oceanic and Atmospheric Administration. We plotted the most extreme years (2001 and 2005) as an indication of the range of variation in locations of the 16°C isotherm for January maxima (Fig. 3). These data suggest that such variation may amount to hundreds of kilometers in the northern range limit of AHB. Such interannual variation may produce a fuzzy range margin that varies across space and time. However, these data also suggest that studies that examine the effect of such yearly variation on the overwintering success of AHB in these ecotones may provide a powerful approach to an understanding of the factors that determine overwintering success of AHB.

A second approach to predicting the northern distribution of AHB in the United States was taken by Southwick and colleagues (1990), who used aspects of AHB behavior and physiology to estimate range limits.

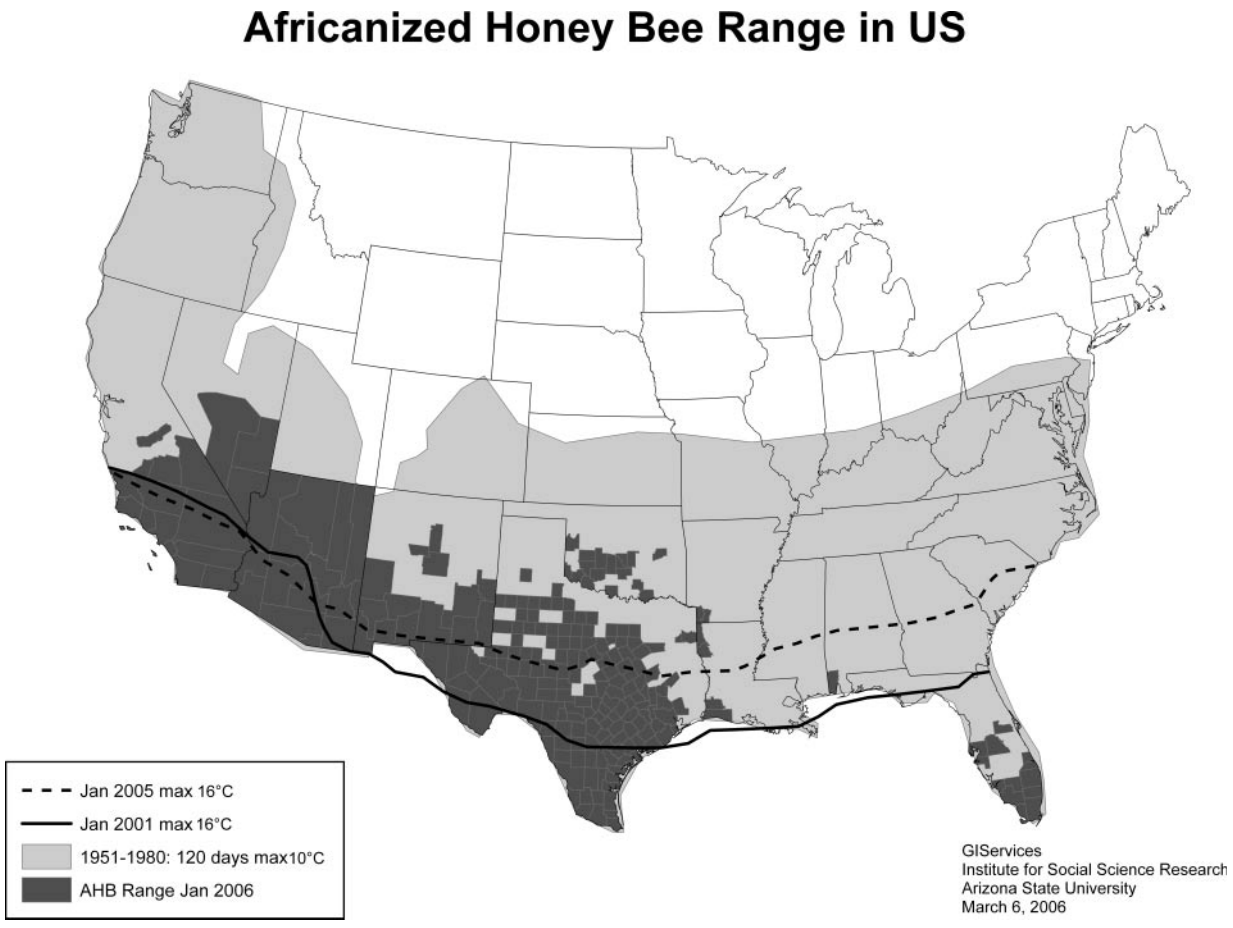

Fig. 3 Comparison of predicted overwintering limits of Africanized honeybees in the United States (Taylor and Spivak 1984; Southwick and others 1990) with reported locations (dark shaded) of Africanized bees as of January 2006 (www.stingshield.com). The 2 southern-most isotherms indicate lines below which January maximum temperatures exceeded 16°C during 2001 and 2005 (data from www.ocs.oregonstate.edu/prism/; plotted by Climate Diagnostics Data Management, National Oceanic and Atmospheric Administration, USA). These years represent the variation in 1995–2005, and thus provide a range of estimates of the northern-most locations where the AHB are predicted to be able to overwinter, based on the climatic analysis of the location of AHB in South America (Taylor and Spivak 1984). The light gray shading indicates locations experiencing more than 120 days of temperatures below 10°C, the range of AHB as predicted by (Southwick and others 1990).

First, they demonstrated that even small AHB clusters thermoregulated well and survived at air temperatures of −15°C for up to 15 h, showing that acute susceptibility to cold is unlikely to be a major factor in range limitation. They also demonstrated, however, that AHB required more energy to thermoregulate at the same air temperature, especially when in smaller clusters. The tendency for the AHB to store less honey (Rinderer and others 1985) combines synergistically with smaller colony size (Winston 1992a) and higher metabolic rate (Southwick and others 1990) to reduce the capacity of the AHB to survive long dearths. Laboratory studies have found that AHB colonies can survive ∼3 months in the cold without foraging (Dietz and others 1988; Villa and others 1991); similarly, studies of AHB at high elevations (2000–3000 m) in Columbia have shown that feral colonies emigrate or die after 4 months of poor foraging conditions (Villa 1987). Villa (1987) reported that AHB did little foraging on cloudy days with air temperatures below 10°C Based on these arguments, Southwick and colleagues (1990) suggested that the northern limit of the AHB in the United States could be estimated by a line that divides regions with more or fewer than 120 consecutive days with maximal temperatures below 10°C. From this prediction, AHB will eventually exist over the majority of the southern half of the United States and extend northward along the west coast to Canada, a dramatically greater range than predicted by Taylor and Spivak (light gray area in Fig. 3).

How do current distributions of the AHB in the United States compare with these predictions? AHB has been reported well north of the Taylor and Spivak

isotherms in the southwestern United States (Fig. 3). They now occur throughout Arizona, through the southern half of New Mexico, throughout most of Texas, and into Oklahoma and Nevada. Since the Taylor and Spivak isotherms predict overwintering regions, these more northern reports may reflect occurrence of migrating colonies that have flown north during the summer from areas where overwintering is possible. Migrating AHB colonies are believed to be able to disperse up to 100 km (Schneider 1995), and this approximates how far the most northern reports of AHB are from the 2005 line indicating a maximum temperature of 16°C in January (Fig. 3). Determination of locations of overwintering, rather than simply reports of AHB will be required to resolve this possibility.

AHB might be extending farther north in the southwestern United States than in South America due to human effects on the environment. Many AHB reports come from cities or agricultural areas, where urban heat islands elevate local temperatures and irrigation and exotic plants provide forage during what would naturally be a flowerless winter period.

Another factor that may move reports of AHB north of the predicted Taylor and Spivak (1984) line is hybridization. Recent measures show contributions of African genome that extend 200 km farther south into Uruguay than the 16°C isotherm of January maximal temperature (Diniz and others 2003). Approximately 20% of honeybees collected at 2 sites in the vicinity of Montevideo, Uruguay (35°S, 15°C January maximal temperature) had African mtDNA, a common diagnostic test for AHB. Our analysis of the available data is that the AHB will eventually extend ~200 km farther north than predicted by Taylor and Spivak (1984) for southwestern United States, but that these northern bees will exhibit decreasing proportions of African genetic, behavioral and physiological traits.

The fact that the northern range of AHB that Southwick and colleagues (1990) predicted far exceeds the distribution of AHB based on its known distribution in South America suggests that some factors involved in that estimate require reanalysis. One possibility is that honeybee foraging is limited at temperatures higher than the 10°C value used by Southwick and colleagues; however, AHB have been reported to have high foraging rates at temperatures of 8–10°C on sunny days at high elevations in Africa (Heinrich 1979). A more accurate analysis of the thermal limits on foraging would require examination of the heat budgets of AHB and EHB under appropriate convective cooling and insolation conditions. Alternatively, limits on resource availability rather than thermal limits on flight determine the length of the winter dearth period for honeybees. In addition, duration of survival of AHB colonies without food may be closer to 90 than the 120 days used by Southwick and colleagues in their analysis (Villa and others 1991).

In contrast to the steady spread of AHB in southwestern United States, the movement of AHB through southeastern United States has been slower than expected by either the Taylor and Spivak or Southwick and colleagues thermal models (Fig. 3). Since AHB are known to have high fitness in humid areas of South America, this reduced invasion rate seems unlikely to be due simply to an interaction with precipitation, which is greater in southeastern United States than in the Southwest. One hypothesis is that interactions with parasites (for example, *Varroa* mites), predators (for example, fire ants) or competitors (high concentrations of EHB) are reducing fitness and the invasion rate of the AHB into southeastern United States, at least temporarily. *Varroa* and tracheal mites were present in both Arizonan and Texan populations taken over by the AHB (Loper and others 1999; Pinto and others 2005). However, it is possible that mites take a stronger toll on honeybees in more humid conditions. One possible explanation for the slower movement of AHB into southeastern United States is that, in contrast to the southwest, this region has experienced little warming (http://www.ncdc.noaa.gov/img/climate/globalwarming/ipcc09.gif). In cool years the predicted isotherm determining successful overwintering for the AHB drops south of the coastline of the southeastern United States (Fig. 3), potentially eliminating a land route for invasion.

In the long run, global warming may result in a greater northern extension of the AHB. Models reviewed by the National Research Council of the United States predict an increase in average temperature of 2.7–4.4°C by 2100 (http://www.ncdc.noaa.gov/oa/climate/globalwarming.html#Q11). Such a warming could extend AHB distributions up to several hundred additional kilometers in the interior, and by a greater distance along both coasts of the United States.

Behavioral and physiological traits underlying ecological differences between AHB and EHB

Foraging preference

At the colony-level, traits that favor the AHB over the EHB in tropical and subtropical areas include higher colonial growth rates, reproduction at smaller colony sizes and the ability to use a wider range of nest sites

(Winston 1992a). The individual-level and genetic bases of these colonial traits remain poorly understood. One of the best documented traits of AHB is a greater foraging preference for pollen relative to EHB (Winston 1992a). This may be a central component of the higher growth rates observed in AHB in the tropics, as pollen serves as a nutrient source for brood production. Conversely, a focus on nectar collection allows EHB to accumulate larger stores of honey, enhancing overwintering capacity (Winston 1992a). Differences in preference for pollen versus nectar between AHB and EHB are observed in co-fostered bees (providing a common environment), suggesting a genetic basis to these differences (Fewell and Harrison 2002; Fewell and Bertram 2002). The preference for pollen versus nectar in honeybees is related to differences in chemosensory tuning (Pankiw 2003). Nectar foragers and pollen foragers differ in the concentration of sugar solutions that elicit proboscis extension, with nectar foragers requiring higher concentrations of sucrose to induce consumption. AHB are shifted relative to EHB in their sucrose sensitivity, generally showing shifts consistent with a greater preference for pollen compared to the EHB (Pankiw 2003).

The preference for pollen relative to nectar for AHB versus EHB suggest that a few genes that affect chemosensory tuning might have wide-ranging affects on colonial growth rates and overwintering capacity (Fig. 4). Preference for pollen could directly increase brood production rate by increasing the nutrients available to produce new workers. This mechanism depends on food intake, rather than on oviposition rate by the queen, being the limiting factor for brood production. Since workers can eat a significant fraction of worker eggs in honeybees, this seems possible (Winston 1987). Preference for pollen will reduce nectar intake and honey storage, thereby reducing the capacity of a colony to survive flowerless periods, suggesting that foraging preference could also be an important trait mediating overwintering success for honeybees.

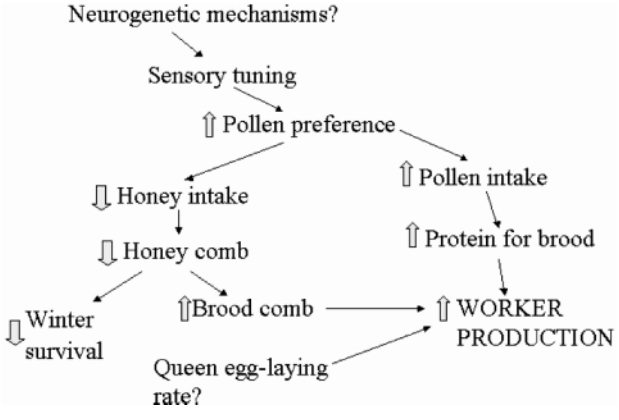

Fig. 4 A hypothesized pathway for higher colonial growth rates and reduced overwintering capacity in AHB relative to EHB (see text for details).

Longevity and overwintering ability

AHB colonies can fail during winter without running out of honey (Villa and others 1991), so inadequate longevity rather than starvation may be responsible for the reduced ability of AHB to survive dearth. AHB have shorter lifespans than EHB in both summer (Winston and Katz 1981) and winter (Woyke 1973). EHB have much higher hemolymph vitellogenin levels than AHB, especially in winter (Amdam and others 2005). High levels of vitellogenin protect honeybees from oxidative stress and thus may be a key physiological trait necessary for extending lifespan sufficiently to survive long, flowerless periods (Seehuus and others 2006).

Differences in flight-related traits among *A. mellifera* races and their potential implications

Subspecies of honeybees in Africa exhibit morphological differences from EHB that suggest a greater flight capacity (Hepburn and others 1999). Morphological examination of 18 African and European subspecies demonstrated that, as a group, the African subspecies were 33% lighter, had 17% greater thorax-to-body mass ratios and 27% lower wing loading (mg/mm) (Hepburn and others 1999). Based on a steady-state aerodynamic model and the assumption that the flight muscle is a constant proportion of the thorax, Hepburn and colleagues estimated that African subspecies have a 25% advantage in generating aerodynamic power based on morphology. *A. m. scutellata*, *A. m. ligustica* and *A. m. mellifera*, the main subspecies present in the Americas, were relatively typical of the subspecies examined, with *A. m. scutellata* collected from South Africa having a 20% greater thorax-to-body mass ratio than these European subspecies when collected from their natal regions (Hepburn and others 1999).

We compared thorax-to-body mass ratios of AHB and EHB workers in March 1992 in Zamorano, Honduras, and for queens and drones in July 1993 in Linaris, Mexico. Colonies were classified as AHB if they possessed African mtDNA; for the reproductives, hexokinase and Mdh allozymes were also used to assign subspecies. All comparisons indicate greater ratios of thorax mass-to-body mass for AHB (Table 1). However, the morphological differences between AHB and EHB workers were much smaller

Table 1 Comparison of African and European honeybees in terms of the ratio of thorax mass to body mass (T/B) and thorax-specific metabolic rates during flight (MR, W g^{-1})

Caste	T/B AHB	T/B EHB	%	MR AHB	MR EHB	%	Reference
Queens	0.47	0.425	+11*	1.25	0.99	+27*	Harrison and others 2005
Drones	0.50	0.43	+23*	1.15	0.84	+36*	Harrison and others 2005
Workers	0.51	0.49	+4%*	1.77	1.62	+9*	Harrison and Hall 1993
Workers	0.53[a]	0.44[b], 0.44[c]	+20%*				Hepburn and others 1999
Workers	0.35 ± 0.0055	0.34 ± 0.0045	+3%[NS]	1.72 ± 0.0051	1.64 ± 0.0029	+5*	This study

Means ± SEM provided for data from the current study ($n = 70$ for AHB, $n = 97$ for EHB) Asterisk indicates significant difference (*t*-test, $P < 0.05$), NS = nonsignificant.
[a]Indicates values measured for *A. m. scutellata* in South Africa.
[b]Indicates values for *A. m. ligustica* collected from Italy.
[c]Indicates values for *A. m. mellifera* collected from Norway (Hepburn and others 1999). Thorax masses in this study were measured after removing the legs, while all other studies kept the legs on.

than reported for AHB and EHB collected from their natal regions (Table 1), suggesting that hybridization has reduced differentiation. Since bees in these American studies were reared on commercial comb designed for European bees, it is possible that American AHB and EHB would be more dissimilar in morphology if reared on natal comb. Reproductives of Neotropical AHB and EHB differed more in thorax-to-body-mass ratios than did workers (Table 1).

Neotropical AHB also exhibit higher mass-specific metabolic rates during flight than do EHB. AHB workers measured in Honduras had 9% greater thorax-specific metabolic rates than did EHB, while queens and drones had 27 and 36% higher rates, respectively (Table 1). These higher rates of metabolism suggest higher rates of generation of aerodynamic power, but this important question has not yet been tested. Together these morphological and metabolic differences suggest that AHB invest a greater fraction of their resources in traits that support flight capacity than do EHB.

AHB in Arizona (Fig. 2) and Texas (Pinto and others 2005) have a considerable (20–35%) European nuclear genetic component, and the lower percentage of AHB mitotypes in Arizona than in Central America or South America suggest that hybridization might cause reduced differentiation between AHB and EHB in these areas. To test this hypothesis, we collected outgoing foragers from 3 AHB and 4 EHB colonies established at Page Ranch, Pinal County, Arizona, in April 2002. AHB colonies were established from locally caught swarms, while EHB colonies were purchased from Allen's Bee Ranch in northern California, where AHB do not occur. All colonies were 2 super colonies (>30 000 bees) that had been established for ~1 year. Measurements were taken during the spring bloom, so colonies were actively foraging.

Table 2 Carbon dioxide emission rates [ml h^{-1}, mean ± 95% confidence interval (*n*)] for bees with different behavioral ratings

Behavior rating	Carbon dioxide emission rate
1	8.24 ± 0.218 (121)
2	7.47 ± 0.632 (29)
3	5.59 ± 1.273 (17)

1 = continuous, unprovoked flight; 2 = intermittent flight, agitation necessary; 3 = no flight.

Foragers were captured in glass vials and transferred to Lucite metabolic chambers for measurement of carbon dioxide emission rate (V_{CO_2}) and metabolic rate as previously described (Harrison and others 2005). Metabolic measurements were made inside a trailer ~30 m from the hives, within which air temperature was regulated at ~22°C. Flight behavior of the bees in the chambers was classified as (1) flew more than 90% of the time with minimal provocation, (2) flew intermittently and required agitation and (3) did not fly. Immediately after the metabolic measures, bees were shaken from the chamber into a plastic bag and thorax temperatures were measured (Harrison and others 2005). Then bees were frozen on dry ice and body and thorax masses were measured as previously described (Harrison and others 2005), except that the legs were removed from the thorax before weighing. To assess the proportion of the thorax that was flight muscle, we split the thorax with a razor and soaked it in 1 mol l^{-1} NaOH for 2 days to digest all noncuticle. The remaining cuticle was rinsed, blotted and reweighed. The proportion of flight muscle in the thorax was calculated as (whole thorax mass − cuticle mass)/(whole thorax mass). Flight muscle mass was calculated by multiplying this proportion times thorax mass.

Bees with better flight behavior exhibited higher V_{CO_2} (Table 2), so for subsequent metabolic analysis

Table 3 Physiological variables during flight for captured outgoing foragers of AHB and EHB colonies measured at Page Ranch, Arizona, April 2002

Variable	AHB	EHB
Body mass (mg)	78.5 ± 0.16	86.6 ± 0.07*
Thorax mass (mg)	27.4 ± 0.36	30.1 ± 0.40*
PFM	0.86 ± 0.011	0.87 ± 0.005
Flight muscle mass (mg)	23.5 ± 0.50	26.3 ± 0.0.42*
Thorax temperature (°C)	37.3 ± 0.29	38.6 ± 0.0.27*
Air temperature (°C)	22.4 ± 0.53^{NS}	22.2 ± 0.18
V_{CO_2} (ml h^{-1})	8.04 ± 0.18	8.38 ± 0.14^{NS}
V_{CO_2} [ml (g body h)$^{-1}$]	103.3 ± 2.34*	97.3 ± 1.63
V_{CO_2} [ml (g thorax h)$^{-1}$]	294.6 ± 7.68*	279.6 ± 4.85
V_{CO_2} [ml (g muscle h)$^{-1}$]	355.2 ± 1.66*	321.5 ± 5.89

Only bees exhibiting continuous unprovoked flight (behavior code = 1) were used for this analysis. PFM = proportion of thorax that was flight muscle. All values presented as the mean ± SEM. $N = 47$ for AHB and $N = 74$ for EHB. Asterisk indicates a significantly higher value (t-test, $P < 0.05$), NS = nonsignificant difference.

we only used bees with a behavior rating of 1. AHB were lighter and had smaller thorax masses, but did not differ significantly in thorax-to-body mass ratios from EHB (Tables 1, 3). The proportion of thorax mass that was flight muscle did not differ between AHB and EHB, so EHB had greater flight muscle masses (Table 3). Despite the greater flight muscle masses, AHB and EHB did not differ in total V_{CO_2} (ml h^{-1}), and AHB had higher mass-specific V_{CO_2} and metabolic rates (Tables 1 and 3).

While Arizonan AHB workers had higher mass-specific metabolic rates during flight than did EHB workers, the differences were smaller than those measured in Honduras (Table 1). Although 100% of the colonies we designated as AHB in both Honduras and Arizona had African mtDNA, bees in Arizona may have had a greater European genetic contribution. Alternatively, this difference could be due to environmentally mediated plasticity.

In Arizona, bees from AHB colonies had consistently smaller bodies and flight-muscle masses and lower thorax temperatures than did bees from EHB colonies, but body mass-specific emission rates of carbon dioxide during flight were more variably distributed among races and colonies (Fig. 5). Among-colony variation in metabolic rates during flight is considerable in EHB (Harrison and others 1996) and AHB (Fig. 5), suggesting that the flight metabolic rates may vary continuously among these subspecies.

In other studies, AHB have been shown to have greater or similar thorax temperatures as EHB, despite

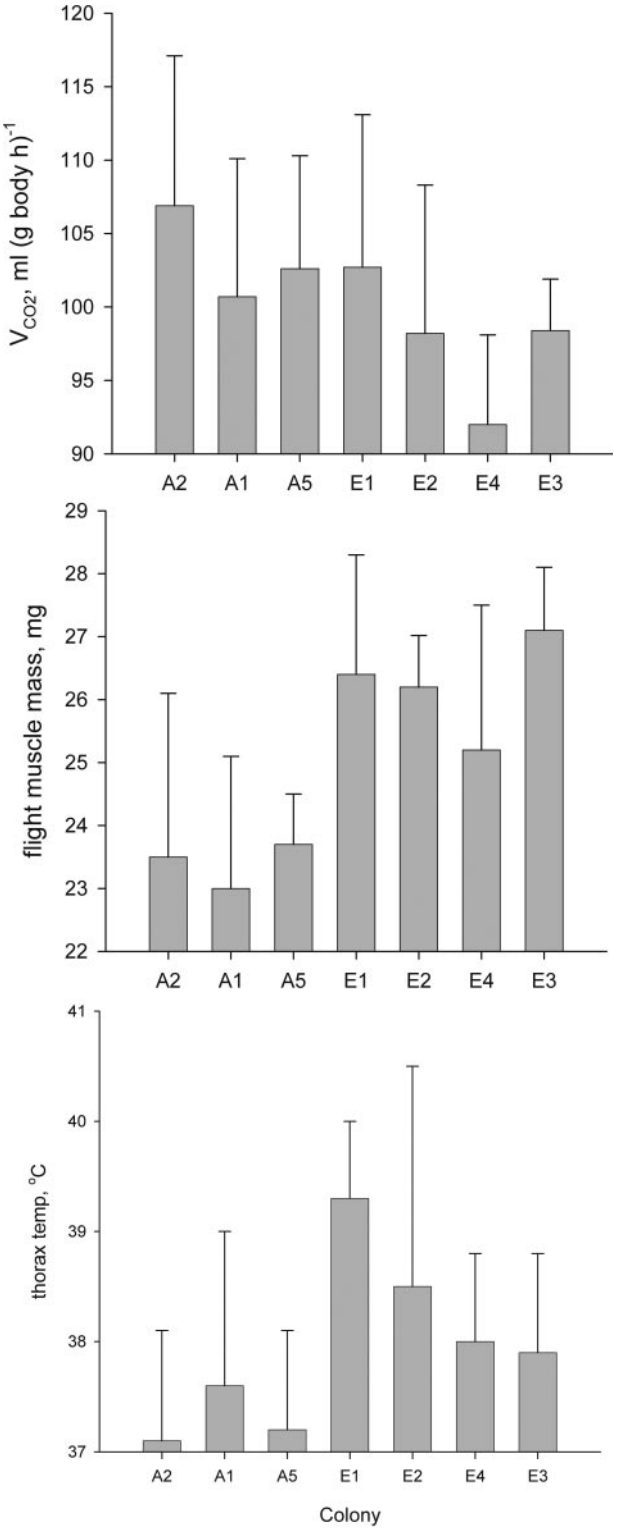

Fig. 5 Variation among hives in V_{CO_2}, flight muscle mass and thorax temperatures during flight, for outgoing foragers at Page Ranch, Arizona, April 2002. Data only shown for bees that exhibited continuous, unprovoked flight (behavior code = 1). AHB colonies: A1 ($n = 12$), A2 ($n = 14$), A5 ($n = 20$); EHB colonies: E1 ($n = 12$), E2 ($n = 16$), E3 ($n = 26$), E4 ($n = 20$). Means and 95% confidence limits shown.

their smaller size (Heinrich 1979; Harrison and others 2005). In this study, AHB workers had consistently lower thorax temperatures than EHB (Table 3, Fig. 5), consistent with their smaller size and relatively similar rates of heat production.

There is evidence that differences in flight metabolic rate between AHB and EHB are genetically based. The queens used by Harrison and colleagues (2005) were reared in common colonies, controlling environmental aspects such as larval nutrition and temperature. Similarly, AHB workers have higher flight metabolic rates than do EHB workers when co-fostered since eclosion in common hives, and this difference only occurs in bees of foraging age (Fewell and Harrison 2002).

What is the ecological significance of the greater investment in flight capacity in AHB? Multiple hypotheses can be generated, but as yet there are few data. Higher flight metabolic rates could enable greater mass-specific foraging intake, enhancing colonial growth. Higher flight metabolic rates in drones may also enhance mating performance of AHB drones, thereby contributing to paternal introgression of African genes into EHB populations (Taylor 1999).

Higher flight metabolic rates in queens and workers could enhance dispersal, one of the most significant traits influencing the invasiveness of a biotype (Cox 2004). Throughout most of South America and Central America, the range of AHB expanded by 160–500 km per year (Otis 1991). Waggle dances indicate that AHB reproductive swarms selected nest sites averaging 5 km from their nest entrance, 2–10× greater than typically shown by EHB (Schneider 1995). AHB also exhibit an absconding behavior in response to short-term disturbance, and long-term migratory behavior in response to deteriorating food conditions, both of which are rare in EHB (Winston and others 1979; Schneider 1990). Waggle dances prior to absconding communicate distances of up to 20 km from the hive, suggesting that this is the primary mechanism of range expansion for the AHB (Schneider 1990). A greater flight capacity might be particularly valuable during swarming or migration since bees consume considerable stores of honey prior to swarming and are more likely to experience difficult weather conditions than during foraging.

Summary and future directions

Study of the imminent formation of the hybrid zone between AHB and EHB in North America provides a temporary opportunity to examine the factors that determine range limits in these important species, and to generate models with economically important predictions. Many important questions remain concerning the invasion of the AHB and the trade-offs in ecological success of AHB and EHB across ecotones. What factors are most important to the competitive advantage of AHB in the neotropics? Preference for pollen-foraging by workers?, Colony size? Nest choice? What factors determine the ability of the AHB to overwinter? Longevity? Honey stores? Colony size? Air temperatures at which bees can fly? Advances in honeybee genetics may soon allow the identification of genes responsible for such phenotypic variation, providing an exciting potential for identifying the genetic basis of invasiveness and range limitations.

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