

Nobody Can Tell You What an Africanized Bee Is

There is no agreed definition, no test with a known error rate, and after nearly seventy years nobody has properly measured the one thing everyone is afraid of. Second of two parts.

Part 2 of 2. By: Peter Awram, Canadian Beekeepers Federation Director



Last month I set out the field evidence: the bees have been established across ten American states for three decades, the whole American industry pools most of the colonies in the United States in California every February and trucks them home again, and none of it has produced the devastation that we were told Killer Bees were going to cause. That by itself shows that this issue should have been dismissed long ago.

There is no clear evidence in the pile of research papers. The literature is contradictory, poorly done and inconsistent. What's there is a stack of papers that never define the thing they're warning about, and tests that draw arbitrary lines in the sand with no clear reason why the line sits where it does rather than somewhere else.

The Africanization tests tell you about ancestors, not aggression

Start with how a bee gets the label at all. There are three methods, and all three are still in use somewhere.

The oldest is body measurement, mostly of the wings. African-descended bees are slightly smaller and the veins in the wing sit slightly differently. It's cheap and fast, and it was the standard screening tool across the American south for decades. However, some scientists have been blunt about what that involves:

"Occasionally, additional characters are introduced, but the selection of characters appears widely arbitrary. As comparisons between studies or to reference data rely on characters matching between data sets, these incongruities seriously compromise meaningful analysis."

MEIXNER AND COLLEAGUES, JOURNAL OF APICULTURAL RESEARCH, 2013

In other words, results from different labs cannot be compared. The second is a mitochondrial DNA test. This tests only the maternal line. One small piece of a bee's DNA is passed down only from mother to daughter, so this test tells you whether the queen's female line traces back to Africa. It says nothing about the drones her ancestors mated with, which is to say nothing about most of the ancestry.

The third is a genome scan for small markers in the DNA (SNPs) that are used to estimate what fraction of a bee's ancestors came from Africa and what fraction came from Europe, much the way the ancestry services work for people. When a recent paper calls a bee Africanized, that percentage is usually what it means.

Notice what all three have in common. They answer the question of who a bee's ancestors were. Not one of them measures aggression. It's like deciding a dog is dangerous by reading its pedigree papers without ever looking at the dog. That turns out to be the whole problem.

What the label was actually based on

It gets more awkward when you look at how the papers themselves decided which bees were Africanized in the first place. The morphometric reference standard that every wing measurement in the Americas descends from set its own reference colonies like this:

"...the classification of base-line colonies as Africanized or European is determined by whether or not the general population was considered to be Africanized at the time of collection and the specific history of the queen that produced the colony. This eliminates the bias inherent in making decisions based on field behavior, which probably led to only the most clearly Africanized members of the sampled populations being collected as base-line material."

RINDERER AND COLLEAGUES, APIDOLOGIE, 1993

The reference bees were labelled by where they were collected and who their queen was. **Behaviour was excluded on purpose**, and the authors say why: choosing colonies by behaviour would have filled the reference set with the most obviously Africanized ones. Every morphometric identification made since inherits that labelling.

The genomics inherited it too. The landmark genome-scale study of honey bee lineages took its category straight off the wing measurements:

"After Africanization, bees defined as Africanized by morphology exhibited predominantly African ancestry admixed with M, C, and to a lesser extent, O group bees."

WHITFIELD AND COLLEAGUES, SCIENCE, 2006

I went through thirty-one studies covering forty-three years and recorded what each one used to decide a population was Africanized. Geography or provenance dominates. Wing measurements and maternal-line DNA fill in most of the rest. Where behaviour enters at all, it is used to build deliberately extreme groups for genetic mapping crosses, not to define or check the category. Not one study in that set designated a population Africanized by

measuring its defensiveness. The one place it does happen is older than the set, and it went into the record anyway. Kerr's estimate that 30% of colonies at the southern edge of the range were African was arrived at by counting the ones that were aggressive.

The line in the sand

If you're going to call some bees Africanized and refuse them at a border, you need some reasoning behind where the line goes. So where is it?

There isn't any.

The SNP test that set the standard puts its cutoff at 15% African ancestry. Here is how the paper arrived at the number:

"The threshold that minimizes the number of false positives (European and European-derived individuals being declared Africanized) while preventing any false negatives... occurs when individuals carrying more than 15% African alleles are rejected."

CHAPMAN AND COLLEAGUES, MOLECULAR ECOLOGY RESOURCES, 2015

The cutoff was chosen to sort bees against reference ancestry panels, and it does that job well. The paper contains no behavioural measurement of any kind. And the logic is circular. Its Brazilian bees are Africanized because they were collected for the 1993 morphometric paper, and that paper called them Africanized because they were from Brazil. The number that defines an Africanized bee for regulatory purposes has never been tested against temperament.

And this arbitrariness is widely known. A study comparing wing morphometrics against microsatellites put it plainly:

"There are no objective criteria for distinguishing between pure subspecies and hybrids. The identification requires the use of threshold values which are to some degree arbitrary."

OLEKSA AND TOFILSKI, APIDOLOGIE, 2015

And the newest molecular identification tool, published in 2024, ran the older assignment methods over its own data and reported that the groups will not separate:

"An attempt to use the GeneClass2 assignments or PCA analysis to demarcate genetically similar groups would be largely arbitrary and unsatisfactory."

DONTHU AND COLLEAGUES, BMC BIOINFORMATICS, 2024

Its own accuracy, the paper says, decreases with highly admixed samples.

The researchers who work on these bees have gone further than that, and said the category itself does not survive contact with the data:

"These bees defy 'Africanized' (vs. 'non-Africanized') labels currently used by researchers, beekeepers, and policy makers."

CALFEE AND COLLEAGUES, PLOS GENETICS, 2020

"'Africanized' as a descriptor is frustratingly broad and fails to accurately reflect the diversity of geographic lineages that a hybrid honey bee of the American continents can encompass."

ZÁRATE AND COLLEAGUES, CALIFORNIA AGRICULTURE, 2023

Nobody has shown a DNA percentage at which a colony's aggression changes, because nobody has connected the percentage to the temper. The line is where somebody chose to put it, and that is all.

They cannot reliably link defensiveness to genetics

A study in Brazil went looking for that percentage directly. Researchers scored the defence response of 116 colonies at four apiaries in São Paulo State, took the 15 most and the 15 least defensive, and sequenced them. They found plenty of genetics. Sixty-five regions of the genome, holding 322 genes, separated the two groups. What they did not find was a real link between genetics and defensiveness:

"We found no significant evidence of a genome-wide difference in C-lineage ancestry between highly and less-defensive cohorts across all sites ($t = -0.68$; $P = 0.49$). Repeating this analysis within only defense-associated regions of the genome... we again found no significant evidence of different levels of C-lineage ancestry between the two cohorts ($t = 1.85$; $P = 0.064$). When we compared the level of M-lineage ancestry between the two cohorts, we found that less-defensive colonies (mean $M = 14.1\%$) had a slightly, but a significantly higher level of M-lineage ancestry genome-wide when compared with highly defensive colonies (mean $M = 13.8\%$; $t = 3.37$; $P = 0.0008$)."

Harpur and colleagues, Genome Biology and Evolution, 2020

The gap between the calm colonies and the aggressive ones was three-tenths of one percentage point of western European ancestry, and it ran the wrong way. The calm bees had more of it. African ancestry did not separate the two groups at all. The authors put it plainly: defence response is not simply the product of more A-lineage honey bee ancestry as previously assumed. That assumption is what the 15% cutoff is built on.

The colonies were sorted by how many times they stung a suede ball, and the genetics were measured afterwards. All 116 were Brazilian bees from four yards within 15 km of each other, and there was no European group in the sequencing at all. It is the one study in the pile that let behaviour define the groups, and the ancestry percentage still did not track the temper.

Nobody ever pinned down aggressive behaviour either

As it's the aggression we don't want, you'd expect, after seventy years of alarm, that aggression had been carefully defined and measured.

It hasn't been.

There is no standard test. One lab counts stings left in a leather patch waved at the hive. Another times how long before the first sting. Another counts how many bees chase the observer, or how far. These don't convert into each other, so results from different labs can't be combined, and "more aggressive" means something different in every paper. A recent study spells out how much the protocols vary:

"Some studies preceded their stinging assay with a disturbance while others did not. Of the studies reviewed in Table S6, half used colonies with 9–10 frames, while the rest used colonies with 3–4 frames. In addition, averaging the number of stings on a target over time does not capture potential variation in the rate of stinging during the observation periods, which varied between 20 and 60 s."

ZÁRATE AND COLLEAGUES, SCIENTIFIC REPORTS, 2023

Then there is the weather. A German study held the genetics constant by design, using sister queens inseminated from a single mixed semen pool, and measured the stinging behaviour:

"Eliminating genetic variance, the following meteorological variables account for 92.4% of the variation in defensive behaviour: air temperature, solar radiation intensity, wind velocity, relative humidity, and barometric pressure."

SOUTHWICK AND MORITZ, INTERNATIONAL JOURNAL OF BIOMETEOROLOGY, 1987

Five weather variables explained 92.4% of the variation in sting counts, once the colonies were averaged within each site to take the genetics out. It rests on 20 site averages, and the authors would not push it further than that: because the weather variables move together, they say a causal analysis of the alarm behaviour was not attempted. Nobody has since shown that an outdoor sting count is measuring the bees rather than the afternoon. That was published in 1987 and the field has gone on running the test outdoors ever since.

Season does the same thing. Every beekeeper reading this knows the colony that's a pussycat on a warm morning with a flow on and vicious in a cold dearth. That isn't folklore. It is in the published record, on the same colonies, measured through one year:

"...our scutellata-hybrid colonies stung at a rate ~6 stings/min in the month they were least defensive (May) and ~66 stings/min in the month when they were the most defensive (October)."

ZÁRATE AND COLLEAGUES, SCIENTIFIC REPORTS, 2023

An eleven-fold swing on the same bees. Both the hybrid colonies and the managed European colonies scored low in May. Through the season the hybrids climbed and the managed colonies fell on three of four measures, though the two yards differed in enough ways that the authors refuse to compare them directly. The managed colonies were not calm either. In October they averaged about 27 stings a minute.

That study turned up something the seasonal result nearly buries. The European colonies were not European. They were bought as nucleus colonies from northern California, outside the hybrid range, and requeened every year with Italian queens bred for gentleness. Sequenced, they averaged 19% African ancestry, and one of them reached 38%. That is the average of the feral swarms caught in San Diego County. The authors dealt with it by renaming the group "predominantly European".

"Overall, defensive behaviors of our scutellata-hybrid and managed colonies (pEHB) significantly changed over time, and thus judging a colony's ancestry based on its behavior at any given moment is unreliable."

ZÁRATE AND COLLEAGUES, SCIENTIFIC REPORTS, 2023

They also note that they could only run the assay on days with negligible cloud cover, which is the weather problem again, this time built into the sampling.

Recent history matters too, and not in the direction anyone expected. Researchers deliberately threatened colonies to see whether the bees would become more aggressive:

"Experimentally induced predation threats modified behavior, but the effect was opposite of our predictions: disturbed colonies showed decreased aggression."

RITTSCHOF AND ROBINSON, GENES, BRAIN AND BEHAVIOR, 2013

A colony's recent disturbance history changes the score. How long it takes to wear off is not something anyone has pinned down, but Southwick and Moritz left 5 to 7 days between tests on the same colonies, an interval they said was needed to allow the bees to return to a completely calm state and to replace those individuals lost in the stinging episode.

And the test misclassifies in both directions. A University of Georgia survey of Argentina, funded by the USDA, found this in the south of Buenos Aires province:

"During our survey, we encountered highly aggressive colonies of bees in the southern part of the province of Buenos Aires... However, morphometric analysis indicates that these bees are clearly European even though they were as aggressive as the most aggressive Africanized bees encountered in Formosa and other areas."

DIETZ, KRELL AND EISCHEN, APIDOLOGIE, 1985

Colonies the wing measurements called European matched the worst Africanized colonies in the same survey.

The authors concluded that the behaviour test cannot stand on its own; the morphometrics overruled it: "While the defensive behavior test is an excellent method of field identification of aggressive honey bee colonies, it must be supported by morphometric analysis for definitive taxonomic determinations." In other words, a mean colony was not necessarily an Africanized one. A year earlier, commenting on an estimate that 30% of colonies in the marginal zone at the southern edge of the range were aggressive and therefore African, Taylor and Spivak had already said as much: "not all African colonies are highly defensive". That was 1984.

On top of this, the person doing the counting almost always knows which colony is supposed to be the mean one. A 2021 study scored 211 colonies for calmness and gentleness by having experts look at them and rate them. That kind of scoring is open to heavy bias.

The direct hunt for the aggression genes has produced findings that do not line up with each other. A cross of 172 colonies in 1998 turned up one region that cleared the statistical bar and four more that did not. The field went on to test the one and two of the four. A follow-up looked for the strongest region in a single colony from each of two backcrosses, found the effect in one and nothing in the other, and read the empty half as dominance, when it is equally consistent with the region doing nothing. When the same group remapped the trait fourteen years later with markers sitting inside genes, they found three new regions and reported "the lack of overlap between

these regions and previous defensive behavior QTL". By then they had also changed the trait. The bees were being shocked one at a time in the laboratory, with the colony taken out of it.

Sequencing has not fixed it. A 2020 study sampled 13 colonies, kept 9, and tested about 3 million markers. Not one locus was associated with aggression in individual bees. Colony allele frequencies gave strong associations, and the authors judged that most of those were probably riding along with one real signal rather than doing anything themselves. A 2021 study of 211 colonies found five regions, and none of them overlapped the earlier work. It was scoring calmness and gentleness by eye, and its authors write that "due to the small sample collection and the subjective scoring of the colonies, we cannot exclude the possibility that our current GWAS results are strongly biased".

Twenty-eight years, four assays, four populations, and not one region confirmed by anybody else measuring the same thing.

What this means at the border

Where does this leave us?

- **The category has no agreed boundary.** The researchers who study these bees say so in print.
- **The tests measure pedigree, not temper.** They also disagree with each other, and their error rates have never been established.
- **The label those tests were calibrated against came from geography and queen origin.** Aggressive behaviour was hardly considered.
- **The aggression itself has never been measured well enough** for anyone to say how much the ancestry predicts it.

This doesn't prove Africanized genetics are harmless. But it means nobody has produced evidence to the standard that a border closure requires. That is not to say the work in these papers is worthless, but it does bring to mind the old phrase 'garbage in, garbage out'.

And all of this is academic. The real science experiment has been running in the southern US as outlined in Part 1. And that one has made it clear that Africanized honey bees are no more of a worry than any other honey bee.

A regulation is supposed to rest on the evidence. This one rests on the reputation of twenty-six queens that got loose in Brazil in 1957 and a lot of hyped-up Hollywood scare flicks.

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