

Genetic Control of the Honey Bee (*Apis mellifera*) Dance Language: Segregating Dance Forms in a Backcrossed Colony

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We studied the genetic control of the dance dialects that exist in the different subspecies of honey bees (*Apis mellifera*) by observing the variation in dance form observed in a backcross between two lines that showed widely different dance dialects. To do this we generated the reciprocal of the cross performed by Rinderer and Beaman (1995), thus producing phenotypic segregation of dance forms within a single colony rather than between colonies. Our results are consistent with Rinderer and Beaman (1995) in that inheritance of the transition point from round dancing → waggle dancing is consistent with control by a single locus with more than one allele. That is, we found one dance type to be dominant in the F_1 , and observed a 1:1 segregation of dance in a backcross involving the F_1 and the recessive parent. However, we found some minor differences in dance dialect inheritance, with the most significant being an apparent reversal of dominance between our cross (for us “black” is the dominant dialect) and that of Rinderer and Beaman (1995) (they report “yellow” to be the dominant dialect). We also found that our black bees do not perform a distinct sickle dance, whereas the black bees used by Rinderer and Beaman (1995) did perform such a dance. However, our difference in dominance need not contradict the results of Rinderer and Beaman (1995), as there is no evidence that body color and dominance for dance dialect are linked.

Honey bee foragers communicate the location of rich forage patches to their nestmates via a well-characterized symbolic dance language (Lindauer 1985; von Frisch 1993). The dance encodes both distance and directional information. The information contained within the dance is used by recruits to help them locate new sources of forage.

Dances can be classified into three broad forms (von Frisch 1993). The type of dance performed is related to the distance of the forage source (Figure 1). For sources near the colony, a simple round dance is performed. At greater distances, a sickle dance is performed. Finally, at the furthest distances from the nest, a waggle dance is performed. The waggle dance encodes the most complex information of all the dances. The angle (as displaced from vertical in *Apis mellifera*) and number of waggles in the waggling run of the dance translate to direction and distance, respectively, to the food source at which the dancer had most recently foraged (Gould 1974, 1975a; von Frisch 1993).

Dialect Differences in Dance Behavior

The distances at which dances change from one form to the next are variable (for

a review, see von Frisch [1993:293–320]). Dance “dialect” describes the distances at which foragers of each *Apis* subspecies make the transition between dance types, that is, from round → sickle or sickle → waggle. The “transition points” at which the different subspecies change between the different dance types are proposed to have evolved as adaptations to the typical foraging range of each subspecies (Gould [1975b, 1982], but also see Dyer [1991] and Dyer and Seeley [1991]). There also appears to be a relationship between dance tempo and distance of the forage source which varies widely between honeybee races (see von Frisch [1993, Figure 254]). A recent study (Esch et al. 2001) suggested that these differences may be environmental rather than genetic in origin. However, Rinderer and Beaman (1995) studied the genetic basis for some of the observed differences in dance dialect. Their results strongly suggest that each of the transition points (round → sickle, sickle → waggle) is controlled by a single gene with multiple alleles (at least two).

In the Rinderer and Beaman study, the direction of the cross generated the segregation of phenotypes among colonies. In

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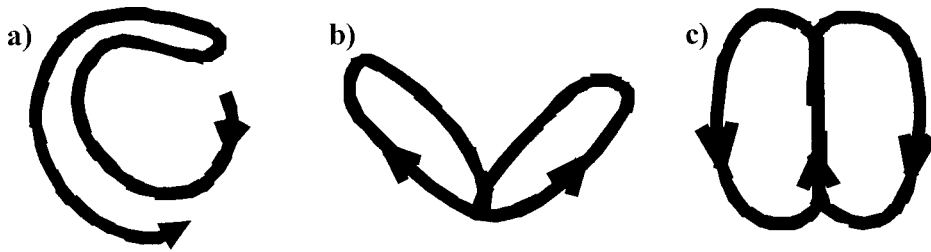


Figure 1. (a) Round dance. (b) Sickle dance. (c) Waggle dance.

our study we performed the reciprocal cross so that the segregation of dance phenotypes occurred within a single colony. Figure 2 shows the differences between the two crossing protocols. Our design has the advantage of standardizing the environment in which foragers dance. That is, all foragers experience an identical environment, which has recently been shown to be very important for these kinds of studies (Esch et al. 2001). Therefore any phenotypic differences in dance form are more likely to be genetically based.

The age of a bee is known to affect waggle speed when communicating long distances (von Frisch 1993). Older bees waggle significantly more slowly than new foragers for a given forage source. We also investigated whether age had an effect on the transition dance distance.

Materials and Methods

Crossing

Two parent colonies were obtained for this study. One colony of yellow bees was classified purebred *A. m. ligustica* by the supplier (J. Horner and Sons, Rylston), who uses controlled mating to provide breeding stock to the Australian honey industry. The second colony of black bees was asserted to be purebred *A. m. carnica* (also supplied by J. Horner and Sons, Rylston). After determining the dance characteristics of 25–35 foragers of these two colonies, two virgin queens were reared from the yellow parent and each instrumentally inseminated with semen of single sons of the black parent (Figure 2). An F_1 queen was reared from one of these colonies and this queen was backcrossed to a single son of the yellow parent, following indications from the dance characteristics of 57 individuals from the F_1 colonies that the black parental phenotype was genetically dominant.

Dance Characteristics

Individual workers from the two parental colonies and the F_1 were all evaluated for

the foraging distance at which they changed from transition dances to waggle dances (Figure 1). This was done by studying the dance behavior of bees that had recently fed at a feeding station containing sugar solution (2.0 M sucrose solution with 1 μ l orange essence per liter: 1×10^{-6} dilution) at each 10 m interval from their colony up to 70 m. For the backcross colony, dance forms were evaluated at the 56 m distance only. The same observation hive and location were used for each colony and bees were required to follow the same trajectory to reach the feeding station. The 56 m distance was determined by evaluating dance behavior at 10 m intervals (10–70 m) from the hive. Fifty-six meters was determined to be the distance at which foraging individuals appeared to perform one of two dance types (classified as round or waggle).

To perform the evaluation, individual workers found feeding at the dish were marked using numbered queen-marking disks (Opalithplättchen mit). After marked bees made several successful foraging trips, we scored them for dance phenotype immediately after they had returned to the observation hive from a successful trip to the feeder. As there is well documented intrabee variation (Rinderer and Beaman 1995) in dance forms, a bee was only scored for dance phenotype after it executed at least five continuous circuits of the dance on at least three separate occasions.

Results

Parental and F_1 Phenotypes

The parental colonies (yellow and black) showed distinct dance types (Figure 3). With the yellow parent making the transition from round to sickle at 20 m and sickle to waggle at 40 m (Figure 3a). Conversely, the black parent was still performing a round dance at distances beyond 30 m, not performing a definite sickle dance, and wagging at distances beyond 60 m (Figure 3b). Both replicate F_1 colonies behaved predominantly like the black parent (most

consistent with an *A. m. carnica* phenotype), and so the black phenotype was deemed “dominant” (Figure 3c). Only the yellow parent performed a distinct sickle dance, whereas it was absent in the black parent (consistent with previous observations of *A. m. carnica*; von Frisch [1993, Figure 254]).

Backcross Phenotype

In the backcross colony there was segregation in dancing behavior among backcross foragers into two broad classes when workers were foraging at a distance of 56 m from the observation hive. At this discriminating distance, 202 individuals were characterized, of which 111 bees performed round dances and 91 performed waggle dances. Dance scoring was found to be consistent across days and was independently confirmed by three separate observers at the 56 m distance. At distances beyond 60 m, all bees from the F_2 backcross performed a waggle dance.

A 1:1 ratio is expected if inheritance follows a single-gene dominant/recessive model per transition point, as proposed by Rinderer and Beaman (1995). The number of round and waggle dancers were not significantly different from a 1:1 ratio of dance types when compared using a chi-square test ($\chi^2 = 0.99$; $.75 < P < .5$).

Age Effect Results

The majority of individuals (81%) tracked for their entire foraging history did not change their dance type over the duration of the experiment. Of the bees that did appear to change, 6% of workers changed from round to waggle and 13% from waggle to round.

Discussion

We confirm previous work (Rinderer and Beaman 1995) that suggests the waggle transition point is inherited as a single gene showing simple Mendelian inheritance. By crossing two colonies with distinct dance dialects we established that one dialect was dominant and used queens from this F_1 to backcross with recessive males. The backcross progeny showed both parental dance dialects (in the form of round and waggle dances) when we fed them at a 56 m discriminating distance (Figure 3).

Investigations into age effects on dance phenotype at the 56 m discriminating distance indicate age does not affect the distance at which bees make the transition between dance types. The proportion of

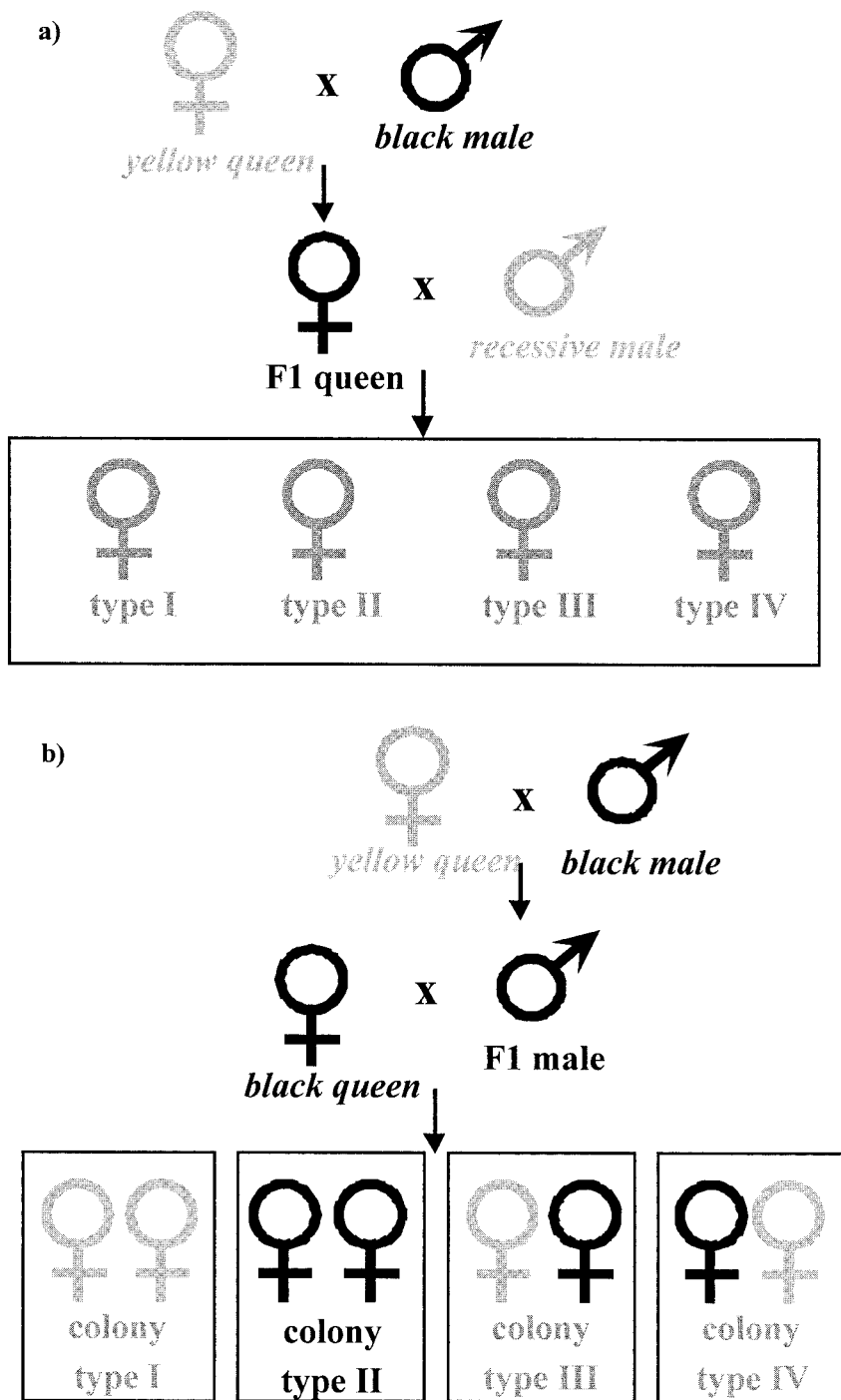


Figure 2. For comparison of the two crosses: (a) Diagrammatic representation of the reciprocal crosses performed in this study. We raised queens from the F₁ and backcrossed to the putative recessive male. Following this, the F₂ backcross progeny should exhibit both parental transition points in a 1:1:1:1 ratio (i.e., type I, both transition distances as for the yellow parent; type II, both transition distances as for the black parent; type III, round → sickle transition distance like that of the yellow parent and sickle → waggle distance like that of the black parent; type IV, round → sickle transition distance of the black parent and sickle → waggle distance like that of the yellow parent). (b) An abbreviated diagram of the Rinderer and Beaman (1995) crosses. In contrast to ours, Rinderer and Beaman raised F₁ males and crossed them to queens from the recessive parental type. This resulted in four possible colony types (represented as separate boxes below; colony type I, both transition distances as for the yellow parent; colony type II, both transition distances as for the black parent; colony type III, round → sickle transition distance like that of the yellow parent and sickle → waggle distance like that of the black parent; colony type IV, round → sickle transition distance of the black parent and sickle → waggle distance like that of the yellow parent).

bees that changed over the course of our experiment included bees that changed from round to waggle and from waggle to round, indicating no directionality. We believe that by only scoring individuals who performed the same dance three times, age effect in our sample is negligible, and for the majority of individuals, transition distance is extremely stable. Contrasting with the apparent stability of transition distance (i.e., 56 m in our cross), dance tempo was found to be variable within a hive at longer foraging distances (results not shown).

Our yellow (*A. m. carnica*) bees behaved as we expected based on previous work (Boch 1957; Rinderer and Beaman 1995; von Frisch 1993). However, our black (*A. m. carnica*) bees not only had a longer dialect (i.e., only perform the waggle dance after having traveled a longer distance) than that observed by Rinderer and Beaman, but also lacked the sickle dance reported in the same study. Conversely, our black bees had a shorter dialect than that observed by Boch (1957) and von Frisch (1993), who found that their bees lacked a sickle dance, as did ours. Our F₁ and F₂ colonies did not perform sickle dances, and so the transition point between round dances and sickle dances could not be evaluated. Regardless, we still observed within-colony variation in the F₂ backcross for dance type at the discriminating distance.

As for dominance of a particular dialect, Rinderer and Beaman found that the yellow subspecies (*A. m. ligustica*) with the shorter dialect was dominant when crossed with their black bees. In contrast, we found that black bees (*A. m. carnica*) exhibiting the longer dialect showed the dominant dance phenotype when crossed with our yellow bees. Since there is no apparent linkage between dominance for dance phenotype and body color, it could be reasonably expected that these two scorable characteristics segregate independently. Body color is a poor measure of racial origin in honeybees, and we regard our results as entirely consistent with those of Rinderer and Beaman (1995), as we are working with completely different genetic material.

From our results and those of Rinderer and Beaman, it is reasonable to suppose that there are a series of alleles determining the threshold distance for the waggle → round transition, and that these have a dominance hierarchy reminiscent of that known from the mouse agouti locus (Siracusa et al. 1987). Our black bees are thus

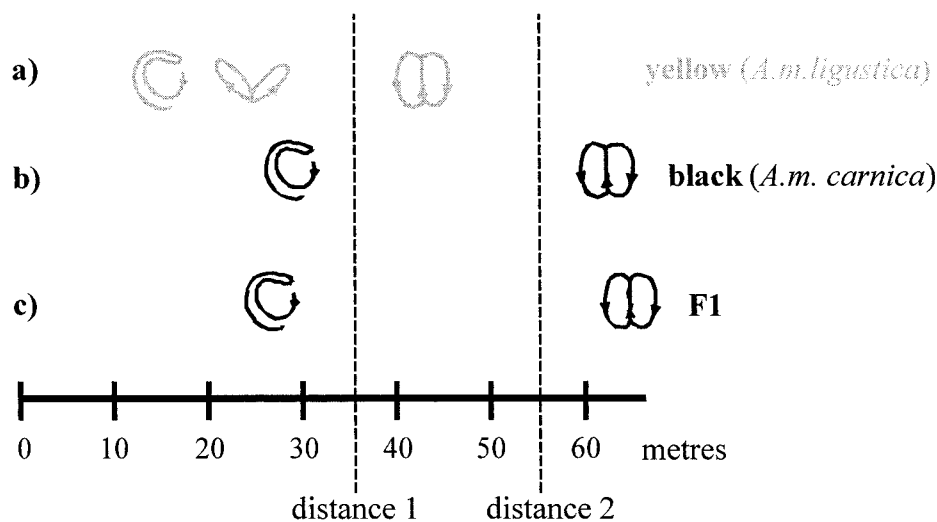


Figure 3. Dance dialects for each of the parental types, yellow and black, used for the F_1 and F_2 backcrosses. The F_1 dance dialect is also shown. The dashed lines indicate two theoretical distances that discriminate between the two parental types (distance 1, round \rightarrow sickle; distance 2, round \rightarrow waggle).

interpretable as carrying an allele dominant over that carried by the yellow bees used, whereas the black bees used by Rinderer and Beaman are interpretable as carrying an allele recessive to that of their yellow bees.

Our results are most consistent with that expected by a single locus. As the next most parsimonious explanation, we consider a model involving two unlinked loci where a hypothetical yellow parent is double recessive (aabb) and a black parent is double dominant (AABB). An F_1 would be a double heterozygote (AaBb) and could be backcrossed to the double recessive (aabb) to create an F_2 backcross. Under this hypothesis, the expected ratio of round to waggle in the F_2 backcross progeny would be 1:3 at the 56 m distance (if the homozygote recessive behaves like that of the yellow parent, the two single heterozygotes at some intermediate distance, and the double heterozygote like that of the black parent). Under a two-locus model, the round phenotype would be expected 25% of the time at the 56 m discriminating distance. We report the round phenotype at a frequency of 0.55, with upper and lower confidence limits of 0.62 and 0.48, respectively, significantly lower than that expected under the two-locus model. Therefore, the two-locus model, as

the next most plausible model, can be rejected.

The results reported here are in agreement with previous findings that dance is a complex honey bee behavior under simple genetic control. This is not unusual in honeybees, as there are several examples of strong genetic influence of behavior in the genus *Apis*. These include such behaviors as hygienic behavior (Moritz 1988; Rothernbuhler 1964), defensive behavior (Hunt et al. 1997), foraging behavior (Hunt et al. 1995), stinging behavior (Hunt et al. 1998), and tracheal mite resistance (Nasr et al. 2001). The male-haploid mechanism of sex determination in Hymenoptera greatly facilitates such studies. Cornerstone work on *A. mellifera* dance behavior by Boch (1956, 1957) and von Frisch (1993) identified a wide range of dialects across a large number of subspecies (but see Esch et al. [2001], where it is proposed that racial differences may be based on environment). Remarkably the amount of genetic variance required to account for all the dialect variance of *A. mellifera* can still be adequately explained by a simple model involving two or three loci with multiple alleles. Hopefully future work will reveal the full complement, as studies to date, including ours, have only

identified a minimal number of these alleles.

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