DIFFERENCES IN LEARNING PERFORMANCE AND RELATED BEHAVIORS ACROSS THREE HONEY BEE SUBSPECIES FROM ANATOLIA

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ABSTRACT

There are at least five honey bee subspecies found in different ecological regions in Turkey, such as Carniolan Honey Bee (*A. m. carnica*) in temperate, north-west, Syrian Honey Bee (*A. m. syriaca*) in subtropical south, and Caucasian Honey Bee (*A. m. caucasica*) in subarctic north-east. Members of these subspecies are living in different environments, and probably differentiated over 10s of thousands of years. The subspecies exhibit traits, potentially indicative of local adaptation. Besides morphological differences, there are life history differences that may relate to foraging environment. In this study we examined differences in learning performance and locomotor activity across these three subspecies. To this end, we used three laboratory experimental setups: We monitored daily locomotor activities of honey bees in LAMs (Large Activity Monitors), we used electric shock avoidance (ESA) conditioning assay, and a Proboscis Extension Response (PER) conditioning reversal learning assay. First, in monitor experiments, we measured the daily activity of honey bees in dark, optimum (hive-like) humidity, temperature conditions for 24 hours. Second, in ESA experiment we studied punishment learning performance of honey bees. Third, in PER reversal learning task we examined changes in stimulus-response association as a result of changes in stimulus–reward contingencies. Overall results show differences across these three subspecies in learning performance and locomotor behavior.

INTRODUCTION

Five different subspecies of A. mellifera - which are *A. m. meda, A. m. syriaca, A. m. caucasica, A. m. anatoliaca* and *A. m. carnica* found in Turkey (Ruttner 1988, Kandemir *et al.,* 2005). Their differentiation should be affected with glaciation and deglaciation events, then they probably differentiated over 10s of thousands of years. The subspecies exhibit traits, potentially indicative of local adaptation, determined by diverse climatic, topographical and floristic variations available. As a result of these local adaptation, morphological differences can be observed across the subspecies likewise differences in body size, colorization, vein pattern of wings (Ruttner 1988, Nawrocka *et al*., 2017). Besides morphological differences, there are life history differences that may relate to foraging environment. In this study we examined differences in learning performance and locomotor activity across three subspecies, these are Carniolan Honey Bee (*A. m. carnica*) found in temperate, north-west, Syrian Honey Bee (*A. m. syriaca*) found in subtropical south, and Caucasian Honey Bee (*A. m. caucasica*) found in subarctic north-east. To this end, we used three laboratory experimental setups: We monitored daily locomotor activities of honey bees in LAMs (Large Activity Monitors), we used electric shock avoidance (ESA) conditioning assay, and a

Proboscis Extension Response (PER) conditioning reversal learning assay. First, in monitor experiments, we measured the daily activity of honey bees in dark, optimum (hive-like) humidity, temperature conditions for 24 hours. We used modified *Drosophila* activity monitoring (DAM) system that called as LAM and it was used previous researches (Giannoni-Guzmán *et al*., 2014), allowed us to measure locomotor rhythms of the three honey bee subspecies. Second, in ESA experiment we studied punishment learning performance of honey bees. This test also was used previous researches (Agarval *et al*., 2011), basically in test individuals were presented two colors, one color was paired with electric shock and the other was not paired with electric shock. Third, in PER reversal learning task, which was commonly used on both *Drosophila* and honey bees (Abramson *et al*., 2015), we examined changes in stimulus-response association as a result of changes in stimulus–reward contingencies. Overall results show differences across these three subspecies in learning performance and locomotor behavior.

MATERIALS AND METHODS

Activity Monitoring

We used Trikinetics Inc. (Waltham, MA, USA) locomotor activity monitors Each unit has 32 independent activity channels, which measure activity by using three infrared beams and sensors to ensure that recordings are accurate. Activity data was collected for 24 hours by each minute. Honey bees were accommodated in dark, optimum (hive-like) humidity, temperature conditions with *ad libitum* feeding in 15 ml centrifuge tubes, placed to experiment setup.

Electric Shock Avoidance

We used a shuttle box apparatus measured 15 cm long by 2 cm wide and contained an electric shock grid with wires spaced .35 cm apart. The shock was presented blue colored side of the apparatus. Shock intensity was 6 V 50 mA DC from an analog power supply. Yellow colored side of apparatus was not paired with the electric shock. An observer recorded the time spent on the shock side, non-shock side and counted the number of the crossing border between the sides.

PER Reversal Learning

Strawberry and lemon odors used as conditioned stimulus (CS). Odors are conveyed to honey bees with air flow from 50 cc plastic syringes, used as “odor cartridge”, contain 1 cm2 piece of filter paper absorbed essential oil of single fruit. One of the conditional stimulus paired with sucrose feeding (CS+) and the other not (CS-). PER reversal learning experiment consist of two phases. In acquisition phase, initial discrimination training was realized. Then, reversal phase, odor used as CS- shifted to CS+ and *vice versa* for odor used as CS+ in acquisition phase. Each phase contains 12 trails with sequence of CS+, CS-, CS-, CS+, CS-, CS+, CS+, CS-, CS+, CS-, CS-, CS+.

RESULTS

Activity Monitoring

Experiments begins at 06:00 pm and data collected from three subspecies as *A. m. caucasi*ca (n=34), *A. m. carnica* (n=34) and *A. m. syriaca* (n=24) for 24 hours. Averages and standard errors calculated for each hour. According to 24 hours activity monitoring graph Caucasian subspecies’ activity is higher than others between 08:00 am – 09:00 am and between 04:00 pm – 07:00 pm. However, between 10:00 am – 02:00 pm, Syrian subspecies' activity is higher than other subspecies. Carniolan Honey Bees almost have the lowest activity than other 2 subspecies. Also, most active hours differentiate between the subspecies, to illustrate Syrian subspecies reaches higher activity at 02:00 pm, Carniolan Bees reach maximum activity at 04:00 pm and Caucasian reaches at 05:00 pm. According to daily activity comparison of subspecies, daily rhythm patterns prominently differs.

(Figure 1)

Electric Shock Avoidance

Individuals from each subspecies of honeybees, *A. m. caucasi*ca (n=31), *A. m. carnica* (n=60) and *A. m. syriaca* (n=39) were observed in 5 min experiment session. Time spent on the shock side and non-shock side were recorded and the number of the crossing border between the sides was counted. We develop a formulation to calculate the learning performance scores of individuals, which is

$$\frac{\sum t_n - \sum t_s}{cn * t_e}$$

t_n is elapsed time in non-shock area, t_s is elapsed time in shock area, *cn* is the total count of the crossing border between the sides, t_e is total duration of experiment, which is 300 seconds. Kolmogorov-Smirnov Test show that the distributions does not fit to the normal distribution. Then, we applied Kruskal-Wallis Test (H = 18.29, DF = 2, P = 0.000), according to Kruskal-Wallis test Caucasian Honey Bees statistically differ from the other subspecies. Thus, because of Caucasian Honey Bees have higher scores, it can be said that *A. m. caucasica* has higher learning performance then other subspecies.

(Figure 2)

PER Reversal Learning

We record the proboscis extension responses for each trail then we divide them to sample size of the subspecies', which are *A. m. caucasi*ca (n=29), *A. m. carnica* (n=37) and *A. m. syriaca* (n=28). Then, we create a proboscis extension responses ratio for each trail. Thus, it can be seen that, all subspecies has a similar learning rate for the A+ in the acquisition phase (Figure 3A), however Syrian Honey Bees falsely give significantly more proboscis extension response to B- in acquisition phase (Figure 3B). In the reversal phase, Syrian Honey Bees gives more response to both B+ and A- (Figure 3C and 3D). It can be said that; Syrian Honey Bees have poor selectivity to positive conditioned stimulus. Also we used the sensitivity scores (d'), which is an index of the detectability or discriminability of a signal, calculated as d' = Z(hit rate) − Z(false alarm rate). In our case hit is proboscis extended with CS+ and false alarm is proboscis extended with CS-. We applied Kolmogorov-Smirnov Test on d' scores, distribution of the scores of samples fit the normal distribution. Comparison for acquisition phase, ANOVA test used (F = 4.19, P = 0.018) and Syrian

Honey Bees have lowest success, however when ANOVA test used for reversal phase there is no difference found across subspecies ($F = 2.81$, $P = 0.066$).

REFERENCES

Abramson, C. I., Craig, D. P. A., Varnon, C. A., & Wells, H. (2015). The effect of ethanol on reversal learning in honey bees (Apis mellifera anatolica): Response inhibition in a social insect model. *Alcohol*, *49*(3), 245–258.

Agarwal, M., Guzmán, M. G., Morales-Matos, C., Del Valle Díaz, R. A., Abramson, C. I., & Giray, T. (2011). Dopamine and octopamine influence avoidance learning of honey bees in a place preference assay. *PLoS ONE*, *6*(9), 1–9.

Giannoni-Guzmán, M. A., Avalos, A., Perez, J. M., Loperena, E. J. O., Kayım, M., Medina, J. A., Massey, S. E., Kence, M., Kence, A., Giray, T., & Agosto-Rivera, J. L. (2014). Measuring individual locomotor rhythms in honey bees, paper wasps and other similar-sized insects. *The Journal of Experimental Biology*, *217*(8), 1307–1315.

Kandemir, I., Kence, M., & Kence, A. (2005). Morphometric and electrophoretic variation in different honey bee (Apis mellifera L.) populations. *Turk J Vet Anim Sci, 29*, 885-890

Nawrocka, A., Kandemir, İ., Fuchs, S., & Tofilski, A. (2017). Computer software for identification of honey bee subspecies and evolutionary lineages. *Apidologie*.

Ruttner, F. (1988). *Biogeography and taxonomy of honey bees*. Springer-Verlag.

FIGURES

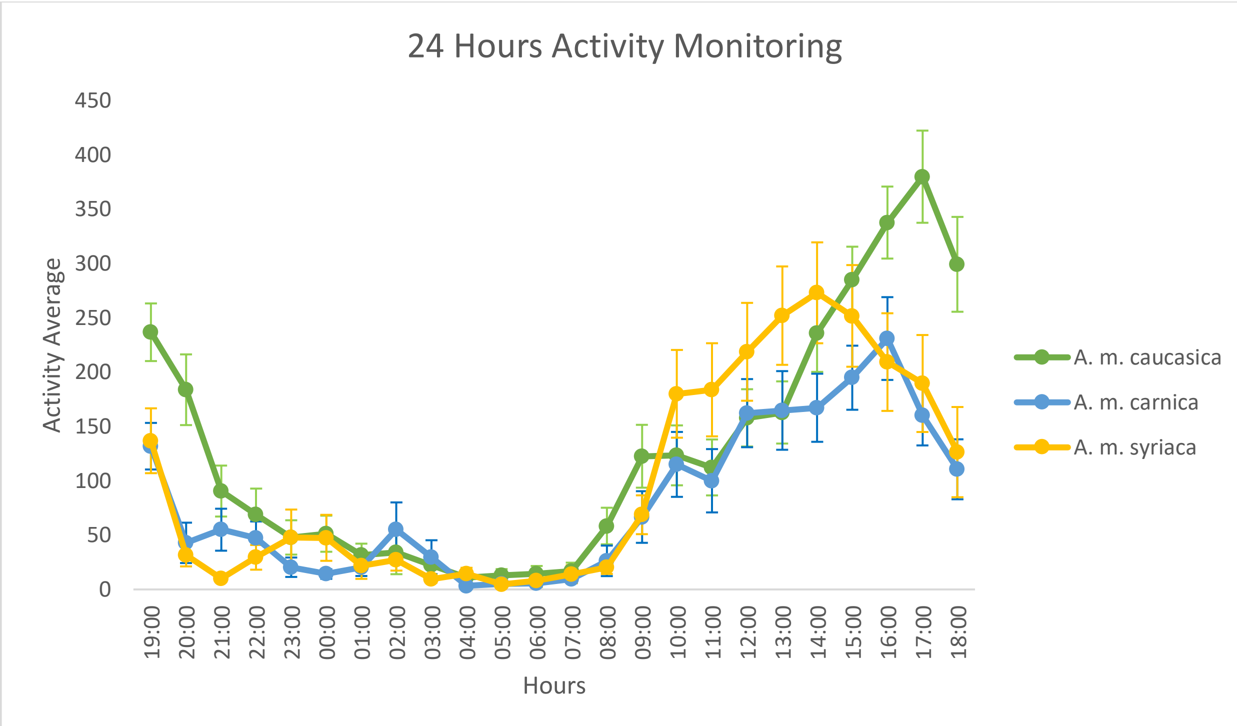

Figure 1

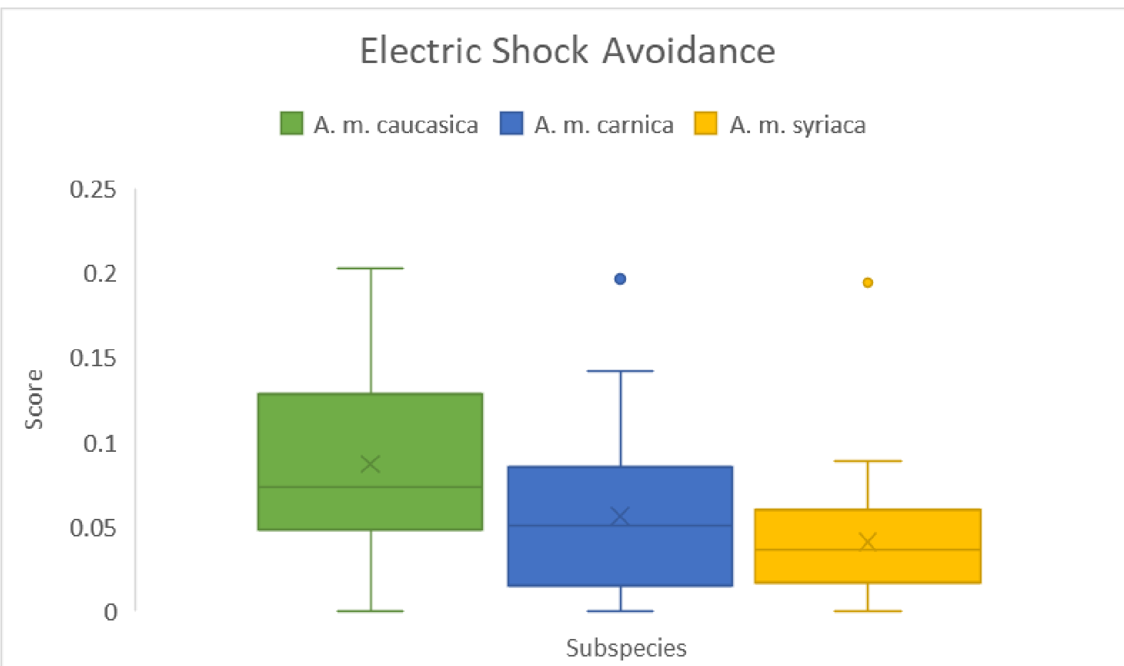

Figure 2

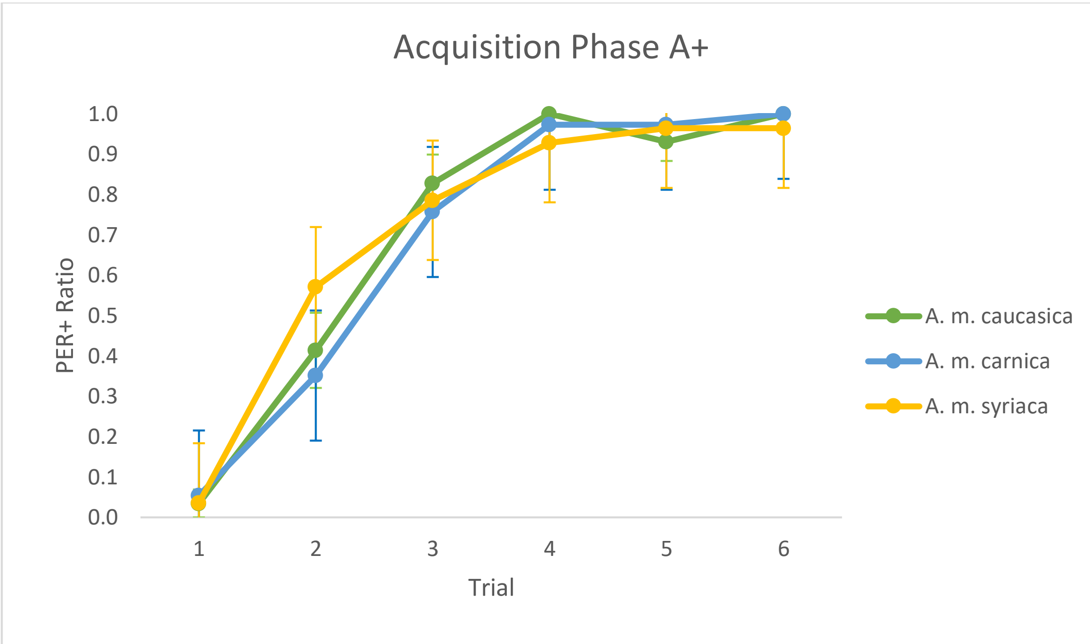

Figure 3A

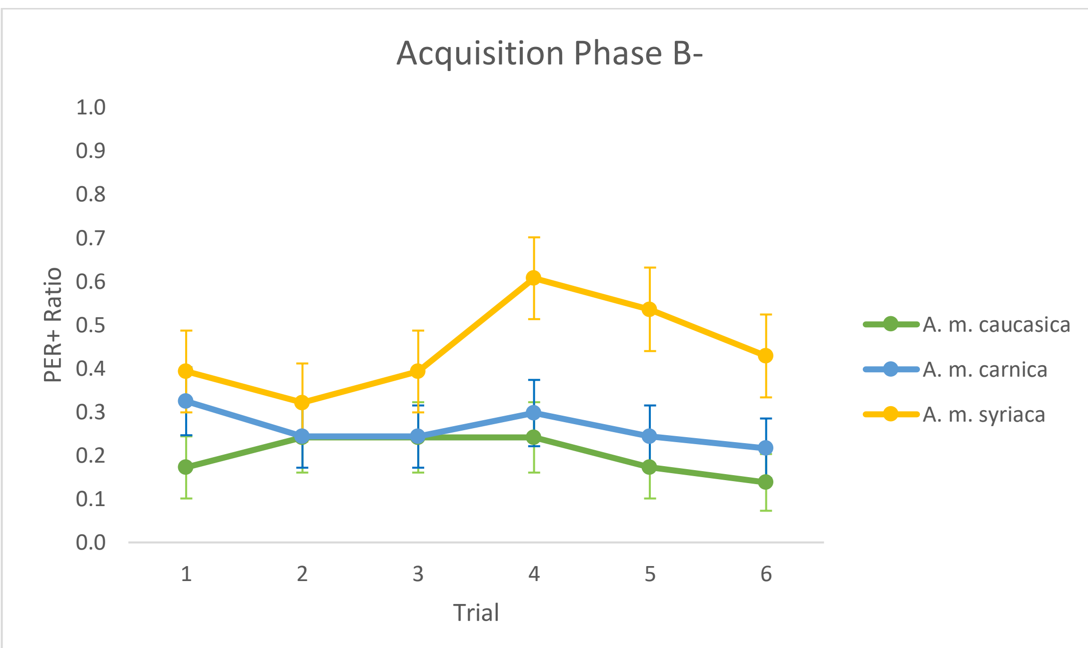

Figure 3B

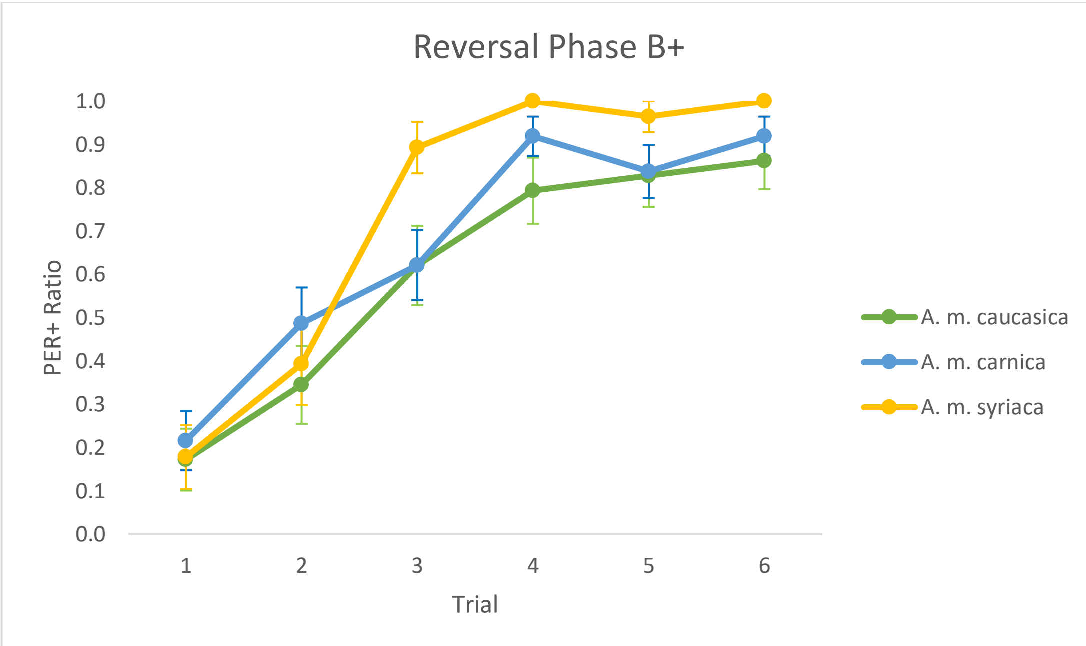

Figure 3C

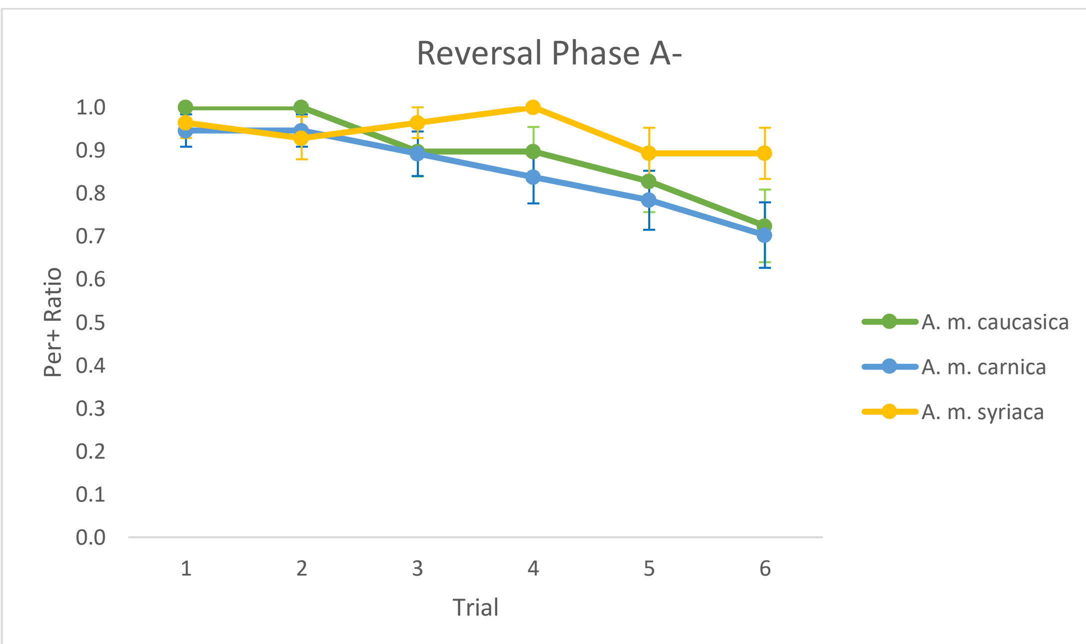

Figure 3D