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Artificial Feeding and Wing Symmetry in Drone Honeybees

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ABSTRACT

Wing asymmetry was used to measure the possible stress which could be created when we feed honeybee colony with cane and beet sugar syrup in comparison with feeding with honey (control). For this purpose, 25 traits of wing venation pattern on left and right forewing of drone honey bees were used. The results indicated that directional asymmetry (DA) was not related to the type of feeding. Fluctuating asymmetry (FA) differed between characters and between feeding treatments, but no interaction was found between them. The individual characters were combined and analyzed as composite fluctuating asymmetry (CFA) to maximize the probability of detecting (FA)-stress relationship when it exists. The result showed that (CFA) of vein angels were significantly higher in sugar-feeding than in honey-feeding colonies.

Keywords: Honeybees, drones, forewing venation, vein distance, vein angels, polar coordination, fluctuating asymmetry, directional asymmetry, composite fluctuating asymmetry.

INTRODUCTION

Adequate feeding of honey bee colonies is an important and essential factor to produce sexually mature drones in high quantity and quality, which ensure an adequate mating with virgin queens and consequently producing strong honeybee colonies. So, nutrition is a honey bee colony's basis for growth, development and reproduction. The natural carbohydrate source of honey bees is nectar or honeydew, collected by foragers from plants, transported to the hive and finally stored in sealed cells as honey. The transformation from nectar to honey is a gradual process and begins in the foragers during their returning flight (Nicolson and Human, 2008). Nectar is very important to honey bee drones as a source of energy especially during their flying when they are feeding on it after its transformation to honey. During unsuitable condition, or shortage of natural sources of nectar, beekeepers tend to feed their colonies with alternatives to nectar (i.e. sugar feeding, such as, sucrose solution, invert sugars, high fructose solution or various fruit syrups (Neupane and Thapa, 2005) inside the hive.) to stimulate brood rearing whether worker or drone brood. .

During development of organisms, there are many unfavorable conditions, to which they may expose and could weak their optimal growth or some physiological functions (Møller and Thornhill 1998; Field and Yuval 1999; Schmid-Hempel 2003) So, the phenotype of them may not able to reflect accurately their expected genotype (Palmer and Strobeck 1986). Accordingly, departures from the ideal expression of particular traits are expected which could be used as indicator that organisms have been exposed to sub-optimal resources as mediated through environmental or genetic constraints (Schmid-Hempel, 2003). Mature honeybee drones (*Apis mellifera*) fly to specific areas, called drone congregation area (DCA), to mate with virgin queens. Honeybee colonies rear a large number of drones, from which only few number succeed in copulating with a queen. Accordingly, one expects that strong selection is being acting on adult drones during their mating flights, and any specific character which may be found in drones in these areas may give an indirect indication of the characters on which the selection acts.

One of the most relevant morphological characteristics being used as stress-bioindicator for measuring developmental instability in many insects is wing fluctuating asymmetry (FA) (Parsons and Hoffmann 1991; Clarke and McKenzie 1992; Bjorksten *et al*. 2000; Rantala, *et al*. 2004). So, the aim of our study was to describe the possible effect of artificial feeding on the fore wing asymmetry of honeybee drones.

MATERIALS AND METHODS

This experiment was carried out in the Apiary of Beekeeping Research Center at the Ministry of Agriculture in 2016 and 2017. The treatments were applied on 12 colonies belonging to Carniolan race (*A.m carnica)* each supplied with one kilogram of bee worker on 3 empty combs. The queens were all sisters and mated naturally in Manzala (where Carniolan bee are preserved purely) at the apiary, from which the experimental colonies were prepared. The colonies were divided randomly into 4 groups each of three colonies, and were fed with different types of sugar nutrition:

I. Sugar cane syrup: Sucrose-water solution 1:1.
II. Sugar beet syrup: Sucrose-water solution 1:1.
III. Sugar beet & Citrus honey syrup: Honey-Sucrose-water solution 0.5: 0.5 :1.
IV. Citrus honey syrup (Control): Honey-water solution 1:1.

Each colony was fed twice a week by one-liter solution. Each group was fed exclusively with its treatment for 6 weeks before taking of bee samples. From each colony in each group, a drone sealed brood comb were taken and put in a cage into their colony until emerging of drone bees, then about 15 drones were collected from each group and preserved in ethanol 70% until dissection.

From each honeybee drone, the pair of forewings was dissected, and then they were air-dried to the glass slides while ethanol evaporated, and the dry mounts were digitally photographed by a slide-scanner.

1. Asymmetry of wing venation:

The forewing measuring characters were chosen according to Kauhausen (2002) and Szymula, *et al*. (2010), who used polar coordinate system for description the location of the different intervenation points in terms of lengths and angles between these points. The number of points chosen was 17 (Fig. 1). The first point has no values on the coordinate system (X = 0 and Y = 0). The second point however was represented by one value on relative to the first reference point.

All the remaining points are represented by two values, so the number of the coordinates is (2 X 17) – 3 = 31 points, which represent the measured characters (Table, 1). The coordinates characters measured represent 16 intervention distances and 15 angles coordinates. For technical reasons and to ensure precision of the measurements values, 6 characters were excluded from the results.

Table 1. Characters measured on the forewing

No	Distance (L) and Angles (A) between coordinates	Morphometrical characters in relation to the first coordinates (1,2) (Fig 1)	No	Distance (L) and Angles (A) between coordinates	Morphometrical characters in relation to the first coordinates (1,2) (Fig 1)
1	L1	1-2	17	A9	*2-1-10
2	L2	1-3	18	L10	*1-11
3	A2	2-1-3	19	A10	*2-1-11
4	L3	1-4	20	L11	*1-12
5	A3	2-1-4	21	A11	*2-1-12
6	L4	1-5	22	L12	1-13
7	A4	2-1-5	23	A12	2-1-13
8	L5	1-6	24	L13	1-14
9	A5	2-1-6	25	A13	2-1-14
10	L6	1-7	26	L14	1-15
11	A6	2-1-7	27	A14	2-1-15
12	L7	1-8	28	L15	1-16
13	A7	2-1-8	29	A15	2-1-16
14	L8	1-9	30	L16	1-17
15	A8	2-1-9	31	A16	2-1-17
16	*L9	1-10			

*** characters being excluded from the analysis**

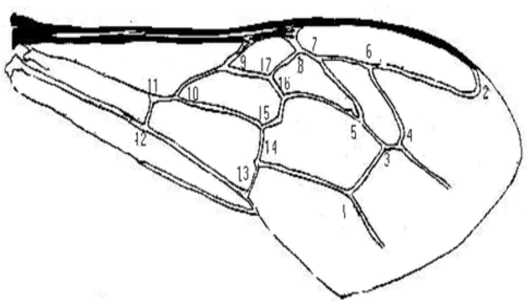

Fig. 1. Forewing of worker bees showing 17 points used to establish wing coordinates

1. Equipment and measurement procedure

The morphometrical system used consisted of the following parts:

1. Computer unit with a suitable analyzing program developed by Kauhausen (2002).
2. A Slide-Scanner (Minolta Dimâge Scan Dual ll).

The wing measurements were made by using a 35 mm slide projector to project the images of mounted wings onto a monitor screen. A particular type of slide known as a Gepe consists of two plastic half frames containing thin metal masks and each half frame is separately glazed.

From each honeybee drone, the left and right forewings were cut off at the base with a fine forceps and dry-mounted onto slides. Each slide bear 15 wings and each 4 slides were put in a slide mount holder, which was then put inside the Scanner, in order to scan the mounted wings. After setting a suitable display-program, the wings were displayed on the screen monitor of a computer, and by the computer-mouse, the different intervention points were marked. The measuring-program converted these coordinate points into actual lengths and angles of the different intervention points relative to the first two points. The converted measurements are then stored in a new file.

For every bee sample, the converted data, the information about its origin, the type of bees, to which it belongs, and the date of collection were registered in a data-bank.

The bees were prepared and the forewing characters were measured at Bee Section of Faculty of Agriculture, Cairo University.

2. Statistical analysis:

To obtain the measure of directional asymmetry (DA), paired t-test between left and right wing for each trait was carried out according to Palmer (1994). Because we have several comparisons (31), and because the individual tests may not be independent, the decisions based on conventional levels of significance might be in doubt, so we employ a conservative approach in which one lower the type 1 error of the statistic of significance for each comparison so that the probability of making any type 1 error at all in the entire series of tests does not exceed α. For all characters, a significant p value was set at 0.002 based on Bonferroni's correction for 25 comparisons (Sokal & Rohlf, 1995).

As stated by Palmer and Strobeck (2003), It was advised that characters that show directional asymmetry should not be used for analysis of fluctuating asymmetry, because its presence make the interpretation of fluctuating asymmetry more difficult), so the characters showing DA in workers were excluded from our analysis when calculating (FA).

(FA) was calculated as (FA)1 of (Palmer 1994), which is the (FA) measure reported in most of studies as the mean of absolute value of the difference in trait size between the right and the left sides of the body |R - L|.

To test the hypothesis that the means of (AF0 of the four groups come from a population with equal μ, we carry out significance test for absolute asymmetry difference by using multiple traits simultaneously by applying two-way ANOVA and testing the effect of both traits and feeding treatments on the value of (FA).

Composite variable (CFA):

Individual data of absolute asymmetry were pooled to make composite variable of all 25 variables (Clarke and McKenzie 1992; Whitlock 1993; Leung, *et al*. 2000). The composite variable is the sum of absolute (FA) values for all traits for each individual. Absolute (FA) values are used because (FA) of individuals should be directionally random, and it is the magnitude of asymmetry in either direction that may indicate stress. The new composite variable was analyzed by using t-test. All the analyses were carried out by using Almo-Statistik-System, Version 15 (Holm, 2015).

RESULTS AND DISCUSSION

Testing of directional asymmetry (DA):

The results of T-Paired test of 25 characters in the four groups are shown in table (2). In drone bees, non of the 4 groups showed any indication of significant (DA).

Table 2. Effect of different feeding on directional asymmetry of the forewing venation characters of drone honey bees (t- Paired test).

Venation characters	Sugar Cane feeding		Sugar Beet feeding		Sugar Beet & Citrus honey feeding		Citrus honey feeding	
	t-value	Sig. (2-tailed)	t-value	Sig. (2-tailed)	t-value	Sig. (2-tailed)	t-value	Sig. (2-tailed)
L1	-3.405	.004	-2.634	.023	-1.598	.149	1.076	.310
L2	-1.815	.090	-.852	.412	-1.000	.347	.491	.635
A2	1.564	.139	.090	.930	1.817	.107	-.751	.472
L3	-1.013	.327	-2.264	.045	-1.414	.195	.937	.373
A3	2.076	.055	.301	.769	.921	.384	.475	.646
L4	-1.266	.225	-2.017	.069	-.841	.425	.829	.428
A4	.007	.995	.597	.563	.232	.822	-.751	.472
L5	-.164	.872	-1.738	.110	-2.031	.077	1.152	.279
A5	1.860	.083	1.455	.174	-.371	.721	-.205	.842
L6	-.433	.671	-1.628	.132	-1.019	.338	1.705	.122
A6	.146	.886	-.162	.874	.495	.634	-.211	.838
L7	-.607	.553	-1.320	.214	-.268	.796	1.049	.322
A7	.432	.672	2.090	.061	.670	.522	-.645	.535
L8	.117	.909	.689	.505	-1.021	.337	1.335	.215
A8	-.576	.573	-.363	.723	-1.105	.301	-.693	.506
L12	1.766	.098	.788	.447	1.061	.319	1.278	.233
A12	-1.303	.212	-1.469	.170	-1.054	.322	1.032	.329
L13	.657	.521	.699	.499	1.156	.281	1.014	.337
A13	-.090	.930	-1.889	.086	1.016	.340	-.554	.593
L14	.273	.788	.422	.681	-.331	.749	1.652	.133
A14	-.472	.644	-.539	.600	.926	.382	-.791	.450
L15	-.726	.479	-.062	.952	.809	.442	1.600	.144
A15	-.121	.906	.779	.453	1.013	.341	-.699	.502
L16	1.015	.326	-2.567	.026	-.706	.500	1.288	.230
A16	-.364	.721	-.517	.615	-.229	.824	-.889	.397

Fluctuating asymmetry (FA).

Results from two-way ANOVA test that combined information from different characters to test the difference in FA among feeding groups are shown in table (3).

As shown in table (3) and concerning (FA) in drones, the effect of characters is significant on the value of (FA). Also, there is a significant effect of feeding-type on (FA). No Interaction was found between traits and feeding groups.

Table 3. ANOVA table for analyzing the effect of trait and feeding treatment on FA of forewing venation in workers

S. V	df	MS	F
Traits	24	13.89	63.02^{**}
Feeding treatments	3	2.32	3.03^{*}
Interaction	72	0.456	0.624^{ns}
Error	1050	0.418	

$*$ p < 0.05

For summarizing the results of (FA), we use an index of (FA) across traits and the results of t test were shown in figure (2 and 3). This index is composite fluctuating asymmetry. As shown in figure (2 and 3), the composite FA (CFA) of distances between vein junctions were not influenced by various feeding treatments in drones, but the angels between veins were affected by it, since it was higher in cane sugar feeding treatment than honey one.

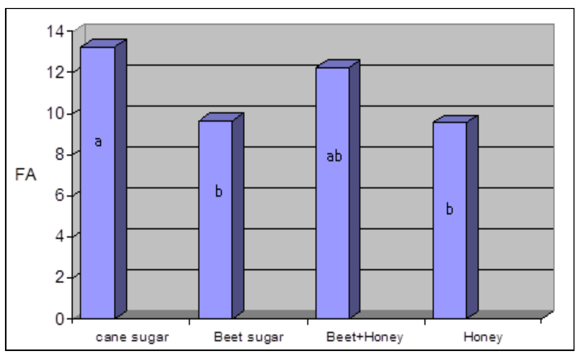

Fig. 2. Drones average values of composite fluctuating asymmetry in veins angles of forewing in different treatments (the same letters indicate non-significant)

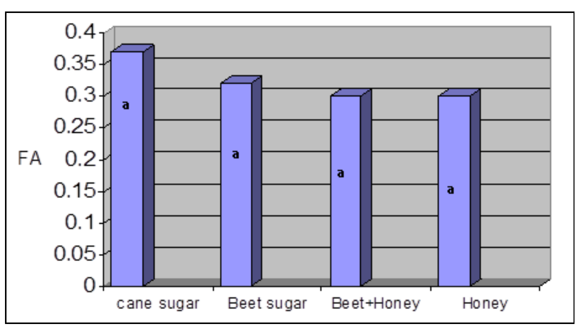

Fig. 3. Drones average values of composite fluctuating asymmetry in veins distances of forewing in different treatments

The presence of directional asymmetry of honeybee wings was reported in some studies (Clarke, 1998; Clarke, and Oldroyd,1996; Smith, *et al*. 1997; Clarke 1997; Schneider *et al*. 2003), but in most of them it was not significant (Jones *et al*. 2005; Ondo Zue Abaga *et al*. 2011).

In our results, no wing characters showed significant directional asymmetry in drone honeybee. So, it could be concluded that (DA) is not associated with the type of feeding under study. This result is in agree with that of Mazeed, *et al*. (2015), who found that (DA) was not related to the type of food. It was earlier suggested, that directional asymmetry is genetically determined and adaptive (VanValen, 1962; Windig & Nylin, 1999), therefore, it is suggested, that it should not be used as a measure of developmental stability (Palmer and Strobeck 1992) or it may related to specific factors which wanted to be investigated.

As the results of (FA) analysis indicated, all characters under study showed difference in sensibility to the different feeding treatments So, all traits are not the same at revealing presumed differences in developmental stability among samples, since some traits are repeatably less stable developmentally than others. The results showed also, that, the four treatments had different effect of the fluctuating asymmetry of the pooled characters, suggesting that the level of developmental stability varies among feeding groups.

The different values of (FA) found between distances between vein junctions may result from the fact that the impact of stress on fluctuating asymmetry seems to be trait- or stressor-specific (De-Anna,, *et al*.2013) or simply be a consequence of their under sampling (Babbitt, *et al*. 2006).

The non-significant of interaction indicated that the (FA) variation among treatments do not depend on the trait, and that the variation of (FA) of the traits do not depend on feeding type.According to the results of composite asymmetry, it could be concluded that, the wing angels of drone are more sensitive to the feeding treatments than distances between vein junctions and consequently caused higher (FA) values of vein angles in colonies fed with sugar cane syrup than those fed with honey.

Many studies concentrated of the effect of internal or external factors on (FA) (Brueckner 1976, but see Clarke 1997; Jones *et al*. 2005; Ondo Zue Abaga *et al*. 2011; De-Anna,, *et al*. 2013), but few studies, however, had dealt with the impact of feeding on the (FA) of members of honeybee colonies. Mazeed, *et al*. (2015) stated that sugar feeding caused significantly higher (FA) in wing venation pattern in comparison to feeding with honey, and this effect was not influenced by neither the time of year nor the genotype of the colonies.

Szentgyörgyi, *et al*. (2016), stated that, limited access to pollen caused some differences in the fluctuating asymmetry of size and shape in pollen-deprived workers and drones compared to the control bees, and there are more pronounced differences in (FA) due to replication than to pollen deprivation itself.

In all the previous (FA)'s Studies, the aim was to investigate the effect of specific factor on the value of (FA) to see to what extent the specific factor could be considered as a parameter for the existence of (FA). But only few studies were done which tell us the biological meaning of the presence of (FA) in terms of its effect of specific behavior or its linking with important behavior of the organism, such as Jaffé and Moritz (2010) who stated that wing (FA) was found to be significantly lower in the drones collected at the drone congregation areas than in those collected at the hives suggesting the a strong selective pressure act on drones to ensure mating the queen with high quality drones. So, individuals with more symmetric wings may have a higher mating success due to flight mechanistic reasons as suggested in damselfly (Harvey and Walsh 1993; De-Block and Stoks 2007),

Comparing the sugar cane with honey, it could detect that the sugar cane has only sucrose, in comparison with honey which has many types of sugars, amino acids, minerals, enzymes, hormones, vitamins, organic acids, and natural antibiotics in its components (Herold, 1982). So, sucrose syrup as artificial feeding might be one of nutritional stresses which are responsible of development instability (Polak, 1993). However, the supply of white sugar (sucrose) to honey bee colonies can be a valuable management tool for beekeepers. It is used to supplement a shortage of stored honey to prevent starvation of the colony, or to stimulate a colony to artificially promote breeding. Accordingly, more studies are needed in order to investigate the correlation between the presence of (FA) and other abnormal behavior on drones or any deleterious effect when feeding continuously with sugar cane in order to determine if (FA) had a biological meaning for bees or it would be only used as a marker to distinguish sugar-fed from honey-fed bees.

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التغذية السكرية وتماثل الجناح فى ذكور نحل العسل

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تم دراسه امكانيه إستخدم عدم التماثل فى جناح ذكور نحل العسل كتأثير محتمل عند اجراء تغذية طوائف نحل العسل على محلول سكر القصب والبنجر مقارنه بالتغذية على العسل ككنترول. لتحقيق هذا الغرض تم إستخدام ٢٥ صفه من صفات تعريق الجناح الأمامى الايمن والايسر لذكور نحل العسل، وقد اوضحت النتائج الى أن عدم التماثل ذو الاتجاه الواحد لم يوجد فى المعاملات تحت الدراسة وبالتالى فهو لا يرتبط بأختلاف نوع التغذية. وعلى الجانب الاخر إختلفت قيم عدم التماثل ذو الاتجاهين بأختلاف نوع التغذيه ونوع الصفة مع عدم وجود تفاعل بينهما.بالاضافه الى ذلك فعند تجميع قيم عدم التماثل ذو الاتجاهين لكل الصفات تحت الدراسه فى صورة متغير واحد مركب وتحلليها احصائيا فقد أظهرت النتائج أن عدم التماثل بين الجناحين الايمن والايسر بالنسبة للزوايا بين العروق كانت عالية بشكل معنوى فى التغذية السكرية بقصب السكر عن التغذية بالعسل .