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► To cite this version:

Ashley N. Mortensen, James D. Ellis. A scientific note on the prevalence of the cordovan phenotype in the African-derived honey bee population in the Southeastern United States. *Apidologie*, 2015, 46 (1), pp.46-48. 10.1007/s13592-014-0294-1 . hal-01284418

HAL Id: hal-01284418

<https://hal.science/hal-01284418>

Submitted on 7 Mar 2016

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A scientific note on the prevalence of the cordovan phenotype in the African-derived honey bee population in the Southeastern United States

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Received 11 February 2014 – Revised 14 April 2014 – Accepted 2 May 2014

Readily identifiable phenotypic markers, such as color, are useful research tools in an array of disciplines. In the western honey bee, *Apis mellifera* L., a recessive phenotype known as cordovan (*cd*, Mackensen 1951; Laidlaw et al. 1953) has been used in numerous genetic and behavior studies. These include studies on the spatial dynamics of the mating system, kinship recognition, and the discovery of haplodiploid sex determination (Mackensen 1951; Rowell et al. 1992; Breed et al. 1994).

Cordovan individuals express brown cuticular coloration in all areas that otherwise would be black (Mackensen 1951, Figure 1). There is considerable variation in the amount of black cuticular color expressed in all subspecies of the western honey bee. However, the sixth abdominal tergum is always black even in the most yellow of wild-type workers and drones (Tucker 1986). Thus, *cd* mutants are differentiated easily from their wild-type counterparts, allowing them to be informative in laboratory and field studies.

The cordovan marker has been used in a variety of comparative studies between African- and European-derived honey bees since the introduction of African honey bees, *A.m. scutellata* Lepeletier, into the Americas (Kerr 1967; Rinderer et al. 1987; DeGrandi-Hoffman et al. 1998a, b; Schneider and DeGrandi-Hoffman 2002, 2003; Schneider et al. 2003, 2004). However, we suspect that the *cd* phenotype occurs in both the African- and

European-derived populations in Florida. Therefore, we suggest the *cd* phenotype be used cautiously, particularly as an indicator of European descent in the USA.

In June and July of 2012, we conducted a survey in which 400 drones were sampled from each of six drone congregation areas (DCAs) for a total of 2,400 drones collected in Orange and Osceola counties, Florida, USA. Three of the DCAs were located within 0.25 km of 96, 10-frame, commercial European-derived honey bee colonies headed by *cd* queens. The three remaining DCAs were located >2.8 km from any managed honey bee colonies. Since drones typically travel <2.0 km to a DCA (Rowell et al. 1992), drones trapped >2.8 km from managed colonies were considered representative of the feral population.

Drones were trapped via an aerial (Williams 1987) trap, baited with a synthetic 9-oxo-2-decenoic (9-ODA) queen lure (Gary 1962), and suspended 10–30 m above the ground using a 1.2-m chloroprene balloon filled with helium. Sampled drones were preserved in 95 % ethanol for transport to the laboratory where they were kept at –80 °C until molecular processing. Total DNA was extracted from a hind leg of each drone with 10 % Chelex extraction (Walsh et al. 1991) and maternal ancestry determined using a PCR-RFLP technique (Pinto et al. 2003) for a mtDNA Cytochrome b gene diagnostic marker (Crozier et al. 1991). Additionally, drones were determined visually to be wild type or *cd*.

In total, 829 *cd* drones were collected (Table 1). One hundred twenty-five (125) *cd* drones were trapped at DCAs >2.8 km from any managed colonies. Fourteen of the 125 *cd* individuals (or 11.2 %) had an African matriline. Moreover, 7 of the 22 (or 31.82 %) African matriline drones trapped at DCAs within 0.25 km of the managed colonies were *cd*. These data demonstrate that

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Figure 1 Feral drones trapped at drone congregation areas in central Florida. Drones **a**, **b**, **c**, and **d** have European mtDNA. Drones **e**, **f**, **g**, and **h** have African mtDNA. Examples of *cd* (**a**, **b**, **e**, **f**) and wildtype (**c**, **d**, **g**, **h**) cuticular colorations are pictured for both European and African matrilines.

Table 1. Characteristics of drones trapped at drone congregation areas (DCAs) >2.8 km from or within 0.25 km of 96 managed European-derived honey bee colonies headed by cordovan (*cd*) queens.

Proximity to managed <i>cd</i> colonies (km)	# drones collected	# <i>cd</i>	# African matriline	# <i>cd</i> and African matriline	% Percentage African matriline + <i>cd</i>
>2.8	1,200	125	412	14	3.40
0.25	1,200	704	22	7	31.82
Total	2,400	829	434	21	4.84

Data are the number of drones collected at the respective DCA (# *drones collected*), the number of those drones with the *cd* phenotype (#*cd*), African matriline (# *African matriline*), and both (# *cd* and *African matriline*). The percentage of African drones that were *cd* (% *African matriline* + *cd*) was determined by dividing the number of African matriline drones that were *cd* by the total number of African matriline drones collected $\times 100$

the *cd* phenotype can be associated with both African and European matriline, and the *cd* mutation has been integrated into the feral African-derived population in central Florida.

There is a high degree of hybridization between African-derived and European-derived honey bees at the southern front of the African expansion in Argentina (Taylor 1977; Sheppard et al. 1991). We suspect a similar pattern at the northern front in Florida where African-derived honey bees have not expanded northward since the boundary's establishment in 2005 (Agriculture Research Services 2011). We hypothesize that this hybridization led to the incorporation of the *cd* phenotype into the feral African-derived population in the Southeastern United States.

Under carefully controlled experimental designs, the *cd* allele will continue to be an informative research tool. However, care should be taken to ensure that feral *cd* individuals are not misinterpreted or misidentified within experimental conditions, especially in regions where beekeepers manage *cd* queens and feral African-derived colonies exist.

ACKNOWLEDGMENTS

We graciously thank Arthur Mathisen for coordination and use of the experimental apiary locations and D&J Apiary for providing and managing European-derived honey bee colonies. Funding for this project was provided by the Florida Department of Agricultural and Consumer Services (FDACS) through the guidance of the Honey Bee Technical Council.

Note scientifique sur la prévalence du phénotype 'cordovan' dans la population d'abeilles africanisées du Sud-Est des Etats-Unis

Eine wissenschaftliche Notiz zur Vorherrschaft des Cordovan-Phänotyps in afrikanisierten Honigbienenpopulationen im Südwesten der Vereinigten Staaten

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