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Africanized honeybees are slower learners than their European counterparts

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Abstract

Does cognitive ability always correlate with a positive fitness consequence? Previous research in both vertebrates and invertebrates provides mixed results. Here, we compare the learning and memory abilities of Africanized honeybees (*Apis mellifera scutellata* hybrid) and European honeybees (*Apis mellifera ligustica*). The range of the Africanized honeybee continues to expand, superseding the European honeybee, which led us to hypothesize that they might possess greater cognitive capabilities as revealed by a classical conditioning assay. Surprisingly, we found that fewer Africanized honeybees learn to associate an odor with a reward. Additionally, fewer Africanized honeybees remembered the association a day later. While Africanized honeybees are replacing European honeybees, our results show that they do so despite displaying a relatively poorer performance on an associative learning paradigm.

Keywords

Apis mellifera; Africanized honeybees; Associative learning; Proboscis extension response

Introduction

Learning, defined as an animal's behavioral adjustment based on previous experience, is traditionally assumed to confer some positive benefit on the animal's fitness. Learning should potentially help an organism in many contexts, such as selecting a superior nest site (Dukas 2008; Dukas and Duan 2000), recognizing individuals that "owe" an altruistic

benefit (Wilkinson 1984), singing a better song to attract mates (Nowicki et al. 2002), foraging for higher quality (Dukas 1999b; Dukas and Bernays 2000; Papaj and Lewis 1993) and quantity (Raine and Chittka 2008) of food, or learning cues from conspecifics to avoid predation (Kelley et al. 2003). However, while previous research has focused on these adaptive benefits of learning, there is little experimental evidence to show that superior learning abilities might directly benefit an animal competing with another animal. This issue is especially interesting when the animals are closely related as this usually results in niche overlap and therefore, competition for the same resources with competitors using similar physical and behavioral traits.

Honeybees are a well-established, invertebrate model for the study of learning, which is a key attribute important for the competitive exploitation of floral resources (Gould 1986). Bees must learn to associate a location of a foraging site with numerous characteristics and remember profitable sites from day to day (Giurfa 2003; Menzel and Muller 1996). As appetitive learning is a robust and rapidly occurring phenomenon in bees (Menzel 2001; Menzel and Giurfa 2001), it has been an important laboratory tool in learning research for over 50 years (Giurfa 2007; Menzel and Erber 1978).

Honeybees (*Apis mellifera*) have been managed by humans for thousands of years. While most European honeybee (EHB) subspecies are relatively docile, native African honeybees (*Apis mellifera scutellata*) are more aggressive. African honeybees were introduced into Brazil in 1956 where they were hybridized with the present EHBs to produce “Africanized” honeybees (AHBs; Whitfield et al. 2006), also known as “killer bees.” Previous studies have focused on agricultural aspects [identification (Daly 1991; Rinderer et al. 1993; Spivak et al. 1987), life history (Benson 1985), honey production (De Jong 1984), pollination (Danka and Rinderer 1986), and response to insecticides (Danka et al. 1986)] and their infamous defensive behavior (Balderrama et al. 1987; Villa 1988). Despite their public fame, relatively little research has been conducted on their cognitive capabilities. It was shown that AHBs are good laboratory subjects, capable of training in a classical conditioning paradigm to associate various odors and other stimuli with a reward (Abramson and Aquino 2002; Abramson et al. 1997).

AHBs are ecologically successful, superseding EHBs throughout Central America and southern USA (Nogueira-Neto 1964; Pinto et al. 2005; Schneider et al. 2004; Whitfield et al. 2006). Their range continues to expand. As AHBs have outcompeted EHBs, we wished to compare the cognitive abilities of the two to see if superior learning performance of AHBs might correlate with ecological success. Additionally, domesticated animals usually have reduced cognitive abilities compared to their wild con-specifics (Kruska 2005), which would also lead us to hypothesize the “feral” AHB to perform better in learning assays than the more “domesticated” EHB.

Here, we compare the performance of EHBs (Fig. 1b) and AHBs (Fig. 1c) in a standard learning paradigm where bees learned to associate an odor (jasmine) with a sugar reward (sucrose solution). We hypothesize that there may be differences in the learning ability between the two races of honeybee (EHB and AHB) that could confer a fitness advantage.

Materials and methods

Study organisms and collection of bees

The European honeybee (predominantly *Apis mellifera ligustica*) colonies used in this study were headed by commercially reared queens (Big Island Queens in Captain Cook, Hawaii) that were mated in Hawaii where only EHBs are present. The Africanized honeybee colonies (*A. m. scutellata* hybrids) were collected from swarms in Tucson, Arizona, USA, as southern Arizona is home to a feral population of Africanized bees (Fewell and Bertram 2002; Loper et al. 1999; Rabe et al. 2005; Schneider et al. 2004). Both colonies of EHBs and AHBs are maintained at the United States Department of Agriculture Carl Hayden Bee Laboratory in Tucson, Arizona, USA. All colonies were queenright and housed in either a standard Langstroth hive of at least one deep box or a smaller mating nucleus hive. All colonies had a visible entrance from which we were able to observe returning foragers and collect bees.

As sucrose responsiveness and associative learning differ between the different types of foragers (nectar/pollen/water; Page et al. 1998; Scheiner et al. 2003), we collected returning foragers without pollen loads outside the entrance of their colony. We made six collecting trips between December 7, 2007 and January 30, 2008, and on each trip, we selected a different AHB and EHB colony, making a total of 12 different colonies, six of each race. We tested a total of 232 bees with an average of 18.2 AHBs (± 0.6 SE) and 20.5 EHBs (± 0.4 SE) per colony/collecting trip.

Bees were transported back to the laboratory where they were chilled and mounted in plastic tubes. At this point, we verified that sample bees demonstrated a clear proboscis extension to the reward solution (50% v/v sucrose in water) as a basic requirement for the conditioning procedure (Frings 1944). Such a high concentration sucrose solution is easily perceived by a human observer and considerably higher than reported thresholds for honeybees. Immobilized bees were fed to satiation at 17:00 h and then left without food overnight, as it is important in any learning protocol to account for appetitive motivation (Giurfa 2007). There was no noticeable behavioral difference between the two races of bees in the testing apparatus (i.e., one race did not appear stressed), as was previously reported (Abramson et al. 1997). Both races showed seemingly similar respiration rates and were able to feed until satiation. Additionally, AHBs had previously been shown to demonstrate reliable gustatory responsiveness and odor discrimination using this classical conditioning assay (McCabe et al. 2007), so we felt confident that neither race was adversely affected by the training regime. Data collection began at approximately 10:00 h the next morning.

Data collection—associative learning by proboscis extension reflex

The next morning, bees were trained in a standard paradigm of classical conditioning (Bitterman et al. 1983; Kuwabara 1957) where a jasmine-laden air current (conditioned stimulus, CS) was presented for 5 s; a vacuum removed the olfactory stimulus from the training area (flow rate ca. 5 l/min). AHBs, like EHBs, are capable of associatively learning a variety of odors (Abramson and Aquino 2002). We chose a floral odor because honeybees use them as a cue to distinguish between flower species when foraging. Also, we repeated

this experiment with an additional EHB/AHB colony pair ($n=30$ bees total) using another floral odor as CS (lily of the valley).

The antennae were touched with a toothpick soaked in the sucrose solution (unconditioned stimulus, US) 3 s after odor onset, upon which the bees reflexively extended their proboscis to receive the reward (unconditioned response). Bees that spontaneously responded to the first odor presentation or that did not respond with a proboscis extension reflex to the first sucrose stimulus were discarded, as both sets of bees would be untrainable in the forward pairing of an odor with a reward; however, this was an infrequent occurrence. For the entire experiment, only a total of four (4%) AHBs and three (2.7%) EHBs were discarded for the former reason and eight (8%) AHBs and ten (8.8%) EHBs for the latter reason.

After 30 min, bees were submitted to another training cycle of successive presentation of CS and US. This was repeated seven times, a training regime similar to the spaced protocol used in learning studies with *Drosophila* (Mery et al. 2007; Mery and Kawecki 2005). Bees responding with a proboscis extension during the first 3 s of the odor presentation before the sucrose application to the antennae were considered as having showed the correct Proboscis Extension Reflex (PER). AHBs and EHBs were tested in random order to account for different levels of hunger/motivation, and the tester was blind to the race of the bee.

Data collection—memory by proboscis extension reflex

After the seventh trial, bees were fed sucrose solution ad libitum. The odor was presented again 24 h later. Bees with at least two of seven PER were used in the memory trial. Of these bees, those that responded with a proboscis extension to the odor 24 h later were considered as having remembered the association.

Data collection—weight and size distribution of workers

As learning generally correlates with body size across species (Rensch 1956), we wished to see if a difference in size between the two bee races might correlate with PER performance. After the memory trial, we chilled each bee and measured her head width with digital calipers to the nearest hundredth millimeters under magnification of 8 $\times$. We weighed each bee using a digital scale (Scientech SA80), which was calibrated to the nearest 0.1 mg. Furthermore, we weighed the bee with the abdomen removed to minimize any potential variation from the volume of fluid in the crop.

Statistical analyses

All statistics were performed using Minitab (Student version 14). To analyze differences in learning and memory between AHBs and EHBs, we used a binary logistic regression because our response variable was yes/no (PER or no PER). Additionally, we expected the data, as it is learning data, to fit a nonlinear function, which is permissible in this model.

The model analyzed the response variable against the factors of race (AHB versus EHB), colony (1–12), and trials (1–7) as well as interactions between factors like race and trial. We did not include the factor of day in the model because of redundancy—we would expect “colony” and “day” to covary completely because each day we selected two new colonies,

one of each race. However, for both learning and retention data, we reran models substituting “day” for “colony” to confirm that significance levels that remained the same.

Size data were analyzed using analysis of variance (ANOVA) and potential correlations between size and learning was determined using Pearson’s correlations.

Results

More EHBs than AHBs learned the odor association

In every trial, a significantly larger percentage of EHBs showed a learned proboscis response to the odor (CS) compared to the AHBs (binary logistic regression model, odds ratio=0.43, $p<0.001$, Fig. 1a). For both races, the rate of change in the number of bees responding to the CS during the first three conditioning trials was similar until trial 3, at which point a median of 75% EHBs and 50% AHBs demonstrated to have learned the odor association by a proboscis extension (Fig. 1a). This difference in the percentage of correct responses between the two races represents the statistically significant difference in the level of learning performance in EHBs and AHBs (Binary Logistic Regression Model, Odds Ratio=0.43, $p<0.001$). This superior performance was seen in every independent test on 6 different days, which represents six different pairs of colonies. This effect did not differ between colonies within a race (Binary Logistic Regression Model, Odds Ratio=1.08, $p=0.514$). As expected, trial number was significant (binary logistic regression model, odds ratio=1.18, $p<0.001$), demonstrating that both races learned (i.e., demonstrated more correct proboscis extensions from trials one to seven; Fig. 1a). The interaction between race and trial was nonsignificant (binary logistic regression model, odds ratio=0.94, $p=0.231$), showing that the learning curves of AHBs and EHBs were similar (i.e., of the ones that did learn, the rates were similar).

Furthermore, a significantly larger percentage of EHBs showed a learned proboscis response to lily of the valley (second CS) compared to the AHBs (binary logistic regression model, odds ratio=0.10, $p<0.001$; Fig. 1a, triangles), demonstrating that the outperformance of AHBs by EHBs was general to learned associations with floral odors.

Lastly, for both types of bees, most of the learning happened by trial 3 and very little learning happened in trials 3–7. This is visible in the figure and was verified by the nonsignificant difference in correct responses of trial 3 versus trial 7 (binary logistic regression model, odds ratio=0.98, $p=0.760$).

More EHBs than AHBs remembered the odor association

In addition to comprising a higher proportion of learners, EHBs also displayed significantly superior retention of learned associations between the odor and the reward after 24 h (79% of EHBs versus 51% of AHBs, binary logistic regression model, odds ratio=0.36, $p=0.001$; Fig. 2). Retention was considered for bees that had previously showed the learned association (PER to two or more of the seven learning trials). Colony/day (binary logistic regression model, odds ratio=0.85, $p=0.642$) were nonsignificant factors. Additionally, this significance was maintained when we reran this analysis using different thresholds for learning: for bees displaying PER to four or greater of seven learning trials, a significantly

higher number of EHBs retained the learned association after 24 h with the jasmine odor than for AHBs (88% EHBs versus 69% AHBs, binary logistic regression model, odds ratio=0.33, $p=0.02$). Colony/day remained nonsignificant factors (binary logistic regression model, odds ratio=0.90, 1.01, $p=0.82$, 0.76).

EHBs are larger than AHBs; however, size and learning do not correlate

As previously reported (DeGrandi-Hoffman et al. 2008; Michelette and Engles 1995), EHBs were significantly larger than AHBs: mean head width (3.71 mm versus 3.66 mm, $n=113$, 100, $SE=0.0072$, 0.0087; one-way ANOVA, $F_{1,211}=20.78$, $p<0.001$), body weight with abdomen (0.1107 g versus 0.1098 g, $n=113$, 100, $SE=0.0012$, 0.0016; one-way ANOVA, $F_{1,211}=18.45$, $p<0.001$), and body weight without abdomen (0.0506 g versus 0.0479 g, $n=113$, 100, $SE=0.0004$, 0.0004; one-way ANOVA, $F_{1,211}=23.13$, $p<0.001$). However, across all bees, there was no significant correlation between learning (as percent correct PER) and body size (head size, Pearson's correlation, $r=-0.009$, $n=213$, $p=0.892$; body mass, Pearson's correlation, $r=0.041$, $n=213$, $p=0.549$). Comparing the size of the best learners (6/7 or 7/7 PER) to the worst learners (1/7 or 0/7 PER) also revealed no significant difference (head size, one-way ANOVA, $F_{1,89}=0.12$, $p=0.734$; body mass, one-way ANOVA, $F_{1,89}=0.03$, $p=0.864$). We conclude that the differences in learning ability are not due to the slightly smaller size of AHB.

Discussion

Our study compared the learning and memory of Africanized and European honeybees in a classical conditioning assay where the bee learns to associate a stimulus with a reward. Surprisingly, a consistent and significant smaller proportion of AHBs than EHBs learned the association ($p<0.001$). Additionally, a significant smaller proportion of AHBs than EHBs remembered the association a day later ($p=0.001$). This result was seen across independent trials and was not affected by colonies of the same race. Of the bees that did learn the association, both races required on average three trials out of seven to associate the reward with the odor. This means that the races learned at similar "rates," which was confirmed by the nonsignificant interaction between race and trial, demonstrating that the learning rate was comparable between the races.

Although there was no control for age of returning foragers, we would expect any variation in learning performance from age to occur in both races. More importantly, we know that both honeybee races display temporal polyethism in which bees initiate foraging in the later stages of life. Previous work has already demonstrated that there is no significant difference between the age of nectar foraging initiation between AHBs and EHBs (Fewell and Bertram 2002).

In a general way, behavioral repertoire and learning ability correlate with body size across comparable species, as larger animals generally have bigger brains (Rensch 1956). We therefore compared the body size of our bees to determine whether this factor was linked with the differential performance between honeybee races. Our data confirmed that EHBs are slightly larger than AHBs ($p<0.001$). However, there was no significant correlation between size and learning, demonstrating that in honeybees, differences in learning are not

due to body size, a result that was also reported in bumblebees, which show pronounced intraspecific size differences (Raine and Chittka 2008; but see Worden et al. 2005).

A similar learning pattern between AHBs and EHBs has been suggested based on a small sample of 18 bees from a single colony (Abramson et al. 1997). To our knowledge, ours is the first comprehensive study comparing not only the learning abilities of these two races of honeybees, but also the first to examine memory 24 h later. Previous work comparing learning in various contexts with other bee races has generated mixed results. For example, there have been reports of differences not only between species of the genus *Apis*, but also within the species: in a test between *A. m. ligustica*, *Apis mellifera lamarckii*, *Apis mellifera carnica*, and *Apis cerana*, *A. cerana* outperformed the others, learning the quickest and reaching the highest level in horizontal and vertical color cross paradigms (Menzel et al. 1973). Additionally, *A. m. ligustica*, which was the EHB in this study, performed relatively poorly compared to the other subspecies. However, no significant difference was found in learning rates and odor discrimination ability between *Apis mellifera carnica*, *Apis mellifera syriaca*, and *Apis mellifera caucasica* (Abramson et al. 2008), and there were no real differences found between *A. m. carnica* and *A. m. ligustica* (Hoefer and Lindauer 1975). None of the above studies tested either pure or hybrid *A. m. scutellata*, which we have now done.

Although we do not believe there were any differences between races in how they respond to jasmine, we did repeat the experiment using another floral odor (lily of the valley) as our CS. The quantitative results of this test confirmed our results (Fig. 1a, triangles) that a higher percentage of EHBs learn the association between an odor and a reward than AHBs. We also did not investigate if the presence of the parasitic mite *Varroa* affected learning and memory. However, as EHBs are reported to be more susceptible to *Varroa* compared to AHBs (Guzmán-Novoa et al. 1996), our results would suggest that the parasitic mite did not adversely influence learning in honeybees.

Other studies have demonstrated within-species variation in learning. In bumblebees, previous reports have shown that some bumblebee races (e.g., *Bombus terrestris audax*) outperform others (*Bombus terrestris dalmatinus*) in onetrial learning or color discrimination tasks, although results were sometimes weak and affected by innate color preferences (Chittka et al. 2004; Ings et al. 2009). Perhaps more relevant to our work, *B. terrestris* was found to display massive differences between colonies in a laboratory associative learning task (Raine and Chittka 2008) where the best learners were almost five times better at the trials than the worst. Interestingly, the colonies that were better learners in the laboratory were also better foragers in the field, which served as a proxy for fitness. These data suggest a strong adaptive selection for faster learning in *B. terrestris* and represents one of the first experimental tests showing how within-species learning variation might correlate with fitness (Dukas and Duan 2000; Papaj and Prokopy 1989; Raine and Chittka 2008).

Our study also demonstrated pronounced within-species/across race variations in learning ability. However, whereas Raine and Chittka (2008) found differences in learning speed, we found pronounced differences in the ultimate performance of the different races. More

importantly, our data demonstrate that EHBs were the superior learners in the assay, although during the past 50 years, AHBs have outcompeted EHBs throughout the Americas with continued range expansion. Is it possible that AHBs possess less olfactory learning and memory abilities, yet are ecologically more successful than EHBs? We did not test this question explicitly, but it has inspired two, interesting, and nonexclusive hypotheses.

If learning and memory were cost-free, then there would be universal selection for superior learning between and across species. Instead, a high degree of variation is maintained in natural populations (McGuire and Hirsch 1977), suggesting that learning could theoretically incur a fitness cost (Dukas 1999a; Johnston 1982; Laughlin 2001). Recent experimental evidence demonstrating an evolutionary trade-off between learning ability and competitive ability in *Drosophila melanogaster* strongly supports this hypothesis: selection for improved learning is consistently linked with a decreased competitive ability for limited food resources in larvae (Mery and Kawecki 2003). Perhaps trade-offs are also occurring in the honeybee between improved learning and successful, aggressive strategies.

Secondly, if it is assumed that differences in learning abilities occur in response to the natural conditions under which animals live (Dukas 1998; Raine and Chittka 2008; Shettleworth 1998), some different ecological strategies that AHBs adopt might make it less important for them to be good olfactory learners. Specifically, might AHBs be subject to lower selective pressure to learn associations between odors and rewards? To answer this directly, one should compare the scouting and foraging behavior of the two races in the same ecological conditions. These data are not currently available; however, there are some individual studies in AHBs and EHBs to suggest differences in how the two races scout and forage and the distance over which they fly.

EHBs forage at the level of the colony (Seeley 1985; Seeley 1986), relying on information flow between foragers and recruits to direct to forage locations. Additionally, EHBs are adapted for temperate regions, and their colonies need large honey stores to survive winter. EHB foragers tend to fly long distances (median distance=6.1 km) to find flowers (Beekman and Ratnieks 2000). On their return, they must remember foraging site locations and resource properties, as well as the route back to the nest. This requires substantial associative learning and memory. In contrast, AHBs forage more as individuals (Rinderer and Collins 1991; Winston 1992), relying less on strong associations between odors and rewards as foragers are more likely to be visiting new sites. AHBs have been reported to fly shorter distances (mean distance=600 m) when foraging (Schneider and McNally 1993). When flowering plants become unavailable in one area, colonies abscond to another where plants are in bloom (Schneider and McNally 1992). Additionally, AHBs demonstrate weaker flower color fidelity than EHBs (Cakmak et al. 1999), which also correlates with their propensity to abscond. AHBs also tend to recruit more and might even displace other bees at floral patches (Núñez 1979). EHBs rarely abscond, but instead might rely on their memory to recall previously profitable sites or to fly further to discover new ones.

Although the above studies suggest that AHBs might experience less selective pressures to learn associatively foraging sites compared to EHBs, it is important to note that the studies do not directly compare the two races in the same ecological condition. As foraging ranges

are strongly influenced by flower distribution, these data are necessary to make direct comparisons. Additionally, future studies should concentrate on the costs of learning abilities (e.g., potentially higher metabolic costs to support larger, more sophisticated neural structures, or the time required to acquire learned behaviors as compared to innate behaviors) and explicitly explore this link in these bees between learning performance and ecological success to aid our understanding of the evolutionary advantages and constraints of learning.

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Fig. 1.

European honeybees (EHB; *b*, red) perform better in a learning assay compared to Africanized honeybees (AHB; *c*, gray). Data (*a*) represent percentage of bees of each race demonstrating a learned associative response (proboscis extension) to an odor (jasmine) for trials 1–7. Odors (CS) used were either jasmine (*boxplots*—data pooled for colonies) or lily of the valley (*triangles*; $n=30$ bees from one colony). *Boxes* represent interquartile ranges (25–75%) with the median marked, and *whiskers* indicate 10–90%. Coloration of bees (*b*, *c*) might differ under field conditions

Fig. 2.

European honeybees (*red*) are better at remembering the odor association 24 h after learning compared to Africanized honeybees (*gray*). We only tested memory in bees that demonstrated having learned the association in the previous day. Data are for all bees of the two races. Each study day corresponds to a new pair of AHB/EHB colonies