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Morphometric variation among the populations of white grub, *Holotrichia consanguinea* Blanchard, (Coleoptera: Scarabaeidae) in India

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Abstract
The white grub species *Holotrichia consanguinea* Blanchard, is cosmopolitan in distribution and a major pest of several economic crops. The present studies have been carried out to document the intraspecific variations among three different geographical populations of *H. consanguinea* in India. Morphometrics of 23 characters in males and 19 in females were studied from each population and the data were subjected to different statistical analyses *viz*., univariate, multivariate, principal component analysis and discriminant function analysis. The various analyses revealed that maximum selected character states showed significant differences among all three populations. The principal component analysis revealed that PC1 and PC2 could explain 42.64% variation in males and 63.57% in females, which had loadings of five characters that can be useful in differentiating the three populations. The discriminant function analysis confirmed the worthiness of selected characters in differentiating the three populations of both male and female *H. consanguinea*. The territorial map drawn from two canonical discriminant functions showed the plots of three distinct populations indicating that significant differences exist among the three populations of *H. consanguinea*, which need to be explored further.

Keywords: *Holotrichia consanguinea*, intraspecific variation, morphometrics, white grub

Introduction
White grubs are the serious insect pests of several economic crops that belong to subfamilies Melolonthinae and Rutelinae of Scarabaeidae (Coleoptera). The genera *Holotrichia* and *Anomala* are the speciose of all in India that belongs to Melolonthinae and Rutelinae, respectively. The genus *Holotrichia* consists of more than 100 species in India that are widely distributed [1]. Of all, *H. consanguinea* is the major important pest that infests several crops such as groundnut, sugarcane, sorghum, maize, etc. It is the predominant species present in plains across the country. Several geographical populations of the species exhibit differences among or within populations that are considered as systematic uniqueness [2]. The distinctness can be generally associated with the variability of fitness in different individuals of the population [3]. Subsequently, examination of variability within and among various populations helps to understand the extent to which the contrasts among individuals lead to different races [4]. The correlation of differences in variability of similar organs and structures in individuals of various populations will yield a key to play major role in studies related to variability in populations [5]. Insects are great subjects for studies on morphological variety [6] and hence, the present study has been carried out to understand the intraspecific variation among different *H. consanguinea* populations through different statistical analyses.

Material and methods
Collection and preparation of specimens
Different populations of *H. consanguinea* were collected from the plains across the country *i.e.* parts of Andhra Pradesh, Rajasthan and Uttar Pradesh during May-July 2015. Collection of adult beetles was made during the night by using light traps with black and mercury light sources. Collected specimens were sorted out to remove damaged specimens and then sorted specimens were subjected to little warm water at 60 ˚C temperature in Sonicator for 5 minutes, then cleaned with camel hair brush to remove soil particles adhered to the body of the specimens. After cleaning, the specimens were kept for relaxation overnight in relaxation boxes. Next day morning, the specimens were pinned, stretched, labelled and placed in hot air oven at temperature about 60 ^{0}C for proper drying.

Around 20 male and 30 female specimens,which are good in condition regarding their cleanliness, proper stretching and presence of all morphological characters selected randomly from each population for the present study. All specimens numbered individually and kept in insect box for further studies.

Selection of characters

A sum of 23 characters in males and 19 characters in females were studied for each population. All the ordered characters, includes both diagnostic key characters like clypeus, tibial spurs and general characters like tarsal segments, elytra etc.

Measurement of characters

Measurements were taken for all the characters separately for male and females of *H. consanguinea.* The calibration factor was derived by taking readings of ocular meter and stage micrometer to express it in mm. 20 specimens of males and 30 specimens of females of each species from each location were selected for this morphometric study. The length of full body was taken from tip of clypeus to end of the pygidium, length of head was taken from tip of clypeus to end of vertex, width of head was recorded inclusive of compound eyes, length of antennal segments were taken individually, width of clypeus was measured at middle of the clypeus, length and width of pronotum was measured across the centre, length of elytra was taken along the elytral suture, width of elytra was taken at the middle point, all tarsal segments measurement were taken individually, length of tibial spurs were taken from the base to tip. For genitalia studies, measurements of phallobase and parameres were carried out after extracting genitalia. The genitalia, after the morphometric measurements were put in a genital vial and pinned along with the adult specimen.

Statistical analysis

The different statistical analyses *viz*., univariate analysis, multivariate analysis, principal component analysis (PCA) and discriminant function analysis (DFA) were carried for intraspecific variation studies.

Univariate study is a type of measurable, quantitative, assessment. This examination has been by utilized for investigation of every character independently in data set to discover noteworthy characters. Its sole object for existing is to depict one character at once. Sometimes, univariate investigations are inadequate in the evaluation of variability in natural populations because they don't reflect the conceivable relationships among characters in an individual in the populations [2]. Thus the characters that showed significance at $P<0.01$ were subjected to multivariate investigation (MANOVA), Principal component analysis (PCA) and discriminant function analysis (DFA).

Principal Component Analysis (PCA), most widely utilized strategy for dimensionality decline with broad applications to information reduction, The targets of this analysis, are to control or to reduce the dimensionality of the information set and to distinguish novel important characters. The first principal component signifies much of the variation in the data, and every after segment represents as a great part of the rest of the changeability as could reasonably be expected. In present study PCA was worked out up to six loadings to explain the degree of variety among the populations. Discriminant function analysis (DFA) was performed to estimate the utility of the characters selected [7].

Softwares used were MS Excel for univariate analysis, SPSS for PCA and DFA and SAS for MANOVA.

Result and discussion

Univariate analysis done for each character for male and female to find out the significance has been presented in Table 1. The results showed that in male, all characters except length of head, length of pronotum, length of 1st tarsal segment and hind leg inner tibial spur exhibited significant differences among three populations and in female, all selected characters showed significant differences among three populations at the 5% level of significance.

Further, all 23 characters of males and 19 characters of females were subjected to multivariate analysis to test the significant differences among the three populations of male and female *H. consanguinea.* The results further confirmed that all the selected characters were significant, as evidenced by various statistical indices *viz.*, Wilks' Lambda, Pillai's Trace, Hotelling- Lawley Trace and Roy's greatest root at $P<0.0001$ (Table 2 and 3). This clearly depicted that the tested characters contributed significantly to differentiate the populations.

Every single selected character was subjected to Principal component (PC) analysis to lessen the proportions and discover the major cause of variation among three populations of *H. consanguinea*. In case of male *H. consanguinea*, the first six principal components that showed eigen values more than one accounted for 72.3% variation. Among six PCs, PC1 and PC2 explained 42.64% variation, while others account to less than 10% variation (Table 4), where PC1 that explained 26.1% variation has loadings of nine characters *viz.*, total length of body, length of antennal scape, length of antennal funicle, length of antennal club, width of clypeus, length of elytra, length of phallobase, length of paramere, width of paramere and PC2 that explained 16.5% variation has loadings of five characters namely width of pronotum, length of second, third, fourth and fifth tarsal segment that can contribute to the variation, while others have loadings of less significant variables. The other variables namely length of head, width of head, length of antennal pedicel, length of pronotum, width of elytra at middle, length of the first tarsal segment, length of hind inner and outer tibial spurs, width of phallobase (values < 0.25 in first two PCs) are of lesser significance in explaining the morphological variation.

In case of female *H. consanguinea* populations, when 19 characters subjected to PCA analysis, the first four principal components amounted to 77.29% variation, where eigen values are more than one and PC1 and PC2 explained 63.570% variation, while others account for less than 10% variation (Table 5). The PC1 explained 46.69% variation that had loadings of nine characters *viz.*, total length of body, width of head, length of antennal pedicel, length of antennal funicle, length of antennal club, width of clypeus, length of elytra, length of fourth tarsal segment and length of hind inner tibial spur while PC2 explained 16.88% variation that has loadings of five characters namely length of antennal scape, width of pronotum, length of first, second and third tarsal segment which can contribute to the variation. All others have loadings of less significant variables. The other variables namely length of head, length of pronotum, width of elytra at middle, length of fifth tarsal segment and length of hind outer tibial spur, (values < 0.25 in first two PCs) are of lesser significance in explaining the morphological variation. External variations other than sexual dimorphism among individuals are most ordinarily quantitative as opposed to subjective, as in geographic variety [8].

The discriminant function analysis (DFA) was carried out for male *H. consanguinea* to exploit the variation among the

groups, assessing the utility of characters and separate the groups. For this purpose cross validation of group membership was done to estimate the utility of characters used in analysis. The cross validation results showed that 100% of original grouped cases correctly classified; 100% of Andhra Pradesh population were correctly classified, where as in the case of Rajasthan population, 90% correctly classified and Uttar Pradesh population, 95% correctly classified. Overall 95% of cross-validated grouped cases correctly classified (Table 6), Similarly the cross validation results of female *H. consanguinea* showed that 100% of original grouped cases correctly classified, 100% of all three populations were correctly classified, where Overall 100% of cross-validated grouped cases correctly classified (Table 7), this indicated high degree of utility of the characters used in grouping the population. The territorial map drawn from two canonical discriminant functions showed the plots of three distinct populations in male (Fig. 1) and female (Fig. 2) of *H. consanguinea*, which need to be explored further.

Table 1: Univariate analysis for three populations of *H. consanguinea*

S. No	Character	Male			Female		
		Mean square	F-value	Pr > F	Mean square	F-value	Pr > F
1	Total length of body	12.782	25.42	<.0001	29.38	35.3	<.0001
2	Length of head	0.023	0.71	0.4947	0.34	16.31	<.0001
3	Width of head	0.226	14.17	<.0001	7.18	502.8	<.0001
4	Length of antennal scape	0.028	19.51	<.0001	0.05	13.83	<.0001
5	Length of antennal pedicel	0.001	11.34	<.0001	0.05	262.1	<.0001
6	Length of antennal funicle	0.064	40.33	<.0001	1.06	959.2	<.0001
7	Length of antennal club	0.097	33.72	<.0001	0.87	302.5	<.0001
8	Width of clypeus	0.369	75.56	<.0001	2.32	291.1	<.0001
9	Length of pronotum	0.174	2.1	0.132	0.35	7.64	0.0009
10	Width of pronotum	0.772	10.2	0.0002	1.09	24.89	<.0001
11	Length of elytra	6.05	8.96	0.0004	57.6	89.97	<.0001
12	Width of elytra at middle	0.258	5.54	0.0063	0.15	4.03	0.0211
13	Length of 1st tarsal segment	0.002	0.59	0.5581	0.03	5.8	0.0043
14	Length of 2nd tarsal segment	0.187	50.01	<.0001	0.14	16	<.0001
15	Length of 3rd tarsal segment	0.016	4.35	0.0174	0.22	38.46	<.0001
16	Length of 4th tarsal segment	0.033	10.72	0.0001	0.61	119.97	<.0001
17	Length of 5th tarsal segment	0.095	27.11	<.0001	0.05	24.53	<.0001
18	Length of hind inner tibial spur	0.002	0.82	0.4459	0.43	59.12	<.0001
19	Length of hind outer tibial spur	0.029	5.2	0.0084	0.05	4.46	0.0143
20	Length of phallobase	0.056	13.03	<.0001			
21	Width of phallobase	0.022	6.85	0.0022			
22	Length of paramere	0.041	5.54	0.0064			
23	Width of paramere	0.072	45.17	<.0001			

Table 2: Multivariate analysis (MANOVA) for male *H. consanguinea*

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.0062	17.84	46	70	<.0001
Pillai's Trace	1.80	14.48	46	72	<.0001
Hotelling- Lawley Trace	29.57	21.95	46	59.841	<.0001
Roy's Greatest Root	24.129	37.77	23	36	<.0001

Table 3: Multivariate analysis (MANOVA) for female *H. consanguinea*

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.00060	144.52	38	138	<.0001
Pillai's Trace	1.94	118.22	38	140	<.0001
Hotelling-Lawley Trace	98.60	176.77	38	120.78	<.0001
Roy's Greatest Root	78.72	290.03	19	70	<.0001

Table 4: Principal component loadings for 23 characters of *H. consanguinea* male populations

S. No	Character	PC1	PC2	PC3	PC4	PC5	PC6
1	Total length of body	0.271	-0.171	0.214	0.27	-0.144	0.06
2	Length of head	0.042	0.172	-0.059	-0.22	-0.43	0.262
3	Width of head	0.219	0.239	-0.223	-0.003	-0.149	0.208
4	Length of antennal scape	0.283	-0.113	0.131	0.089	-0.158	-0.296
5	Length of antennal pedicel	0.167	-0.182	0.329	0.086	0.233	0.175
6	Length of antennal funicle	0.339	-0.065	-0.05	-0.147	0.149	0.194
7	Length of antennal club	0.302	-0.1	-0.098	-0.15	0.192	-0.042
8	Width of clypeus	0.35	-0.1	-0.13	-0.135	-0.006	0.055
9	Length of pronotum	0.131	0.214	-0.265	0.344	-0.068	-0.082
10	Width of pronotum	0.038	0.375	-0.031	0.268	-0.129	0.114
11	Length of elytra	0.266	-0.065	0.262	0.098	-0.257	0.137
12	Width of elytra at middle	0.205	-0.042	0.065	0.17	0.042	0.507
13	Length of 1st tarsal segment	0.096	0.084	0.376	-0.13	0.341	0.079
14	Length of 2nd tarsal segment	-0.19	0.321	0.256	0.254	0.136	0.121
15	Length of 3rd tarsal segment	0.019	0.284	0.413	0.1	0.161	-0.1
16	Length of 4th tarsal segment	0.102	0.302	0.052	-0.083	0.196	-0.275
17	Length of 5th tarsal segment	-0.012	0.432	-0.105	-0.002	0.103	0.104
18	Length of hind inner tibial spur	0.078	-0.113	-0.247	0.457	0.107	-0.02
19	Length of hind outer tibial spur	-0.078	-0.212	-0.215	0.452	0.288	0.037
20	Length of phallobase	0.291	0.238	0.074	0.052	-0.174	-0.081
21	Width of phallobase	0.118	0.151	-0.269	-0.153	0.406	0.248
22	Length of paramere	0.25	0.08	0.012	0.129	-0.039	-0.415
23	Width of paramere	0.283	0.098	-0.161	-0.137	0.212	-0.264
	Eigen value	6.00	3.8	2.12	1.818	1.604	1.29
	Percentage of variance	26.1	16.5	9.2	7.9	6.97	5.61
	Cumulative percentage	26.1	42.64	51.86	59.76	66.73	72.34

Table 5: Principal component loadings for 19 characters of *H. consanguinea* female populations

S. No	Character	PC1	PC2	PC3	PC4	PC5	PC6
1	Total length of body	0.265	-0.170	0.194	-0.050	0.162	-0.114
2	Length of head	0.149	0.163	0.304	-0.471	0.391	-0.360
3	Width of head	0.258	-0.250	-0.062	0.157	0.064	0.323
4	Length of antennal scape	-0.010	0.458	0.071	0.149	-0.206	-0.043
5	Length of antennal pedicel	0.308	-0.129	-0.129	-0.082	-0.063	-0.034
6	Length of antennal funicle	0.318	-0.060	-0.174	-0.058	-0.092	0.045
7	Length of antennal club	0.308	-0.017	-0.138	-0.102	-0.147	-0.069
8	Width of clypeus	0.319	-0.023	-0.089	-0.078	-0.016	0.037
9	Length of pronotum	0.183	0.138	0.307	-0.096	-0.300	0.654
10	Width of pronotum	0.200	0.281	0.312	-0.247	-0.108	-0.006
11	Length of elytra	0.294	-0.037	-0.037	-0.187	0.016	0.016
12	Width of elytra at middle	0.120	-0.175	0.613	0.151	0.099	0.021
13	Length of 1st tarsal segment	0.139	0.350	-0.065	0.348	0.295	0.045
14	Length of 2nd tarsal segment	0.005	0.513	0.056	0.100	0.075	0.157
15	Length of 3rd tarsal segment	0.228	0.285	-0.215	0.083	0.135	-0.155
16	Length of 4th tarsal segment	0.285	0.155	-0.295	0.062	0.093	0.001
17	Length of 5th tarsal segment	0.184	-0.163	0.070	0.372	0.494	0.166
18	Length of hind inner tibial spur	0.256	-0.056	-0.025	0.059	-0.409	-0.235
19	Length of hind outer tibial spur	0.146	-0.035	0.270	0.539	-0.312	-0.425
	Eigen value	8.872	3.206	1.410	1.198	0.924	0.800
	Percentage of variance	46.690	16.880	7.420	6.300	4.860	4.210
	Cumulative percentage	46.690	63.570	70.990	77.290	82.150	86.360

Table 6: Classification results of cross-validation of group membership for male *H. consanguinea*

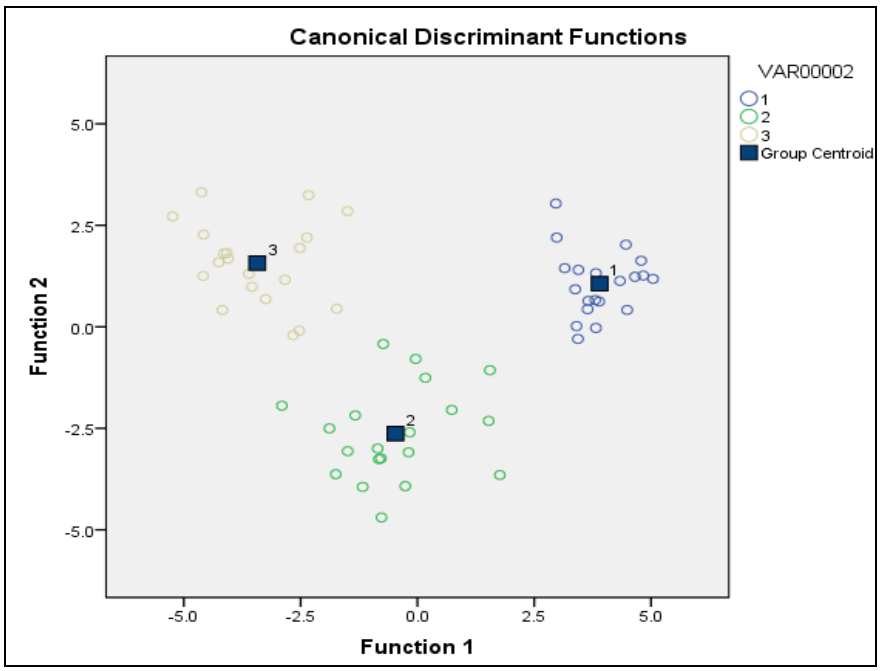
			Predicted Group Membership			Total
			1	2	3	
Original	Count	1	20	0	0	20
		2	0	20	0	20
		3	0	0	20	20
	%	1	100	0	0	100
		2	0	100	0	100
		3	0	0	100	100
Cross-validated[b]	Count	1	20	0	0	20
		2	1	18	1	20
		3	0	1	19	20
	%	1	100	0	0	100
		2	5	90	5	100
		3	0	5	95	100

Note: 1-Andhra Pradesh, 2-Rajasthan, 3-Uttar Pradesh populations

Table 7: Classification results of cross-validation of group membership for female *H. consanguinea*

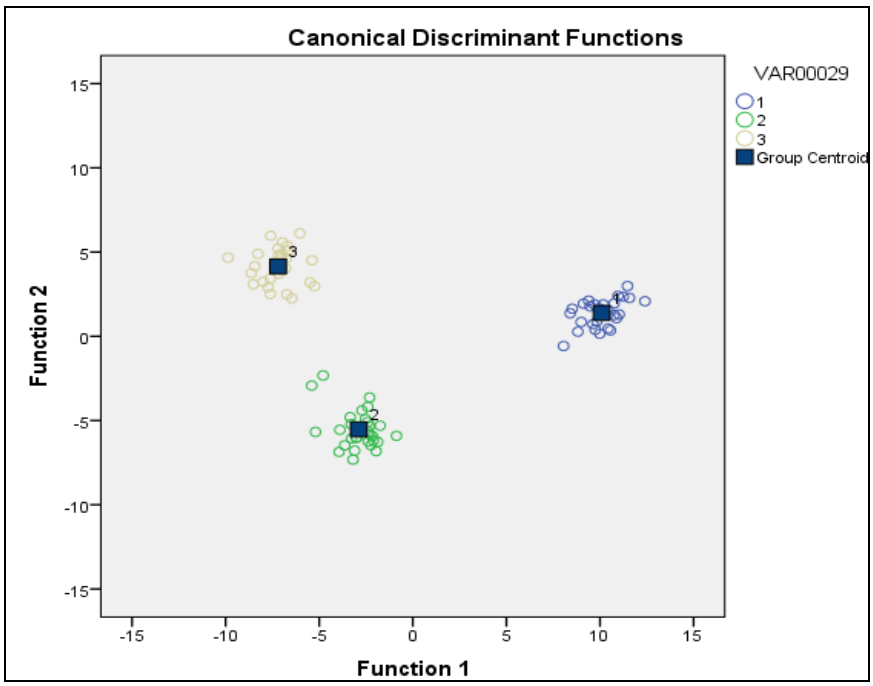
			Predicted Group Membership			Total
			1	2	3	
Original	Count	1	30	0	0	30
		2	0	30	0	30
		3	0	0	30	30
	%	1	100	0	0	100
		2	0	100	0	100
		3	0	0	100	100
Cross-validated	Count	1	30	0	0	30
		2	0	30	0	30
		3	0	0	30	30
	%	1	100	0	0	100
		2	0	100	0	100
		3	0	0	100	100

Note: 1-Andhra Pradesh, 2-Rajasthan, 3-Uttar Pradesh populations



Note: 1-Andhra Pradesh, 2-Rajasthan, 3-Uttar Pradesh populations

Fig 1: Territorial map showing the plots of three populations of male *H. consanguinea*



Note: 1-Andhra Pradesh, 2-Rajasthan, 3-Uttar Pradesh populations

Fig 2: Territorial map showing the plots of three populations of female *H. consanguinea*

Conclusion

The three populations of *H. consanguinea* exhibited significant morphometric variations as evidenced by various statistical analyses. In case of *H. consanguinea* males, 19 selected characters out of 23 characters showed significant differences and around 14 characters viz., total length of body, length of antennal scape, length of antennal funicle, length of antennal club, width of clypeus, width of pronotum, length of elytra, length of second, third, fourth and fifth tarsal segment, length of phallobase, length and width of paramere are identified as main basis that can contribute to the variation through principal component analysis. In case of *H. consanguinea* females, all 19 characters showing significant variation in univariate analysis were further confirmed by MANOVA and 14 characters *viz.*, total length of body, width of head, length of antennal pedicel, length of antennal scape, length of antennal funicle, length of antennal club, width of clypeus, width of pronotum, length of elytra, length of first, second, third, fourth tarsal segment and length of hind inner tibial spur are identified as main basis that can contribute to the variation, which needs to be explored further for its consistency. The three distinct populations as evidenced through territorial map indicates geographical isolation that aids in speciation process in due course of time.

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