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Larval salivary glue protein heterosis and dosage compensation among the interspecific F_1 hybrids of *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans*

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Abstract Reciprocal cross effects with respect to larval salivary secretory protein levels were studied in the interspecific fertile reciprocal hybrids by crossing *Drosophila nasuta nasuta, and Drosophila nasuta albomicans*. These proteins are produced copiously during the third larval instar stage and are believed to play a role in the attachment of pupa to the substratum prior to pupariation as well as in insect immunity. Quantitative variations were encountered among the reciprocal hybrids. Significant heterosis was observed between *D. n. nasuta* and the F_1 hybrid female of a cross between *D. n. albomicans* female and *D. n. nasuta* male (21.39%) while the F_1 hybrids of a cross between *D. n. nasuta* female and *D. n. albomicans* male showed a marginal increase (4.24%) from the mid parent level. The glue secretions were correlated to total cell number but independent of gland size. SDS PAGE revealed a considerable heterosis with respect to X-linked protein fractions. Here we report sex specific biochemical heterosis. However the X-linked fractions undergo dosage compensation in both parents and hybrids indicating strict regulatory control.

Introduction

Heterosis is a well known genetic phenomenon where the mean of the F_1 family exceeds its better parent or mid parental values (Mather and Jinks, 1971). The term was introduced by Shull in 1908 and various theories have been proposed like the dominance, over dominance, epistatic interactions and epigenetic factors for the occurrence of heterosis (*cf.* Seyfried and Yu, 1980). Studies in *Drosophila* provide evidence for heterosis. Hybrid vigor regarding fitness parameters, sex linked enzyme locus and H_1 histone proteins has been documented (Brncic, 1954; Anderson, 1968; Richmond and Powell, 1970; Martinez and Mcdaniel, 1981; Fry et al., 1998; Vaiserman et al., 2013).

The *nasuta* subgroup of *Drosophila* which belongs to the *Immigrans* group includes an assemblage of closely related morphologically almost similar species/subspecies that serve

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as an excellent model for the analysis of patterns and processes of differentiation among them. Various species/subspecies of this subgroup have been used to study salivary glue proteins which are a set of sticky glycoproteins secreted by the larval salivary glands in *Drosophila* that affix the puparium to a solid substratum prior to pupariation (Beckendorf and Kafatos, 1976; Korge, 1977; Ramesh and Kalisch, 1988; Shivanna and Ramesh, 1995; Shivanna et al., 1996; Zajonz et al., 1996; Ramesh and Shivanna, 1998; Aruna and Ranganath, 2006). These proteins are also believed to possess functions in providing insect immunity (Korayem et al., 2004; Syed et al., 2008; Mitchell et al., 2014). Analysis of SDS–PAGE patterns of these larval secretory proteins has shown that they are species specific, wild type strain specific and major protein fractions are X-linked (Ramesh and Kalisch, 1988; Ramesh and Kalisch, 1989a,b; Kalisch and Ramesh, 1997). Thus these secretions serve as biochemical markers.

Among various members of the *nasuta* subgroup, *D. n. nasuta* (2n = 8) and *D. n. albomicans* (2n = 6) are allopatric species belonging to frontal sheen complex that possess identical morphology but divergent karyotypes and the interspecific hybrids of these two species are fertile (Wilson et al., 1969; Kitagawa et al., 1982). The glue proteins in these two species constitute about 58–60% of the glue and the quantity of secretions is independent of the size of the glands in the parents (Shivanna and Ramesh, 1995). The X-chromosome was found to be homosequential and the major fractions of glue proteins are X-linked with a propensity to show dosage compensation (Ramesh and Rajasekarasetty, 1982; Ramesh and Kalisch, 1988; Kalisch and Ramesh, 1997; Aruna and Ranganath, 2005).

Earlier in the *nasuta* subgroup reciprocal cross effects were studied to check for sex-linkage or maternal effects. Reciprocal effects are supposed to be due to the genetic effects of parents (*cf*. Vaiserman et al., 2013). Thus the main purpose of the present study was to check for reciprocal effects in F_1 hybrids using glue proteins as a tissue specific marker. The following issues were addressed-

- Ascertain heterosis phenomenon in the reciprocal hybrids in glue protein levels.
- Correlation studies of glue protein levels with number of cells and gland size.
- Experimentally verify whether the hybrid males undergo dosage compensation or not.

Materials and methods

Drosophila stocks and maintenance

The wild type strain of *Drosophila nasuta nasuta* (Coorg, India; Stock No. 201.001) and *D. n. albomicans* (Okinawa, Japan; Stock No. 202.001) was obtained from Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Mysore, India. Populations of these flies were built up by the serial transfer technique using quarter pint bottles containing standard wheat cream agar medium and maintained in a vivarium at 22 ± 1 °C with 12:12 L:D cycle and 60% humidity.

Virgin females from both the stocks were collected periodically and aged for 6 days. Crosses were conducted between *D. n. nasuta* and *D. n. albomicans* to obtain F_1 progeny.

Cross I: *D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂ (*n*♀ × *a*♂).

Cross II: *D. n. albomicans* ♀♀ × *D. n. nasuta* ♂♂ (*a*♀ × *n*♂).

Synchronized eggs from the parents and the hybrids were collected as per the method of Delcour (1969). The eggs thus collected were transferred into culture medium to which few drops of yeast suspension were added. The eggs were allowed to hatch; the parental larvae and the hybrid larvae obtained from both the crosses were fed with yeast solution till they attained the late larval stage. Uniform conditions with respect to larval population density, quantity of food and temperature were maintained.

Determination of cell number and cell size

Two sets of samples were prepared to determine total cell number and gland size. Well fed third instar male and female larvae were dissected in invertebrate saline to isolate the salivary glands. To determine the cell number a single lobe of salivary glands was briefly fixed in 1 N HCl and later transferred to 2% lactoacetoorcein stain. After 15 min the glands were transferred on a clean slide and squashed to facilitate spreading of the cells. The larval salivary gland cells are uninucleate in *Drosophila* and the number of nuclei was counted according to Shivanna and Ramesh (1995).

Single lobe of salivary glands was imaged and the size of the glands (length × breadth) was determined by means of ImageJ software.

Sample preparation

Male and female late third instar larvae which were about to pupate were selected for preparing the samples. The bloated glands filled with secretions were carefully isolated in the invertebrate saline from the larvae without damaging the glands. The samples were prepared as described by Ramesh and Kalisch (1988). These bloated glands were immediately fixed in 95% ethanol thereby condensing the secretions in the form of plug. The plug was separated from the glandular cells using a pair of extra- fine needles and processed further in 1:1 chloroform and methanol. The processed plugs were allowed to dry and later dissolved in sample buffer (0.0625 M Tris–HCl pH-6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol).

Protein quantification

Total protein concentration in the samples was determined by micromethod (Neuhoff, 1985). The secretions from 5 pairs of male and female salivary glands were precipitated separately in the form of plug and later dissolved in 25 µl of sample buffer. Briefly 25 µl of sample thus prepared was spotted on 2 × 2 cm cellulose acetate strips (Sartorius AG 37070, Germany) and dried at room temperature. Similarly known quantities of BSA dissolved in sample buffer were spotted on cellulose acetate strips for obtaining the calibration curve. The strips were stained with 0.5% amido black in methanol and acetic acid (9:1) and allowed to dry completely. The spots stained with dye were excised from the dried strips, dissolved in 4 ml DMSO and the absorbance was read at 630 nm in Hitachi

U-2900 spectrophotometer. The data obtained were used to calculate Mid Parent Heterosis (MPH) and Better Parent Heterosis (BPH) for 3 parameters: (i) males + females (ii) males and (iii) females. These parameters were computed using the following formula

$$\mathrm{MPH} = \frac{(\mathrm{F_1} - \mathrm{MP})}{\mathrm{MP}} \times 100$$

$$\mathrm{BPH} = \frac{(\mathrm{F_1} - \mathrm{BP})}{\mathrm{BP}} \times 100$$

$\mathrm{MP} = \frac{\mathrm{P_1}+\mathrm{P_2}}{2}$ and BP is mid parent and best parent values respectively. $\mathrm{P_1}$ = mean of *D. n. nasuta*. $\mathrm{P_2}$ = mean of *D. n. albomicans* (Subramanya and Bishop, 2011; Zorgo et al., 2012).

SDS–PAGE

In a parallel experiment, a single pair of salivary plug isolated from bloated glands of parents and hybrids dissolved in 20 µl of sample buffer was heated for 10 mins in boiling water. The samples thus prepared were loaded into the wells of 13.7% SDS–polyacrylamide gels containing 0.8% bisacrylamide and electrophoresed at 70 V initially and later at 100V until tracking dye reached the edge (Ramesh and Kalisch, 1988). After electrophoresis the gels were stained with CBB-R250 for 2 h and destained in a mixture of methanol: glacial acetic acid (3:1). They were documented using Bio Rad gel documentation XR^+ image system (Bio Rad, USA). Volume analysis of the major glue protein fractions was carried out by using Image Lab software (version 2.0.1) to examine changes if any, in the volume of major glue fractions among the parents and hybrids. In the volume mode the software identifies the signal intensity of the bands concerned. The bands were quantified and expressed as volume; the sum of the intensities of the pixels within a defined volume boundary × pixel area (intensity units × mm^2). Background correction was made using the local background subtraction parameter. The volume of the bands was normalized using the volume of the BSA band (Besic et al., 2014). Volumes of these major fractions obtained were categorized into autosomal, X-linked and total (autosomal + X linked) fractions. The data were subjected to normality test and log transformed (Mc Lean et al., 2007).

Statistical analysis

One way ANOVA was performed followed by Tukey's HSD test to determine difference in cell number, gland size and glue protein quantity in parents and hybrids. Pearson's correlation coefficient was calculated to determine the degree of relationship between cell number, gland size and glue protein quantity. The MPH and BPH data were subjected to one way ANOVA followed by Tukey's post hoc test to test the significance of differences obtained. Correlation analysis was made between mid parent value and the reciprocal hybrid glue proteins.

The transformed volume data of the total fractions and X-linked fractions from SDS–PAGE were subjected to One way ANOVA to test the significance. MPH and BPH of the X linked fractions were calculated as mentioned earlier and subjected to One way ANOVA to test the significance. The X-linked glue fractions of the parents and $\mathrm{F_1}$ hybrids were subjected to Independent *t* test to determine the dosage compensation in males (Aruna and Ranganath, 2005).

Results

Cell number, gland size and glue protein estimation

The cell number, gland size and the glue protein quantity were subjected to one way ANOVA followed by Tukey's HSD test. Significant difference was observed in cell number and glue protein quantity between parents and hybrids and insignificant difference with respect to gland size. *D. n. nasuta* male showed a considerable variation in cell number from *D. n. albomicans* female and $\mathrm{F_1}$ hybrid female from the cross of $a♀ \times n♂$. Differences were also observed for cell number between *D. n. albomicans* female and $\mathrm{F_1}$ hybrid male from the cross of $n♀ \times a♂$. Though no differences were observed for protein quantity in parents, glue protein differed considerably between *D. n. nasuta* and female of a cross of $a♀ \times n♂$. There was a marginal increase in the mean of $\mathrm{F_1}$ hybrids of $n♀ \times a♂$ which was found to be statistically insignificant (Table 1). Pearson's correlation coefficient revealed a significant relationship between cell number and quantity of glue secretions ($r = 0.479$, $p < 0.01$). Size of the gland was independent of quantity of secretions ($r = 0.245$, $p > .05$). There was no correlation between glue protein secretion among parents and hybrids.

Mid parent and better parent heterosis data computed were subjected to one way ANOVA followed by Tukey's HSD test. Post hoc test revealed a clear heterosis in $\mathrm{F_1}$ hybrids of $a♀ \times n♂$ from mid parent values in the following parameters (i) males + females, $p < 0.01$ (ii) males (iii) females, $p < 0.01$. Among the hybrids, $\mathrm{F_1}$ $(n♀ \times a♂)$ and $\mathrm{F_1}$ $(a♀ \times n♂)$ statistical differences were observed at $p < 0.05$ for mid parent values in both parents as well as females (Table 2). The data revealed a general quantitative variation in the protein expression levels in reciprocal hybrids. Correlation analysis between mid parent values and the hybrids did not reveal any significant relationship between them.

Evaluation of better parent values (*D. n. albomicans*) and the hybrids with One way ANOVA revealed a difference between $\mathrm{F_1}$ hybrid females and better parent females. However post hoc tests could not confirm differences among the individual groups (Table 3).

SDS–PAGE

The major fractions were resolved in parents and hybrids (Fig. 1, Table 4). Volume of the major bands comprising total and X-linked fractions among the parent and hybrids was analyzed. Post hoc analysis revealed quantitative variation between *D. n. nasuta* and the $\mathrm{F_1}$ $(a♀ \times n♂)$ females in the total and X-linked fractions between the parents and hybrids at $p < 0.01$ respectively (Table 5).

Analysis of X linked fractions by SDS–PAGE

Major glue fractions in *D. n. nasuta* and in *D. n. albomicans* are X- linked (Table 4). Hence the $\mathrm{F_1}$ females of the crosses inherit all the X-linked fractions from their parents while the hybrid males inherit only the X-linked fractions of their female parent. This pattern of inheritance prompted us to examine

Table 1 One way ANOVA of total number of cells, size of salivary gland and quantity of glue secretions in parents and their hybrids.

Groups	No. of cells[a]	Size of glands (mm^2)[b]	Neuhoff Micromethod µg of glue secretions Mean ± S.E.[c]
D. n. n ♂	118.45 ± 1.15[a]	.305 ± .057	7.780 ± .20[a]
D. n. n ♀	119.45 ± 0.86[a,c]	.312 ± .078	7.786 ± .21[a]
D. n. a ♂	121.9 ± 1.65 [a,c]	.315 ± .114	8.73 ± .86[a,b]
D. n. a ♀	124.27 ± 1.50[b,c]	.328 ± .124	8.79 ± .44 [a,b]
F_1 ♂ (*n*♀ × *a*♂)	118.27 ± 1.46 [a,d]	.319 ± .943	8.511 ± .35[a,b]
F_1 ♀ (*n*♀ × *a*♂)	123.27 ± 1.28[a,c]	.325 ± .811	8.74 ± .34[a,b]
F_1 ♂(*a*♀ × *n*♂)	120.36 ± 1.34[a,c]	.329 ± .122	9.67 ± .22[a,b]
F_1 ♀ (*a*♀ × *n*♂)	125.36 ± 0.84[b,c,e]	.332 ± .927	10.42 ± .54[b]

Means having superscripts of different lower case alphabets represent significant difference from one another.
[a] Average number of cells in a single lobe of salivary gland ($N = 20$). Significant at $p < 0.01$.
[b] Size of a single lobe of salivary gland ($N = 20$). Not significant at $p > 0.05$.
[c] Average quantity of protein secretion from salivary glands/individual. 5 sets of replicates containing 5 pairs of salivary glands were used. Significant at $p < 0.01$.

Table 2 Analysis of variance (One way) of mid parent heterosis (MPH) of glue proteins in males and females between parents and the hybrids, males and females separately between parents and the hybrids.

Groups MPH		Mean ± S.E	Heterosis (%)	Sig.
Males + females	*D.n.n* + *D.n.a*	8.28 ± .35[a]		0.006
	F_1 (*n*♀ × *a*♂)	8.63 ± .31[a]	4.24	
	F_1 (*a*♀ × *n*♂)	10.04 ± .33[c]	21.39	
Males	*D.n.n* + *D.n.a*	8.26 ± .50		0.047
	F_1 (*n*♀ × *a*♂)	8.51 ± .35	3.06	
	F_1 (*a*♀ × *n*♂)	9.67 ± .22	17.13	
Females	*D.n.n* + *D.n.a*	8.29 ± .29[a]		0.008
	F_1 (*n*♀ × *a*♂)	8.74 ± .35[a]	5.40	
	F_1 (*a*♀ × *n*♂)	10.42 ± .54[c]	24.62	

Note: Five pairs of plug in 5 replicates were used for parents and hybrids. Significance at $p < 0.05$, $p < 0.01$. Means having superscripts of different lower case alphabets represent significant difference from one another.

Table 3 Analysis of variance (One way) of better parent heterosis (BPH) of glue proteins in males and females between parents and the hybrids, males and females separately between parents and the hybrids.

Groups BPH		Mean ± S.E	Heterosis (%)	Sig.
Males + females	*D.n.a*	8.76 ± .62		0.081
	F_1 (*n*♀ × *a*♂)	8.62 ± .31	0.84	
	F_1 (*a*♀ × *n*♂)	10.04 ± .33	14.59	
Males	*D.n.a*	8.73 ± 0.86		0.323
	F_1 (*n*♀ × *a*♂)	8.51 ± 0.35	−2.57	
	F_1 (*a*♀ × *n*♂)	9.67 ± 0.22	10.73	
Females	*D.n.a*	8.79 ± .45		0.037
	F_1 (*n*♀ × *a*♂)	8.73 ± .35	−0.64	
	F_1 (*a*♀ × *n*♂)	10.42 ± .54	18.42	

Note: Five pairs of plug in 5 replicates were used for parents and hybrids. Significance at $p < 0.05$. Means having superscripts of different lower case alphabets represent significant difference from one another.

whether the hybrid males show heterosis and dosage compensation with respect to X-linked glue protein fractions. By employing the semi quantitative technique the volume of the major bands was determined. The MPH and BPH were calculated for only X-linked fractions and on Post hoc analysis revealed a significant difference in MPH for F_1 hybrids (*a*♀ × *n*♂) at $p < 0.05$ and an insignificant change in BPH (Table 5). The log transformed volume of individual X-linked fractions was subjected to Independent *t* test to check the dosage in males and females. It revealed an insignificant difference among male and female X-linked glue fractions (Table 6).

Discussion

In the present study, reciprocal cross effects were analyzed with respect to larval salivary glue proteins. The F_1 hybrids

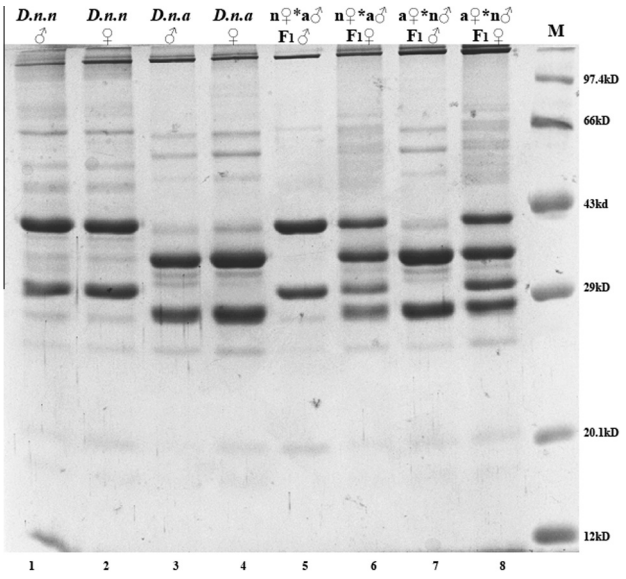

Figure 1 SDS PAGE (13.7%) using glue plug from a single pair gland was performed. Major fractions were resolved using SDS–PAGE and volume analysis was done with 25 replicates by Image Lab software (version 2.0.1). Lanes (1) *D. n. nasuta* ♂ (2) *D. n. nasuta* ♀ (3) *D. n. albomicans* ♂ (4) *D. n. albomicans* ♀ (5) F_1 (*D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂) ♂ (6) F_1 ($n♀ \times a♂$) ♀ (7) F_1 (*D. n. albomicans* ♀♀ × *D. n. nasuta* ♂♂) ♂ (8) F_1 ($a♀ \times n♂$) ♀.

show a higher mean from their parents. The female hybrids of a cross between *D. n. albomicans* female and *D. n. nasuta* parents show a statistically noteworthy heterosis while the F_1 hybrids of a cross between *D. n. nasuta* female and *D. n. albomicans* male parents secreted glue proteins marginally higher than the mid parent values (Tables 1 and 4). Our results illustrate an example of sex specific biochemical heterosis. Hannon et al. (2011) have also shown that F_1 hybrids of one sex reveal clear heterosis whereas the F_1 of the other is intermediary between the means of the parent population. Thus essentially the combination of the parental pair has a major bearing on the extent of heterosis manifested by the hybrids for different traits (Chen, 2010; Baranwal et al., 2012). The MPH and BPH for males, females separately and males + females calculated showed statistically significant heterosis (Tables 2 and 3) while the X-chromosomal fractions showed a considerable heterosis only for MPH and not BPH (Table 5). From the data it is very clear that the quantitative variations are higher in females. This is due to the contributions from both the parents to the hybrids. With respect to fitness parameters F_1 individuals tend to show heterosis followed by breakdown in F_2 progeny (Rajasekarasetty et al., 1987). In case of accessory gland protein secretions the interspecific hybrids do not show any luxuriance or breakdown (Ravi Ram and Ramesh, 2002) whereas Civetta and Singh (1998) have shown luxuriance in hybrids for non sexual traits. In our study the female hybrids of $a♀ \times n♂$ cross showed a considerable heterosis for glue protein expression which is mainly sex linked. Overdominance was observed in females of $a♀ \times n♂$ cross. The physiological basis of the increase in size of the F_1 hybrids has been attributed to derive either from very much smaller percentage increases in the growth rate or due to relaxation of the strict regulatory control in the heterozygotes (*cf.* Milborrow, 1998). Thus the overall difference in growth rates can be further analyzed into small percentage differences between components of growth (Hunt and Cornelissen, 1997).

An important feature of heterosis is that it has a dosage element. In *Drosophila* equalization occurs by the up- regulation of the male X-chromosomal genes. Sex linkage of a gene is established when the females display a codominant pattern and the males express only the maternal pattern in a reciprocal cross (Dickinson and Sullivan, 1975). Dosage compensation of X-linked proteins has been studied earlier by measuring their quantities directly in gels (Korge, 1975; Williamson and Bentley, 1983). In *D. melanogaster* the *sgs4* glue protein gene was found to be dosage compensated in males whereas the same gene of a variant strain in *D. melanogaster* lacks dosage compensation (Korge, 1975; Furia et al., 1992).

Unlike *D. melanogaster* where all except *Sgs4* gene are autosomally linked in case of the *nasuta* subgroup major glue protein fractions were found to be X-linked. The major glue protein fractions in these two species show different patterns as observed on SDS–PAGE which can be easily analyzed in the heterozygous F_1 females. F_1 hybrid analysis confirmed their X chromosome linkage with a codominant inheritance

Table 4 One way ANOVA of major fractions and X-linked fractions in parents and hybrids.

Groups	Major fractions in SDS–PAGE	Molecular mass of glue proteins (kD)	SDS–PAGE Log transformed mean volume ± S.E.(pixels × mm^2)	
			Total	X-linked
D. n. n ♂	5	130, 43, 30/28, 14	$6.98 \pm 0.02^{a,b}$	$6.83 \pm 0.03^{a,b}$
D. n. n ♀	5	130, 43, 30/28, 14	$7.01 \pm 0.02^{a,b}$	$6.93 \pm 0.02^{a,b}$
D. n. a ♂	5	130, 35, 25.5/23, 14	$7.02 \pm 0.02^{a,b,e}$	$6.95 \pm 0.02^{a,b,e}$
D. n. a ♀	5	130, 35, 25.5/23, 14	$7.04 \pm 0.02^{a,b,e}$	$6.97 \pm 0.03^{a,b,e}$
F_1 ♂ ($n♀ \times a♂$)	5	130, 43, 30/28, 14	$7.05 \pm 0.01^{a,b,e}$	$6.96 \pm 0.02^{a,b,e}$
F_1 ♀ ($n♀ \times a♂$)	8	130, 43, 30/28, 35, 25.5/23, 14	$7.04 \pm 0.03^{a,b,e}$	$6.96 \pm 0.03^{a,b,e}$
F_1 ♂($a♀ \times n♂$)	5	130, 35, 25.5/23, 14	$7.07 \pm 0.02^{a,b,e}$	$6.98 \pm 0.02^{a,b,e}$
F_1 ♀ ($a♀ \times n♂$)	8	130, 43, 30/28, 35, 25.5/23, 14	$7.10 \pm 0.02^{c,d,e}$	$7.02 \pm 0.03^{c,d,e}$

Note: Groups: *D. n. nasuta* – *D. n. n*, *D. n. albomicans* – *D. n. a*, *D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂-F_1 ($n♀ \times a♂$), *D. n. albomicans* ♀♀ × *D. n. nasuta* ♂♂-F_1 ($a♀ \times n♂$). Total refers to all the major fractions in parents and hybrids and X-linked refers to all the protein fractions except 14kD. One way ANOVA from 25 replicates revealed a significant difference for total and X-linked fractions at $p < 0.01$. Means having superscripts of different lower case alphabets represent significant difference from one another. Significant difference between *D.n.n* parents and $a♀ \times n♂$-♀.

Table 5 Analysis of variance (One way) of MPH and BPH of only the X-linked fractions in hybrids.

Groups		Log transformed mean volume ± S.E.(pixels × mm^2)	Heterosis (%)	Sig.
MPH	*D.n.n* + *D.n.a*	6.93 ± 0.018^d		0.022
	F_1 (*n*♀ × *a*♂)	6.94 ± 0.017^d	3.48	
	F_1 (*a*♀ × *n*♂)	7.00 ± 0.024^e	19.86	
BPH	*D.n.a*	6.96 ± 0.020		0.094
	F_1 (*n*♀ × *a*♂)	6.94 ± 0.017	1.35	
	F_1 (*a*♀ × *n*♂)	7.00 ± 0.012	13.02	

Note: Single pair of plug in 25 replicates each was used for parents and hybrids. Significance at $p < 0.05$. Means having superscripts of different lower case alphabets represent significant difference from one another.

Table 6 Independent *t* test of X-linked glue protein fractions between parents and hybrid male and females to check for dosage compensation.

Groups	X-linked fractions Log transformed Mean volume ± S.E (pixels × mm^2)	Sig.
D.n.n ♂	6.88 ± 0.024	.287
D.n.n ♀	6.92 ± 0.021	
D.n.a ♂	6.95 ± 0.020	.561
D.n.a ♀	6.97 ± 0.028	
F_1 ♂ (*n*♀ × *a*♂)	6.93 ± 0.012	.628
F_1 ♀ (*n*♀ × *a*♂)	6.95 ± 0.029	
F_1 ♂ (*a*♀ × *n*♂)	6.98 ± 0.025	.404
F_1 ♀ (*a*♀ × *n*♂)	7.02 ± 0.030	

Note: X-linked refers to all the protein fractions except 14kD. Single salivary glue plug in 25 replicates was used. Not significant at $p > 0.05$.

pattern which tends to get compensated. As no recombinant patterns were localized among F_1 hybrids these fractions were considered to be inherited as a single unit (Ramesh and Kalisch, 1988, 1989). From our results based on quantitative analysis we could show that the hybrids especially females showed higher levels of glue proteins than the parents. This finding encouraged us to distinguish whether the hybrid males undergo dosage compensation or not. The F_1 hybrids of the crosses were checked for dosage compensation of X-linked fractions of glue proteins and we observed that there was no significant difference in X-linked fractions indicating compensation of dosage (Table 6). Thus dosage compensation mechanism is being maintained even at the F_1 hybrid level indicating strict regulatory control. Further studies on gene expression at the molecular level might provide an answer regarding the above mentioned occurrence. However at the protein level we could show heterotic effect which is also dosage compensated convincingly.

Thus the fertile F_1 hybrids in the *nasuta* subgroup provide a platform to answer a plethora of questions with respect to heterosis and dosage compensation data. This is the first report on variable heterotic effects in reciprocal crosses with respect to glue protein secretions. From the present experimental data we could infer that overall there is an increase in the glue protein expression in the hybrids which has been compensated in the hybrid males thereby maintaining the integrity of glue protein expression in the crosses. Further experiments should help in elucidating the mechanism behind the observed effects.

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