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Research paper

Geometric Morphometrics Approach on Genotoxicity Evaluation of Ecdysone Agonist, Chromafenozide on Corcyra Cephalonica Stainton (Lepidoptera: Pyralidae)

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Abstract

The present investigation attempted to evaluate the genotoxicity effect of ecdysone agonist, on the wing architecture using the land mark based shape analysis through geometric morphometrics. The study revealed that the fore and hind wings of treated male and female showed significant variation in shape. High significance in shape was observed in male fore and hind wings. But, variation in size was not affected. The analysis of symmetry and asymmetry between left and right wings, suggests that the untreated male exhibit shape difference between left and right forewings and similar variation was also found in forewings of treated female. These findings demonstrate that, chromafenozide treated on the egg masses produced genotoxic effect on the adult wing architecture of *C. cephalonica*. Both the male and female forewing showed high significant shape decomposition at P = 0.0001 level exposed to 1/5 EC_{50} of chromafenozide. The morphological alteration in wing shape suggests that, it affects the aerodynamics pattern of the insect. In the present study, species diagnosis was performed in laboratory reared F_1 generation using multilocus barcoding technique and obtained a sequence of 658bp (CO I) and 563bp (18s) in length.

Keywords: *Corcyra cephalonica; ecdysone agonist; geometric morphometrics; Land mark; PCA*

1. Introduction

In biological system, shape forms an important element since, it provides a link between genotype and the sum of all environmental factors. The existing knowledge provides the information that the study of shape is extensively used for taxonomic classification, analysis of different forms, evolutionary relationship and exploring environmental effects on organisms. The wing morphology is sophisticated system that has great importance in insect's phenotype such as sexual dimorphism and territorial display, foraging, defense mechanism and aerodynamics [1, 2].

The wing architecture, shape of taxonomic importance in particular can be used as an indicator of stressful environmental conditions and evaluate the developmental noise [3, 4]. The landmark based approach of geometric morphometric methods are currently used to test the insect wing morphological similarity and dissimilarity between sexes and differ with the environmental conditions. But, to date a detailed study on the morphology of wings of the most serious lepidopteran pest of stored product, *Corcyra cephalonica*has not been examined. Hence, an attempt has been elucidated to study the genotoxicity effect of chromafenozide on the morphology of wing patterns in rice moth, *Corcyra cephalonica* with geometric morphometric procedure.

Insect identification through molecular systematics had impressive growth over the recent decades. The widely used genes for getting the information of divergence in insects are cytochrome oxidase I, elongation factor-1α, 16srRNA and 18s rRNA. Therefore, insect molecular systematics facilitates fast species diagnosis and better understanding of classical taxonomy without ambiguity. Shape information analysis through geometric morphometrics is a special kind of information which bridge between classical and molecular taxonomy [5-7].

2. Materials and Methods

The present investigation was carried out in stored products insect pest, *Corcyra cephalonica* (Stainton) procured from National Bureau of Agriculture, Bangalore. The obtained stock was reared and maintained at laboratory conditions, at 37±1°C temperature and RH 53±5%, under 12: 12 hours photo period and F_1generation was used for the experiments.

2.1. Multigene DNA Barcoding

The genomic DNA of test organism was isolated from the hind legs of freeze immobilized samples using Nucleospin insect tissue kit (Macherey-Nagel). DNA pellets were dissolved in 50 µl of TE buffer ((10 mMTris–HCl, 1 mM EDTA, pH 8.0) and isolated DNAs were stored at -20^0 C until use. PCR amplification of Cytochrome oxidase I (COI) and the 18S rRNA gene was performed with the universal primers LCO (F) 5'GGTCAACAAATCATAAAGATATTGG 3' and HCO (R) 5'TAAACTTCAGGGTGACCAAAAAATCA3', 18S 1F (F) 5'TACCTGGTTGATCCTG CCAGTAG3' and 4R (R) 5'GAATTACCGCGGCTGCTGG3'. PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700,

Applied Biosystems) with the following programs: 98°C for 30sec; 98°C for 5sec; 45°C for 10sec; 72 °C for 15sec at 10 cycles and at 30 cycles respectively. Similarly, amplification for 18S was performed at 98°C for 35sec; 40 cycles at 54°C for 10sec; 72 °C for 1min; and final extension at 72 °C for 60sec.
The products were subjected to agarose gel electrophoresis and the gels were visualized and documented using Gel documentation system (GBOX F3, Syngene, UK). The nucleotide sequences obtained were submitted to GEN bank database. The sequences were BLAST in the NCBI GenBank database and also examined the phylogenetic relationship.

2.2. Bioassay of Chromafenozide

The nonsteroidal ecdysone agonist chromafenozide, (2'-tert-butyl-5-methyl-2'-(3,5-xyloyl)-chromane-6-carbohydrazide) purchased from Sigma Aldrich were used for the study. The compound was dissolved in acetone and diluted to obtain the required different concentrations. The volume of the acetone was maintained equal in all concentration.

2.3. Treatment of Eggs

The initial toxicity experiment were conducted in the eggs of healthy adults of *Corcyra cephalonica* exposed to a series of concentrations of chromafenozide in order to obtain ED_{50} value (Effective Dose causing 50% adult emergence failure) as indexed by comparing with vehicle control and control groups. The concentration arrived through the toxicity assay was 1.5ppm. For genotoxic assay one third of ED_{50} (0.5ppm) were topically applied using Hamilton micro syringe and reared in sterilized plastic containers. The emerged adults were then subjected to wing patterns analysis using landmark based geometric morphometric analysis.

2.4. Experimental Design for Geometric Morphometric Analysis

Three batches of 20 per numbers of male and female moths were collected from experimental culture and sedated with diethyl ether. The forewings and hindwings were plucked with forceps

3. Results

3.1. Molecular Species Diagnosis

Multilocus gene analysis of the experimental organism was conducted for species level confirmation. The partial mitochondrial Cytochrome oxidase I and 18s rRNA regions amplified using PCR technique and the product obtained were sequenced for the identification of *Corcyra cephalonica.* The resultant sequence obtained is of 658bp and 563bp in length. The sequence of *C. cephalonica* was compared with25of other sequences available in online at the NCBI (www.ncbi.nlm.nih. gov/BLAST) site by multiple sequence alignment tool and showed that 658 bp partial sequence of CO I gene of *Corcyra cephalonica* was 100% similar to that of the same species collected from Canada and India with Genbank accession number KF397934.1 and HQ897685.1. *C. cephalonica* showed 90% similarity with other species like *Tirathabacatharopa, Mecistophylla sp., Hypolophota sp*. and few others.
The 563 bp of 18s sequence of *C. cephalonica* showed 99% similarity with other species. The blast report indicated there is neither 100% similar species reported in NCBI blast program. *Galleria mellonella* showed 99 % similarity with accession number X89491.1 which is a lepidopteran moth of the family Pyralidae. *Helicoverpa sp*., *Heliothis virescens, Tanadema neutral* and many species have 99% similarity with *C. cephalonica.*

3.2. Landmarks of Forewing and Hindwing

Twenty landmarks points of forewing of both male and female moths of taxonomic importance were identified and the shape information was extracted in x, y coordinate system (Fig: 1; Table: 1).Similarly, 13 landmarks points were identified for hindwings of male and female moths (Fig: 7; Table: 2). The acquired data were further processed for size and shape variation through geometric morphometric analysis and the results are depicted in Figs: (1 - 24).
The Procrustes analysis were performed on the acquired landmarks data of wings to determine the average shape within the subgroup of male and female, and respectively among and between both control and treated organisms. The landmarks were superimposed to optimize the distances from a common centroid shape. The outcome of this study provides detailed information on the average shape and variation in wing morphology within the group of *C.cephalonica*, and data were graphically represented. The symmetry among left and right forewings and hindwings of male and female individuals were analyzed among control and experimental groups and graphically represented in canonical variate analysis (CVA) and principal component analysis (PCA). Also, the wing shape variations caused by the ecdysone agonist chromafenozide on *C.cephalonica* was also represented in shape decomposition grid for control and treated group.

Table 1: The landmark of forewing of *Corcyra cephalonica*

Landmark points	Descriptive location	Description
1	SC(B)	Base of the subcosta
2	SC(D)	Distal end of the subcosta
3	R_1(B)	Basal end of radius 1
4	R_1(D)	Distal end of radius 1
5	R_2(B)	Basal end of radius 2
6	R_2(D)	Distal end of radius 2
7	R_3(B)	Basal end of radius 3
8	R_3(D)	Distal end of radius 3
9	R_4(B)	Basal end of radius 4
10	R_4(D)	Distal end of radius 4
11	M_1(B)	Basal end of median 1
12	M_1(D)	Distal end of median 1
13	M_{2+3}(B)	Basal end of median
14	M_{2+3}(D)	Distal end of median
15	CuA_1(B)	Basal end of Cubitus Anterior
16	CuA_1(D)	Distal end of Cubitus Anterior
17	CuA_2(B)	Basal end of Cubitus Anterior
18	CuA_2(D)	Distal end of Cubitus Anterior
19	A(B)	Basal end of anal vein
20	A(D)	Distal end of anal vein

Table 2: The landmark description of hindwing of *C. cephalonica*

Landmarks	Descriptive location	Description
1	SC+R_1(B)	Basal end of SC+R_1
2	SC +R_1(D)	Distal end of SC+R_1
3	RS(B)	Base of RS
4	RS(D)	Distal end of RS
5	M_1(B)	Base of M_1
6	M_1(D)	Distal end of M_1
7	M_{2+3}(B)	Base of M_{2+3}
8	M_{2+3}(D)	Distal end of M_{2+3}
9	CuA_1(D)	Distal end of CuA_1
10	CuA_2(B)	Base of CuA_2
11	CuA_2(D)	Distal end of CuA_2
12	1A(D)	Distal end of 1A
13	2A(D)	Distal end of 2A

Impact of chromafenozide on female, male forewing of *C. cephalonica*

3.3. Procrustes ANOVA

The symmetry and asymmetry among left and right forewings of control and treated individuals were analyzed separately using procrustes ANOVA. The result revealed that the control and treated individuals have no significant size variation (P = 0.4081). Significant P value obtained in forewing shape of female (<0.0001) indicated asymmetry between control and treated individuals (Table.3). The shape variation was also observed between left and right forewings of treated female, which revealed that they showed asymmetry in forewings.

Table 3: Results of Procrustes analysis of variance (ANOVA) and decomposition of shape

Centroid size:, Procrustes ANOVA:		**Female forewing Control and Treated**			
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	15.114462	1.889308	8	0.73	0.6682
Side	1.979074	1.979074	1	0.76	0.4081
Ind * Side	20.771796	2.596474	8	1.89	0.1245
Error 1	24.703675	1.372426	18		
Shape, Procrustes ANOVA:					
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	0.02733291	0.0000949059	288	1.42	0.0016
Side	0.00586935	0.0001630375	36	2.43	<0.0001
Ind * Side	0.01929346	0.0000669912	288	1.02	0.3976
Error 1	0.04235196	0.0000653580	648		
Centroid size:, Procrustes ANOVA:		**Male forewing Control and Treated**			
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	3.492591	0.436574	8	0.74	0.6573
Side	0.134362	0.134362	1	0.23	0.6451
Ind * Side	4.695831	0.586979	8	0.73	0.6678
Residual	12.132507	0.808834	15		
Shape, Procrustes ANOVA:					
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	0.02182447	0.0000757794	288	0.91	0.8007
Side	0.00791303	0.0002198064	36	2.63	<.0001
Ind * Side	0.02411018	0.0000837159	288	0.91	0.8135
Residual	0.04963946	0.0000919249	540		

3.4. Female Forewings Size and Shape Variations

The thin plate deformation grid of the forewings of both treated and untreated females exhibited deviation in almost all landmarks points. The minimum deviation was observed in landmark number 17 (Basal end of Cubitus Anterior) and maximum deviations were seen in landmark number 13, 15 and 19 (Fig: 5 &6). In PCA, first PC has the largest variance between samples and variation decreases with each succeeding components. The morphometric difference has been explained using PCs. The cumulative contribution ratio of the first three principle components of control and treated group of *C.cephalonica* was 57.785% (PC1=32.221%, PC2=14.576%, PC3=10.988%), potentially indicating that the first three components represented the main shape variation. The total shape variation recorded in the PCA was 0.00261090 (Figs:2& 4). The female forewing showed co variation in landmark numbers 3, 5, 4, 6, 9 and 8. Even though, asymmetry was observed between these forewings. The canonical variate analysis (CVA) showed variations in left and right halves of treated individuals with respect to controls (Fig: 6).

3.5. Male Forewings Size and Shape Variation

The differences in forewing shape of male *C. cephalonica* in control and experimental conditions were compared and visualized through graphs. The thin plate deformation grid produced to show the wing shape differences between treated and untreated individuals (Fig: 11&12). The graph revealed that most of the landmark points in the forewing showed deviation. The deviation denotes the average shape deterioration of individuals of same sex. The maximum deviations were observed in the distal region of the wing and minimum deviation was noted at the basal end of the forewing. The landmark points showed maximum deviations at SC (B) (Base of the subcostal), $1CuA_2$ (B) (Basal end of cubitus) and in 1A (B) (Basal end of anal) veins. The obtained data showed that deviations were not observed at two landmark regions atR_4 (D) (Distal end of radius 4) and R3 (B) (Basal end of radius 3). These landmarks are similar in the case of forewing of control and treated individuals. Landmark number 13 (M_{2+3} (B)) and 15(CuA_1 (B)) showed lesser deviation. This implies the position of these landmarks were almost similar in the case of forewings of treated and untreated individuals.

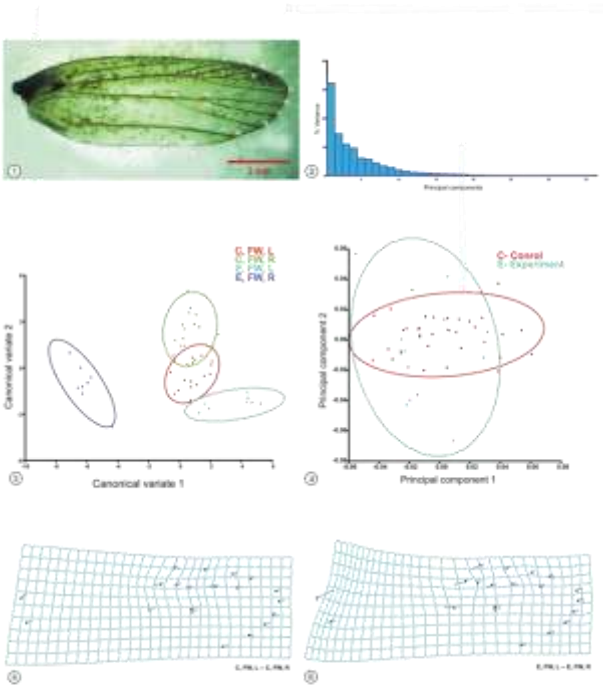


Fig: 1 Digitized image of forewing of *C.cephalonica* with landmark points
Fig: 2 Percentage of variance in Principal components (PCA) of female forewing of *C.cephalonica*
Fig: 3 Canonical variate plot showing distortion of female forewing in control and treated *C.cephalonica*, based on the shape of left and right areas.
Fig: 4 Thin- plate spline analysis representing wing shape deformation in left and right areas of forewing of female *C.cephalonica*.
Fig: 5 Thin plate deformation grid of female forewing, left and right area of *C.cephalonica*
Fig: 6 Thin plate deformation grid of treated female forewing, left and right area of *C. cephalonica*

The observations on Principal Component Analysis (PCA) revealed that the PC1 (27.171%) PC2 (15.649%) and PC3 (11.677%) together share 54.497% variance (Fig: 12). The total percentage of variance recorded was 0.00289535 in PCA (Fig: 8). The procrutes fit plot represents the mean shape of male forewing (Fig: 7).The canonical variate analysis (CVA) was performed to determine the shape features between multiple groups of the individuals. The CVA result showed that the forewings of treated

and untreated individuals exhibited great variation in the shape of left and right areas (Fig: 9)

The ANOVA result of centroid size of combined male treated and untreated forewing showed P value of 0.6451 which is more than 0.05 (Table. 3). Hence, the variation of size is non-significant, the untreated male showed high degree of symmetry (size) in forewing.

The ANOVA result of treated and untreated male forewings shape showed highly significant the P value at <0.0001 (Table.3) and revealed that shape more susceptible than size of the organisms treated with the compound, chromafenozide. So, the values showed high degree of asymmetry in treated groups.

3.6. Female Hind wings Size and Shape Variations

In the thin plate deformation grid, the landmark number 10 (CuA_2 (B)) showed considerable variation in the position. The maximum deviation observed in the landmark number 3 (RS (B)) (Figs: 15& 16).The principal component analysis showed PC 1 (33.362%), PC 2 (21.137%) and PC 3 (15.402%) share variation 69.897% (Fig: 16, 18). The total variance observed is 0.00524185 (Fig: 16). The CV1 and CV2 plot determined an aggregation of the left and right regions in treated samples (Fig: 15). From the ANOVA result it is clear that there is no size variation between treated and untreated female hindwings. The obtained P value (0.9126) is >0.05 and close to 1 that mean almost perfect symmetry and with least asymmetry component (Table.4).

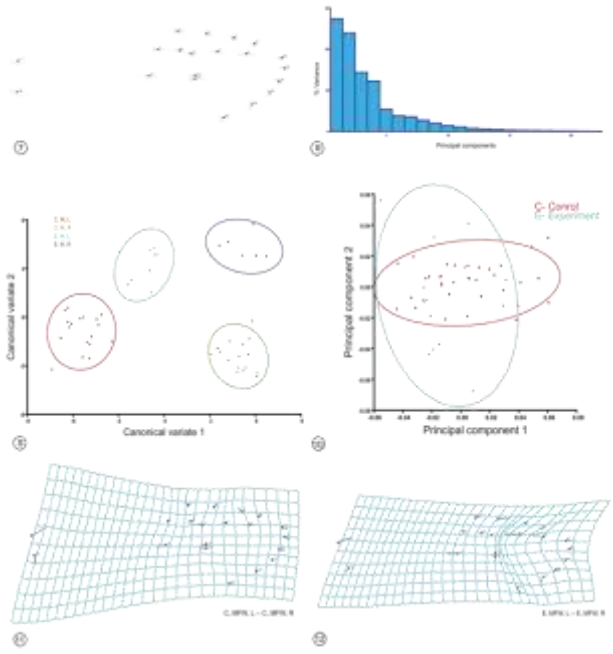


Fig: 7 Procrustes fit displaying the mean shape of male forewing *C.cephalonica*
Fig: 8 Percentage of variance in Principal components (PCA) of male forewing of *C.cephalonica*
Fig: 9 Canonical variate plot showing distortion of forewing in control and treated *C.cephalonica*, based on the shape of left and right areas.
Fig: 10 PCA plot of treated and control male forewings of *C.cephalonica*
Fig: 11 Deformation grid of male forewings exhibiting left and right area
Fig: 12 Deformation grid of treated male forewings exhibiting left and right area

The ANOVA of shape indicated that there is no significant shape variation between treated and untreated female hindwings, with the P value of 0.1736 (Table.4). The insects treated with chromafenozide exhibited a declining trend of the P value from 0.9126 to 0.1736 which indicates that symmetrical nature of the wing property changes.

3.7. Male Hindwing Size and Shape Variations

The distortion of male hind wings of treated and untreated male individuals showed shape variation compared to normal wings (Fig: 23 &24). The principal component analysis showed PC 1 (27.391%) PC 2 (23.906%) and PC 3 (14.336%) share variation of 65.633% (Fig: 22). The total variance observed is 0.00458177 (Fig: 20). The scatter plot of CVA represented great shape variations between the control and treated individuals hindwings (Fig: 21).

The ANOVA result showed that there is no significant size variation between treated and untreated male hindwings. The P value obtained was 0.7196 which is >0.05 (Table.4). Therefore the result was non-significant and wing showed asymmetry.

The ANOVA result of shape showed that there is a significant shape variation between treated and untreated male hindwings. The P value is <0.0001(Table.4). Therefore, the result is highly significant due to the high variation in shape. Among the hindwing only male hindwing showed more susceptible to chromafenozide.

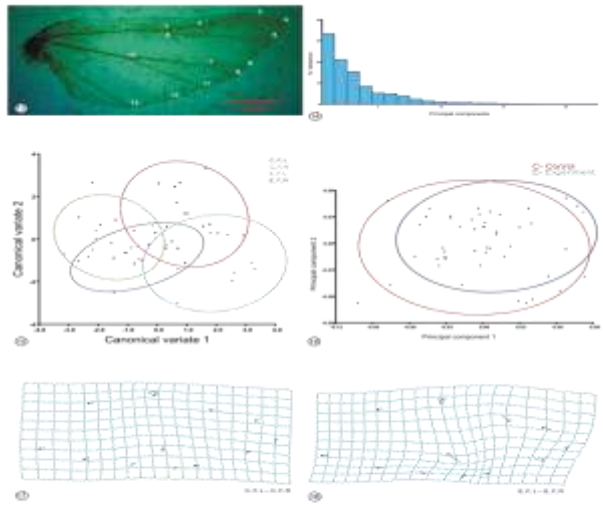


Fig:13 Image of female hindwing of *C.cephalonica* with landmark points
Fig:14 Percentage of variance in Principal components (PCA) of female hindwing of *C.cephalonica*
Fig:15 Canonical variate plot showing distortion of hindwing in control and treated *C.cephalonica*, based on the shape of left and right areas
Fig:16 Percentage of variance in Principal components (PCA) of female hindwing of *C.cephalonica*
Fig:17 Deformation grid of female hindwing exhibiting left and right area
Fig:18 Deformation grid of treated female hindwing exhibiting left and right area

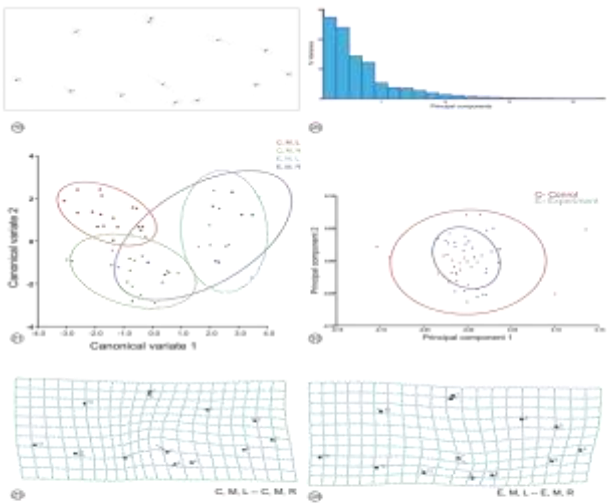


Fig:19 Procrustes fit displaying the mean shape of male hindwing *C.cephalonica*
Fig:20 Percentage of variance in Principal components (PCA) of male hindwing of *C.cephalonica*
Fig:21 Canonical variate plot showing distortion of male hindwing in control and treated *C.cephalonica*, based on the shape of left and right areas
Fig:22 Percentage of variance in Principal components (PCA) of male hindwing of control and treated of *C.cephalonica*
Fig: 23 Deformation grid of male hindwing exhibiting left and right area
Fig:24 Deformation grid of treated male hindwing exhibiting left and right area

Table 4: Results of Procrustes analysis of variance (ANOVA) and decomposition of shapes

Centroid size:, Procrustes ANOVA:		**Female Hindwing Control and Treated**			
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	7.853702	0.872634	9	2.08	0.1446
Side	0.005333	0.005333	1	0.01	0.9126
Ind * Side	3.768638	0.418738	9	0.56	0.8100
Error 1	14.122610	0.743295	19		
Shape, Procrustes ANOVA:					
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	0.04672669	0.0002359934	198	0.97	0.5837
Side	0.00696302	0.0003165008	22	1.30	0.1736
Ind * Side	0.04815377	0.0002432009	198	1.10	0.2180
Error 1	0.09264437	0.0002216373	418		
Centroid size:, Procrustes ANOVA:		**Male Hindwing Control and Treated**			
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	2.366555	0.295819	8	0.90	0.5586
Side	0.045581	0.045581	1	0.14	0.7196
Ind * Side	2.635134	0.329392	8	1.52	0.2185
Error 1	3.899409	0.216634	18		
Shape, Procrustes ANOVA:					
Effect	**Sum of sqrs**	**Mean square**	**df**	**F**	**P (param.)**
Individual	0.02546824	0.0001447059	176	0.70	0.9901
Side	0.01716091	0.0007800415	22	3.79	<.0001
Ind * Side	0.03623353	0.0002058723	176	0.95	0.6518
Error 1	0.08590324	0.0002169274	396		

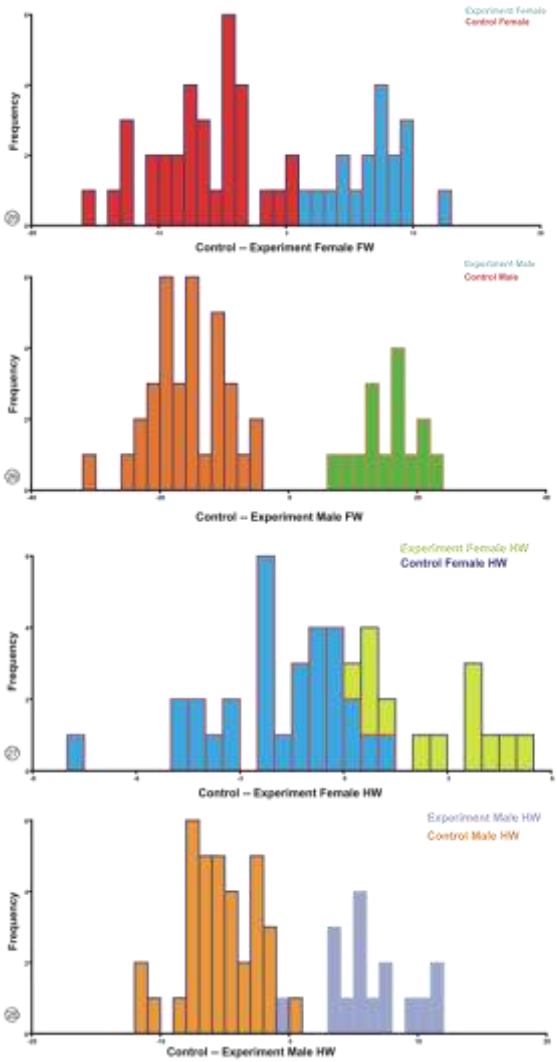
3.8. Canonical Variate and Discriminant Analysis

The canonical variate analysis showed very distinct change in wing morphology of insects exposed to sublethal concentrations of chromafenozide. The data clouds were diagonally distributed so It showed strong variations between control and treated organisms (Figs: 22-28). The Procrustes distance between control left and right wing was 0.01527772 , whereas Procrustes distance for treated left and right wing was 0.03015337 and showed that the magnitude increase twice which indicate the mode of toxicity that affect the wing symmetry (Fig-25). Discriminant analysis graphs showed clear separate block of control (Procrustes distance: 0.02715224) and treated groups (Procrustes distance: 0.03705745). Similarly discriminant analysis for symmetry of male forewing showed variation and prominent change. In the case of female and male hindwing only shape of male hind wing is susceptible to chromafenozide.

4. Discussion

DNA barcoding is one of the wildly used molecular techniques for quick and accurate identification of the biological specimens. 18S rRNA of *C. cephalonica* is the first report in order to understand the confirmation for the species analysis. PCR amplification of mitochondrial COI gene yielded 658 bp long sequence and NCBI blast report showed 100% similarity with the same species reported in China [12] and India [13]. The 563 bp of 18s rRNA was also sequenced and it is a first report on the sequence of 18s rRNA in *C. cephalonica*. The blast result showed 99% similarity with several other species. The data obtained showed that the test sequences were correctly identified using multilocus molecular recognition which is consistent with DNA barcoding studies of Lepidoptera [13]. Hence, the study reinforces the efficacy of DNA barcoding as a tool for species identification.

Currently, it is well known that the morphological variations of insects can be detected using morphometric analysis as a tool for quantitative and multidimensional comparison of morphological characters. Landmark-based geometric morphometrics allows the evaluation of phenotypic differentiations and has a high



discriminatory power at species and even subspecies level [14]. In the present study, the egg masses of *C. cephalonica* were exposed to the treatment of ecdysone agonist, chromafenozide to evaluate the genotoxicity effect of the compound on the wing architecture using landmark-based geometric morphometrics. The traditional morphometrics or multivariate morphometics is the application of multivariate statistical techniques (e.g. discriminate function analysis) to evaluate morphological data sets. Most of the research works in geometric morphometrics have focused on landmark data [15- 18].

The current study examined on the symmetry and comparison of wing pattern in treated and untreated organism. The study had demonstrated that, total 20 landmarks were identified and marked on forewing and 13 landmarks in hindwing of *C. cephalonica.* Procrustes superimposition which is the most important analysis

of geometric morphometrics was performed where only the shape information is considered.

From the result, it is clear that forewing and hindwing of male and female *C. cephalonica* exhibit shape changes when treated with chromafenozide. But, there is no size variation. High significance results were found in male forewings and hindwings. The P value of male forewing and hindwing shape was <0.0001 which indicates high significance in shape variation. In female forewing, the P value is <0.0001 and in hindwing, the value is 0.1736. Based on the PCA and CVA results, the left and right areas of forewings and hindwings of C.*cephalonica* presented large variance in treated ones.

Investigations of insect wings are of recent interest in studying shape change and the ability to discriminate shape differences between the sexes, population and species. Since, geometric morphometrics is an emerging area in biology only few reports are available on the existing study on shape information of insects. Recent study on dimorphism in the shape of the wings in *Lucilia sericata* using geometric morphometric methods showed that the variations observed could be genetic or could be mere reflections of the existence of high phenotypic plasticity due to environmental changes during growth and development of the larvae [19]. Similar study was conducted on the body size and wing shape of *Pieris rapae* [20] and *Lymantria dispar* [21]. The study on the molecular protein chaperone Hsp90 was used to analyze the effects on the variations on the individuals and fluctuating asymmetry of wing shape of *Drosophila melanogaster* and showed that mutations were observed [22]. Earlier few studies have been conducted on the effect of Hsp90 in order examine the quantitative traits of *Drosophila melanogaster* on its wing size and bristle counts and reported that it did not find increase variation [23-25].

The present study also proves that IGR causes characteristic morphological and genetic alteration in the test organism *C. cephalonica*. The investigation concedes that the chromafenozide treated individuals exhibited significant shape variation in the wings, which might influence hindrance in the flight dynamics of the insect. At present, the existing information on the toxicity effect of insecticides on the wing shape aberration of insects are not available. Therefore, more extensive studies in this area have to be explored for future pest control management.

In summary, the present investigation on geometric morphometric study, it is evident that both forewing and hindwing of male and female moths of *C.cephalonica* exhibited variation in size and shape when treated with the ecdysone agonist chromafenozide. The present study revealed that variation of shape is more susceptible than size. Hence, the study depicts that the chromafenozide caused genotoxic effects in the wing shape of male and female moths of *C.cephalonica*.

5. Conclusion

In summary, the present investigation on geometric morphometric study, it is evident that both forewing and hindwing of male and female moths of *C.cephalonica* exhibited variation in size and shape when treated with the ecdysone agonist chromafenozide. The present study revealed that variation of shape is more susceptible than size. Hence, the study depicts that the chromafenozide caused genotoxic effects in the wing shape of male and female moths of *C.cephalonica*.

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