WING SYMMETRY AS AFFECTED BY ARTIFICIAL FEEDING IN HONEYBEES COLONIES

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Abstract

Wing asymmetry was used to measure the possible stress which could be created when we feed honeybee colony with sugar syrup in comparison with feeding with honey (control). By using 31 character of wing venation pattern on left and right forewing it could be stated that fluctuating asymmetry (FA) was higher in colonies fed on sugar syrup than those fed with diluted honey in most of the characters mesured. In contrary, directional asymmetry (DA) was not related to the type of feeding but to the time in which the experiment was conducted. The individual characters were combined and analyzed as composite fluctuating asymmetry (CFA) to maximize the probability of detecting FA-stress relationship when it existes. The result showed that CFA was significantly higher in sugar-feeding than in honey-feeding colonies.

Keywords: Honeybees, forewing venation, polar coordination, fluctuating asymmetry, directional asymmetry, composite fluctuating asymmetry.

1. INTRODUCTION:

During development, individuals of an organism spieces may be exposed to unfavourable conditions which may impair optimal growth or some physiological functions (Thornhill &Moller, 1998, Field and Yuval, 1999, Parsons, 1990 and Schmid-Hempel, 2003). So, they may be unable accurately to develop their expected phenotype given their genotype and the environment (Palmer & Strobeck, 1986). Accordingly, departures from the ideal expression of particular traits are expected which could be used as evidence that organisms have been exposed to sub-optimal resources as mediated through environmental or genetic constraints (Parsons, 1990 and Schmid-Hempel, 2003).

Many insects have a complex life cycle with a discrete larval and adult stage, often separated by metamorphosis. One challenge which is confronted by scientists is to understand whether and how unfavourable conditions in the larval stage could affect adult fitness in such animals (Moran 1994). Because of their short generation times and the relative ease at which environmental conditions can be manipulated, insects are particularly well-suited to studying relationships between morphology and ecological correlates.

One of the most relevant morphological characteristics being used as stress-bioindicator for measuring developmental instability in many insects is wing fluctating asymmetry (FA) (Hoffmann & Parsons, 1991, Clarke and Mckenzie, 1992, Bjorksten, *et al*, 2000 and Ranta et al, 2004). Because corresponding body sides presumably share the same genome and experience similar external effects in a homogeneous environment, differences in their development cannot be explained by direct effect of genetic or environmental factors (Reeve, 1960). Rather, the observed asymmetries are believed to reflect the inability of individuals to buffer their development against small, random perturbations of cellular processes ('developmental noise ' , Palmer, 1994) and, hence, accurately to develop their expected phenotype given their genotype and the environment (Palmer & Strobeck, 1986).

Over the world, many beekeepers tend to feed their honeybee colonies regularly with artificial sugar syrupe basically for activating queens to lay eggs early before the beginning of the season and to supply colonies with carbohydrate as energy source in winter. The bees used the resulted artificial honey more or less as winter storage, for feeding themself during winter and for feeding the offspring of their mother colony. As the quality of the resulted artificial honey differs dramatically from the natural honey in their components (Herold, 1982), we might consider artificial honey as one of stressors facing honeybee colonies during their life. So, we want to investigate the effect of this type of feeding as stress factor on the stability of growth of the growing bee larvae in terms of studying symmetry of wing venation pattern of the resulted worker bees.

2. MATERIALS AND METHODS

This experiment was carried out in the Apiary of Beekeeping Research Center at the Ministry of Agriculture. The treatments were applied on 6 hybrid colonies which were chosen randomly from the apiary. The colonies were divided randomly into 2 groups each of three colonies, and were fed with different types of sugar nutrition:

I **Clover honey syrup (Control)**: Honey-water solution 1:1.

II **Sugar Cane syrup**: Sucrose-water solution 1:1.

Each group received the two treatments but in reverse order. One group received honey-water solution in June ,followed by sugar syrupe in August. The other group received sugar syrupe in June, followed by honey-water solution in August.

At the first time of applying the two treatments (in June), the honey combs were removed from the tested colonies and replaced with empty drawn combs, and then we began to feed them with diluted sugar and honey. Each group was fed exclusevly with its treatment for 6 weeks before taking of bee samples in July

At the second time of applying treatments (in August), the combs of the two groups were exchanged in order to replace the storage of natural with artificial honey storage for one group and *vice versa* for the other group. We began to feed them with the two tratments also for 6 weeks before taking of bee samples in September.

From each colony in each group, a worker sealed brood comb was taken and put in an incubatur at 34 °C, until emerging of worker bees, then about 15-27 workers were collected from each group and preserved in alkohol 70% until dissection.

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

2.1. Asymmetry of wing venation:

The forewing measuring characters were chosen according to Kauhausen (2002 & Szymula *et al.*, 2010), who used polar coordinate system for describtion 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, howeve was represented by one value on 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 intervenation distances and 15 angles coordinates.

2.1.1. 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 II).

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 worker, 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 intervenation points were marked. The measuring-program converted these coordinate points into actual lengths and angles of the different intervenation 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. For each character measured in the forewings of honeybee worker, the absolute value of the difference between right and left forewinsg was calculated to obtain an estimate of fluctuating asymmetry (FA).

2.2. Statistical analysis:

FA was calculated as FA1 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 two means of absolut asymmetry of the two groups come from a population with equal μ, the individual average values of absolute asymmetry of 31 characters measured were compared separately betweeen honey- and sugar-treated colonies by using t-test. Because we have several comparisons (31), and because the individaul 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 dose not exceed α In this study we use the method of Bonferroni sequental method (Sokal and Rohlf, 1995).

To obtain the measure of directional asymmetry (DA), 1-sample t-test on the signed difference (R - L) for each trait was carried out according to Swaddle *et al.* (1994).

Antisymmetry (AS) was tested by departures of absolute differenced (R - L) frequency distribution from normality by Kolmogorov–Smirov test as suggested by Palmer (1994).

After doing individual tests, individual data of absolute asymmetry were pooled to make composite variable of all 31 variables (Clarke and Mckenzie, 1992). The new composite variable (CFA) was analyzed by using t-test. All the analyses were carried out by using Almo-Statistik-System, Version 15 (Holm, 2013).

3. RESULTS

3.1. Testing of normality:

Frequency distributions of signed differences (R -L) did not show bimodal distribution and showed normal distribution in Kolmogorov–Smirov test, suggesting the absence of antiymmetry (AS).

3.2. Testing pf directional asymmetry (DA):

The mean of signed values (R - L) of 10 characters in two groups showed significant different from zero, suggesting the presence of directional asymmetry (DA) in data. These groups are honey-feeding and sugar-feeding colonies which were established in June. In contrary, non of the two groups tested in August showed DA, except one character in sugar-feeding colonies.

3.3. Testing of fluctuating asymmetry (FA):

The average values of absolute asymmetry was used for estimating fluctuating asymmetry (FA) for each bee in each group (Palmer, 1994). The characters showed directional asymmetry were not excluded because they did not reveal greater differences in developmental stability among samples than those exhibiting ideal FA. (Palmer,1994). Correlation of absolute asymmetry with average trait size was not significant for all traits, except V5 and 7, so these characters were excluded from further analysis instead of correcting for size (Windig and Nylin, 1999)

3.3.1 Intervenation distances

The average values of FA for intervenation distances in honey feeding group in June was lower in comparison with the same group when they were fed on sugar in August in most of the characters measured. Using t-test for 16 of wing intervenation distances, the averaged absolute asymmetry values were higher in sugar-feeding colonies in 13 characters than when they were fed on honey (Figure, 2 & 4). In the other group of colonies, and when they were fed on sugar syrupe in June, it was 12 characters which showed higher FA than when the colonies were fed on honey in August.

3.3.2 Intervenation angles

The average values of FA for intervenation angles in honey feeding group in June was lower in comparison with the same group when they were fed on sugar in August in most of the characters measured. By using t-test, All of 15 wing intervenation angles were higher in sugar-feeding colonies than in honey-feeding colonies (Figure, 3 & 5). In the second group, and when the colonies were fed on sugar syrupe in June, it was 12 characters which showed higher FA in comparison with feeding on honey in August.

By applying Bonferroni correction (Sokal and Rholf, 1995) for both of distances and angles coordinates, only one character (L2) showed significant difference in favor of sugar feeding group.

3.4. Composite fluctuating asymmetry (CFA)

After considering 31 traits separately to compare the FA between honey- and sugar-feeding colonies, we compared a sum of standardized absolute FA values between the two groups under study. For this purpose we use z scores of the absolute asymmetry and combine them in each individual, so that the sum of z score of each individual represnted the new composite variable and the mean of z scores represents the average value of each group. A mean of z score of zero means the absolute asymmetry is average value. A large positive mean of z scores means the asymmetry absolute is very high and a large negative mean of z score means the absolute asymmetry is very low.

As shown in figure (6), the average values of composite fluctuating asymmetry differed between sugar and honey-feeding colonies. For both groups of colonies, the composite fluctuating asymmetry was significantly higher in sugar-feeding than honey-feeding colonies irrespective of the time of the year and the genotype of bee colonies ($t = 2.51$, $df=25$, $p< 0.05$ & $t = 2.37$, $df=43$, $p< 0.05$).

4. DISCUSSION

Nutrition is considerd the first line of defense of honeybee colonies and is the key in dealing with major honeybee diseases. Honeybee Larvae fed on a nutritionally poor diet were found to be significantly more susceptible to various diseases (Li *et al*, 2007, Foley *et al*, 2012). In addition, Longevity of worker bees (Li *et al*, 2014).and body size (Daly, *et al*, 1995) decrease when their larvae experince poor naturational conditions.

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.

Comparing the Sugarcane with honey we could detect that the Sugarcane 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).

In this study we investigate the influence of artificial feeding on the symmetry in comparison to honey-fed colonies in terms of the degree of symmetry between right and left wing of honeybees, as many researches established that the use of wings seems to be effective in measuring developmental instability in many insects (Hoffmann & Parsons, 1991, Imasheva *et al.*, 1997, De Block & Stoks, 2007, Berggren and Low, 2009, Mazeed, 2012).

As the results indicated, larvae that had experienced artificial feeding with white sugar reflected more FA in most of the characters measured on the forewings of their adult stages, than those which were fed with diluted honey. On the contrary, DA is less associated with the type of feeding under study.

Also, the results indicated that, although the bee colonies used did not share a common genetic background, the different characters of forewing under study were affected simillay by the treatments under study.

Since most of beekeepers rely on artificial feeding for maintaining their colonies especially in poor-nectar localities, or during the dearch period through season, so our results needed to be supported by further studies to search the possible deterious effect of continous artificial feeding on some biological characters, such as longevity, resistance to diseases, brood and foraging activity. As there is a direct link between FA and fitness, as 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), so, further studies are also needed to know the effect of this characteristics on both queen and drones of honeybees.

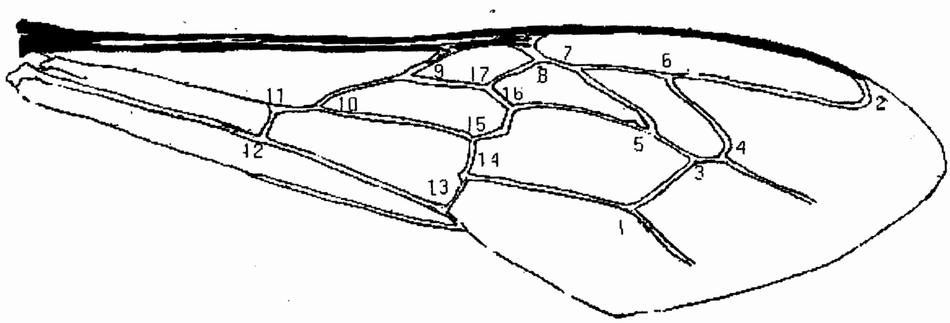

Fig. (1): Forewing of worker bees showing 17 points used to establish wing coordinates

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			

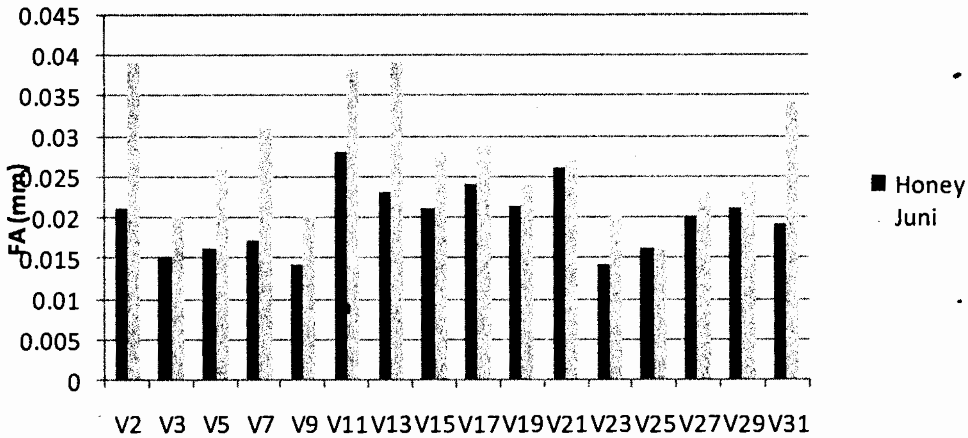

Fig.(2): Average fluctuating asymmetry values (FA) of 16 wing intervenation distances of wings of workers fed with diluted honey in June and sugar syrup in August

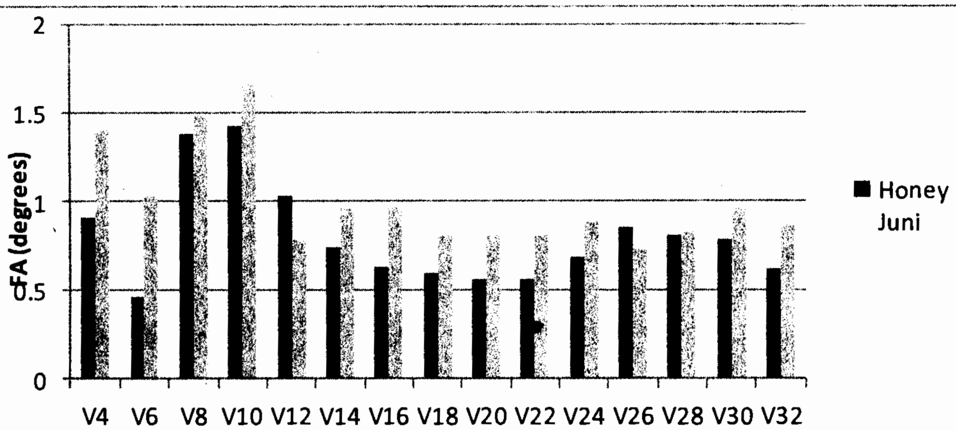

Fig.(3): Average fluctuating asymmetry values (FA) of 15 wing intervenation angels of wings of workers fed with diluted honey in June and sugar syrup in August.

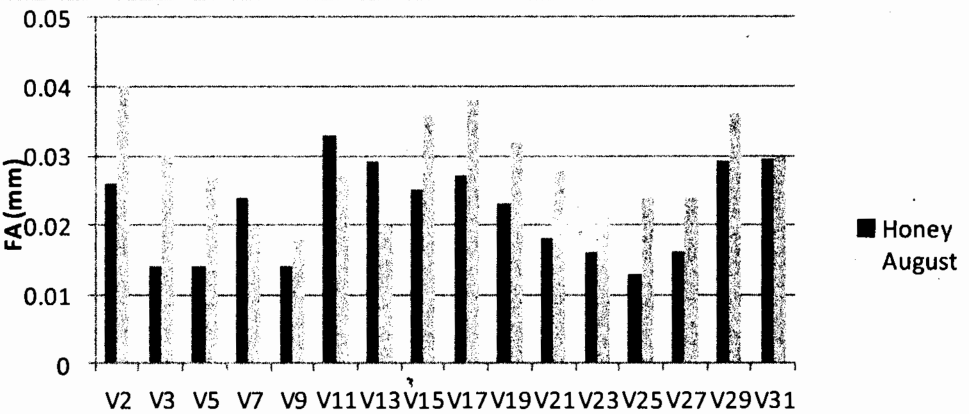

Fig.(4): Average fluctuating asymmetry values (FA) of 16 wing intervenation distances of wings of workers fed with sugar syrurp in June and diluted honey in August

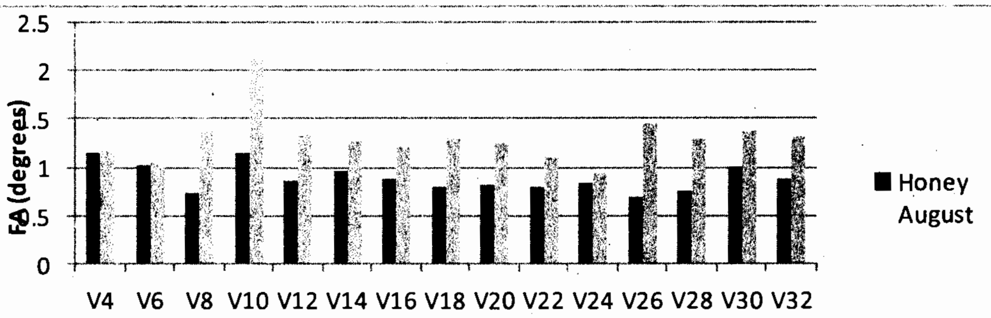

Fig.(5): Average fluctuating asymmetry values (FA) of 15 wing intervenation angels of wings of workers fed with sugar syrurp in June and diluted honey in August

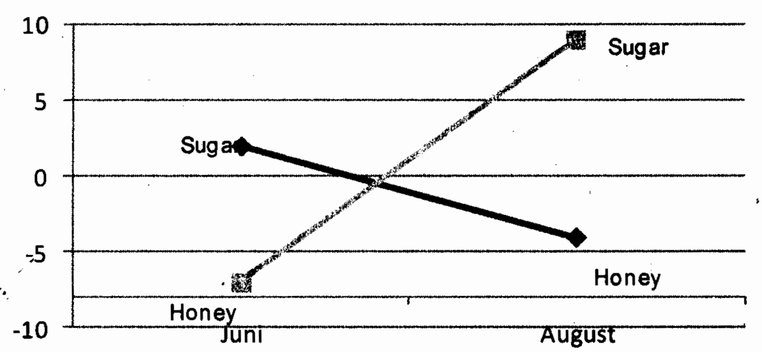

Fig.(6): Changing of composite fluctuating asymmetry (Averages of z values) of both distances and angels between sugar and honey-feeding colonies

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