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Heterosis without selectional coadaptation in *Drosophila ananassae*

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Summary. The relative viabilities of homozygous and heterozygous karyotypes were measured by making crosses between strains of *D. ananassae* homozygous for ST or inverted gene orders in the second and third chromosomes. The strains utilized during the present study originated from widely separated localities in India, Kuala Lumpur and Kota Kinabaru, Malaysia and Chian Mai, Thailand. The presence of heterosis in many interpopulation crosses is evident from the results which show that the inversion heterozygotes formed by chromosomes coming from distant populations exhibit heterosis. On the other hand, heterosis is absent in two intrapopulation crosses. Thus the present results provide evidence that heterozygosis for many genes and gene complexes does produce high fitness without previous selectional coadaptation.

Key words: Inversion karyotypes – Relative fitness – Heterosis in interpopulation crosses – Coadaptation – *Drosophila ananassae*

Introduction

The study of inversion polymorphisms in *Drosophila* has proven that it is an adaptive character and that the inversion heterozygotes often exhibit heterosis (Dobzhansky 1951). The adaptive function of inversions is that of protecting coadapted complexes of polygenes.

It has been shown by Dobzhansky and co-workers (Dobzhansky and Powell 1975) that any two gene arrangements derived from the same population give, as a rule, heterotic heterozygotes in *D. pseudoobscura.* However, the adaptive superiority of heterozygotes formed by chromosomes of different geographic origins may be lost. This shows that the gene arrangements within the inversions are unique to specific populations and are mutually adjusted. Dobzhansky (1950) has called this mutual adjustment of polygene complexes to produce high fitness in inversion heterozygotes 'coadaptation'. The coadaptation of gene complexes of homologous chromosomes must have evolved by natural selection. The development of heterosis for pairs of inversion sequences derived from different localities has been demonstrated by Dobzhansky and Levene (1951) who provide evidence for heterosis due to selectional coadaptation.

Evidence for heterosis due to selectional coadaptation has also been provided in *D. paulistorum* and *D. willistoni* (Dobzhansky and Pavlovsky 1958) and *D. pavani* (Brncic 1961). In these species of *Drosophila,* the frequency of inversion heterozygotes decreases in hybrid populations of mixed geographic origins involving polymorphic strains. This indicates that in hybrid populations heterosis is lost due to breakdown of coadapted polygene complexes by crossing-over. However, the results of similar experiments conducted in *D. ananassae* by the present author (Singh 1972, 1981) are not in agreement with what has been reported in other species. The persistence of heterosis has been observed in interracial crosses involving geographic strains of *D. ananassae* originating from different localities in India.

The present article reports the results of the experiments which were conducted to estimate the relative viabilities of homozygotes and heterozygotes in F_2 generation of crosses involving homozygous strains of *D. ananassae.*

Materials and methods

During the present study the following strains of *D. ananassae* were utilized.

1. GH-ST, homozygous for standard gene sequence in all the chromosomes; origin-Ghazipur, Uttar Pradesh.
2. VN-ST, homozygous for ST gene sequence in all the chromosomes; origin – Varanasi, Uttar Pradesh.
3. Am_6, homozygous for ST gene arrangement in all the chromosomes; origin – Mysore, Karnataka, South India.
4. BH-ST, homozygous for ST gene order in all the chromosomes; origin – Bhagalpur, Bihar.

5. BH-AL, homozygous for AL inversion in 2L and for ST gene arrangement in the third chromosome; origin – Bhagalpur, Bihar.
6. D_{38}, homozygous for ST gene arrangement in all the chromosomes; origin – Chian Mai, Thailand.
7. B_{17}, homozygous for alpha, delta and eta inversions; origin, – Kota Kinabaru, Malaysia.
8. KL 1–4, homozygous for delta and eta inversions but polymorphic in 2L due to occurrence of alpha inversion including a new inversion within it (Singh 1983a); origin – Kuala Lumpur, Malaysia.
9. D_6, homozygous for ST gene arrangement in the third chromosome but polymorphic in 2L (alpha inversion); origin – Kuala Lumpur, Malaysia.

The alpha (subterminal), delta (terminal) and eta (basal) are the three cosmopolitan inversions which have been found to be polymorphic in most of the populations sampled.

The strain homozygous for the standard gene sequence was crossed to the strain homozygous for the inversion. From F_1 hybrids (inversion heterozygotes), 20 females and 20 males were transferred to a fresh culture bottle. In the F_2s, nearly 100 larvae taken directly from the culture bottle were analysed for their karyotypes. The usual acetocarmine method was used for making the preparations of polytene chromosomes. Of several crosses made, two are intrapopulation crosses involving chromosomes derived from the same population. These crosses are BH-ST×BH-AL and D_6×KL1–4. The other crosses are interpopulation crosses.

Results

The presence of heterosis can be demonstrated by a direct method given below. One strain homozygous for ST is crossed to another strain homozygous for inversion. F_1 hybrids (inversion heterozygotes) are placed in culture bottles and allowed to produce progeny. According to the first law of Mendel, the F_2 zygotes will consist of 50% heterozygotes and of 25% of each of the two classes of homozygotes. Due to the differences in viability (a component of fitness) of different karyotypes, these proportions may be modified among the larvae. The relative viabilities of homozygotes and heterozygotes are estimated on the basis of deviations from 1 : 2 : 1 ratio expected in the F_2 offspring. The relative viabilities of karyotypes were estimated by making the viability of heterozygotes always equal unity.

In order to measure the discrepancies between the viabilities of homozygotes and heterozygotes, the χ^2 values were calculated by following the formula suggested by Dobzhansky and Levene (1951). These χ^2 values have one degree of freedom.

The relative viabilities of 2L karyotypes in different crosses have been shown in Table 1. In most of the interstrain crosses, the ST/AL heterozygotes are superior to both homozygotes and the differences are also significant in five crosses. The heterozygotes are slightly inferior to the AL/AL homozygotes in two crosses. The heterozygotes formed by ST and AL chromosomes derived from the same locality (Bhagalpur) do not exhibit heterosis.

The data on the relative viabilities of 3L karyotypes are presented in Table 2. The superiority of inversion heterozygotes over both homozygotes has been found

Table 1. The relative viability of 2L karyotypes in different crosses involving homozygous strains of *Drosophila ananassae*

Crosses		Karyotypes		
		ST/ST	ST/AL	AL/AL
1. BH-ST×BH-AL	Viability	0.46± 0.15	1	1.38±0.30
	χ^2	13.35		1.60
2. VN-ST×BH-AL	Viability	0.63±0.15	1	1.14±0.23
	χ^2	5.8		0.38
3. GH-ST×BH-AL	Viability	0.55±0.14	1	0.48±0.14
	χ^2	9.66		14.86
4. D_{38}×BH-AL	Viability	0.42±0.12	1	0.47±0.12
	χ^2	25.10		18.24
5. Am_6×BH-AL	Viability	0.40±0.13	1	1.06±0.23
	χ^2	22.5		0.07
6. BH-ST×B_{17}	Viability	0.33±0.11	1	0.60±0.16
	χ^2	34.64		6.15
7. VN-ST×B_{17}	Viability	0.65±0.16	1	0.41±0.12
	χ^2	4.86		23.84
8. GH-ST×B_{17}	Viability	0.86±0.19	1	0.51±0.14
	χ^2	0.56		12.99
9. D_{38}×B_{17}	Viability	0.39±0.12	1	0.84±0.20
	χ^2	33.83		0.67
10. Am_6×B_{17}	Viability	0.46±0.15	1	0.46±0.15
	χ^2	13.38		13.38

Table 2. The relative viability of 3L karyotypes in different crosses involving homozygous strains of *Drosophila ananassae*

Crosses		Karyotypes		
		ST/ST	ST/DE	DE/DE
1. BH-AL × B_{17}	Viability	0.84±0.20	1	0.52±0.14
	χ^2	0.67		11.02
2. BH-ST × B_{17}	Viability	0.46±0.15	1	0.92±0.23
	χ^2	13.35		0.12
3. VN-ST × B_{17}	Viability	0.65±0.17	1	0.77±0.19
	χ^2	4.45		1.53
4. GH-ST × B_{17}	Viability	0.77±0.17	1	0.26±0.09
	χ^2	1.96		74.00
5. D_{38} × B_{17}	Viability	1.11±0.25	1	0.59±0.17
	χ^2	0.19		5.92
6. Am_6 × B_{17}	Viability	1.10±0.29	1	0.70±0.22
	χ^2	0.12		1.90
7. D_6 × B_{17}	Viability	1.00±0.24	1	0.92±0.23
	χ^2	–		0.12
8. D_6 × KL1–4	Viability	1.03±0.24	1	0.66±0.18
	χ^2	0.02		3.51
9. BH-ST × KL1–4	Viability	0.83±0.20	1	0.69±0.18
	χ^2	0.72		3.01
10. VN-ST × KL1–4	Viability	0.93±0.21	1	0.40±0.13
	χ^2	0.12		22.50
11. GH-ST × KL1–4	Viability	0.90±0.21	1	0.58±0.16
	χ^2	0.24		7.35
12. D_{38} × KL1–4	Viability	0.66±0.17	1	0.66±0.17
	χ^2	3.91		3.91
13. AM_6 × KL1–4	Viability	0.61±0.15	1	0.42±0.12
	χ^2	6.36		21.59

Table 3. The relative viability of 3R karyotypes in different crosses involving homozygous strains of *Drosophila ananassae*

Crosses		Karyotypes		
		ST/ST	ST/ET	ET/ET
1. BH-AL × B_{17}	Viability	0.84±0.20	1	0.52±0.14
	χ^2	0.67		11.02
2. BH-ST × B_{17}	Viability	0.46±0.15	1	0.92±0.23
	χ^2	13.35		0.12
3. VN-ST × B_{17}	Viability	0.63±0.16	1	0.69±0.17
	χ^2	5.35		3.34
4. GH-ST × B_{17}	Viability	0.77±0.17	1	0.26±0.09
	χ^2	1.96		74.00
5. D_{38} × B_{17}	Viability	1.07±0.24	1	0.50±0.15
	χ^2	0.08		11.36
6. Am_6 × B_{17}	Viability	1.10±0.29	1	0.70±0.22
	χ^2	0.12		1.90
7. D_6 × B_{17}	Viability	1.00±0.24	1	0.92±0.23
	χ^2	–		0.12
8. D_6 × KL1–4	Viability	0.83±0.20	1	0.62±0.17
	χ^2	0.72		5.16
9. BH-ST × KL1–4	Viability	0.83±0.20	1	0.69±0.18
	χ^2	0.72		3.01
10. VN-ST × KL1–4	Viability	0.93±0.21	1	0.40±0.13
	χ^2	0.12		22.50
11. GH-ST × KL1–4	Viability	1.00±0.22	1	0.60±0.16
	χ^2	–		6.15
12. D_{38} × KL1–4	Viability	0.66±0.17	1	0.66±0.17
	χ^2	3.91		3.91
13. Am_6 × KL1–4	Viability	0.69±0.17	1	0.44±0.13
	χ^2	3.34		18.89

in eight crosses involving ST and DE chromosomes derived from distant populations and differences are also significant in some crosses. In one interpopulation cross, the heterozygotes are equal to the ST/ST homozygotes and are inferior to the same homozygotes in two crosses. In the intrapopulation cross (ST and DE from Kuala Lumpur), the heterozygotes no longer exhibit heterosis. Table 3 incorporates the data on relative viabilities of 3R karyotypes. It is evident from the data that the results are more or less similar to those of 3L karyotypes. In the intrapopulation cross ($D_6 \times$ KL1–4), the heterozygotes are superior to both homozygotes. However, the difference between the viabilities of ST/ET and ST/ST is not significant ($\chi^2 = 0.72$).

Discussion

During the present study the relative fitness of homo- and heterokaryotypes has been estimated in intra- and interpopulation crosses involving several strains of *D. ananassae* homozygous for ST or inverted gene orders, originating from distant localities. It is clear from the present results that the inversion heterozygotes formed by chromosomes coming from different populations exhibit heterosis with respect to viability. However, the heterozygotes formed by chromosomes derived from the same population (e.g. Bhagalpur and Kuala Lumpur) are not heterotic with respect to viability. In many interracial crosses, heterosis is evident but the degree of heterosis for a particular inversion varies in different crosses. This is most likely due to variation in allelic contents of the same chromosome in different populations.

If the present results are compared with those of Dobzhansky (1950, 1957) in *D. pseudoobscura, D. ananassae* seems to differ from *D. pseudoobscura* regarding the mechanism of heterosis associated with inversion polymorphisms. In interracial crosses of *D. pseudoobscura,* the superiority of inversion heterozygotes was lost. This suggested that the adaptive superiority of inversion heterozygotes is not necessarily a consequence of heterozygosity for inversions but it is determined by the polygene complexes which they maintain. Polygenes may exhibit two types of balance (Mather 1943; Lerner 1958), namely internal balance and relational balance. The relational balance established between the genes in a pair of homologous chromosomes is responsible for the establishment of balanced polymorphism due to the adaptive superiority inversion heterozygotes. This has also been confirmed by the results of Dobzhansky and Pavlovsky (1953) in *D. pseudoobscura.* Thus, heterosis associated with inversion polymorphism in *D. pseudoobscura* is due to selectional coadaptation.

The results obtained by Dobzhansky and Pavlovsky (1958) in *D. paulistorum* and *D. willistoni* and by Brncic (1961) in *D. pavani* extend evidence for heterosis due to selectional coadaptation. In these species of *Drosophila,* the frequency of inversion heterozygotes decreases in hybrid populations of mixed geographic origins involving polymorphic strains. In such types of crosses the same gene arrangements but of different geographic origins come together and the gene complexes are disrupted owing to recombination with a consequent loss of heterosis. Contrary to this, no breakdown of heterosis has been observed in hybrid populations of mixed geographic origin in *D. ananassae* when polymorphic strains were used (Singh 1972). This provides no evidence for coadaptation in geographic populations of *D. ananassae.* Furthermore, the persistance of chromosomal polymorphism due to adaptive superiority of inversion heterozygotes has also been observed in interracial crosses involving homozygous strains of *D. ananassae* (Singh 1981).

The present results on the relative viabilities of karyotypes and the previously obtained data on the frequencies of inversion heterozygotes in interracial hybridization experiments indicate that our findings in *D. ananassae* clearly conflicts with what has been claimed for other species. *D. ananassae* is a cosmopolitan and domestic species. Due to its close association with man this species is characterised by high incidence of immigration. Based on this, it has been argued (Singh 1981) that coadaptation may not be confined to local groups of this species. However, the present observation on relative viabilities of karyotypes cannot be explained on the basis of coadaptation. Further, the present author (Singh 1983 a, b, c) has also observed that the degree of heterosis for a particular inversion varies in different strains of *D. ananassae.* In some strains the inversion heterozygotes are in significant excess while in others the observed frequencies of heterozygotes are close to their expected frequencies. This is due to variation in allelic contents of the same chromosome in different strains. Thus the heterosis associated with cosmopolitan inversions in *D. ananassae* appears to be simple luxuriance rather than populational heterosis (coadaptation).

Carson (1959, 1965) suggested that most of the chromosomal polymorphisms of widespread species of *Drosophila* are rigid polymorphism maintained by a process of heteroselection which could be explained on the basis of simple luxuriance hypothesis. Heterosis has also been observed in crosses between widely separated populations of *D. hydei* (Stone 1942), *D. pseudoobscura* (Vetukhiv 1953, 1954; Brncic 1954; Anderson 1966, 1968) and *D. melanogaster* (Wallace 1955) which lends support to the luxuriance hypothesis. However, this heterosis disappears in F_2 interpopulation hybrids as crossing-over breaks up the integrity of chromosomes (Wallace and Vetukhiv 1955; Wallace 1959). On the other hand, McFarquhar and Robertson (1963) found neither heterosis in F_1s nor breakdown in F_2s in crosses between geographical races of *D. subobscura,* which led them to suggest that evidence for coadaptation is entirely lacking in *D. subobscura.* Thus the gene pool of *D. subobscura* seems to be more rigid (Parsons 1973).

The experimental results obtained by Carson (1961) in *D. melanogaster* have shown that natural selection favours balanced polymorphism due to autosomal heterosis. The persistence of increased fitness of F_1 individuals for a number of generations suggests that heterosis is of the simple luxuriant sort and coadaptation of chromosomes is not involved. Thus luxuriance can function in the adjustment of organisms to their environments (Carson 1961). This conclusion is further borne out by the results of the experiments of the present author in *D. ananassae.*

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