Heterosis Relating to a Terminal Inversion in Artificial Population of *Drosophila ananassae*

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It is well known that a chromosomal inversion, when it occurs, prevents crossing over of gene blocks included within it. Accordingly, two reciprocal types of chromosomal arrangements resulting from an inversion should act as a pair of allelic genes. In fact, with some of these chromosomal variants in *Drosophila* populations, balanced chromosomal polymorphisms have often been established by "heterosis", where structural heterozygotes are more adaptive than homozygotes.

In *Drosophila ananassae*, when two gene arrangements by a subterminal inversion in the left arm of the second chromosome were mixed in a population cage, their relative frequencies always attained a constant equilibrium value as the result of heterosis (Moriwaki *et al.* 1955). On the other hand, in the same species, another inversion, *In3L*, a terminal inversion in the left arm of the third chromosome has long been known and it constitutes a suggestive case where the inversion may not suppress crossing over (Kikkawa 1938; Moriwaki 1950).

In the present experiments, this inversion, *In3L*, was studied to examine if it could establish a balanced chromosomal polymorphism. Clarification of the following three major points were attempted.

- 1) Success or failure of establishment of balanced chromosomal polymorphism regarding this terminal inversion in population cages.
- 2) Comparison of phenotypic differences among the karyotypes, with respect to each life cycle component.
- 3) Estimation of relative adaptive values of gene arrangements on the basis of the components of the life cycle, and comparison of them with those computed by population cage technique.

Materials and Methods

The two reciprocal gene arrangements, *In3L*-A and -B, were respectively fixed as homozygotes, isolated from "Hawaii" strain of *Drosophila ananassae*, which was sent by the University of Texas and has been kept in our laboratory since 1950. Four cages were set up in which *In3L*-A and -B were mixed with different initial frequencies. The length of generation was estimated as 15 days. The bottom of the cage had 15 holes for insertion of food cups. 14 cups were used for feeding, among which, since

2 cups with fresh medium were inserted every third day, each cup remained in the cage for a period of 21 days. The central cup was used for obtaining samples. Sampling was performed once a month for first several months and once in 2 months thereafter. One sample consisted of 150 larvae collected on 6 successive days, 25 larvae for each day. The eggs were allowed to develop at optimal condition in culture bottles and 150 of the larvae were used for microscopic examination of the salivary gland chromosomes.

For the analysis of heterosis, the examination of life cycle components was made. The following experimental procedure was employed in order to minimize the effect of inbreeding and obtain flies possibly isogenic for the genetic background other than A- or B-type of the third chromosome. Ten strains of A-homozygotes and 10 of B-homozygotes were isolated at random from a population cage in which an equilibrium between *In3L*-A and -B has been established. Then equal number of virgin females and males from each strain were collected and grouped to be used for crosses as follows:

AA♀♀ (from 10 strains) × BB♂♂ (from 10 strains) — AB heterozygotes

BB♀♀ (from 10 strains) × AA♂♂ (from 10 strains) — BA heterozygotes

AA♀♀ (from 10 strains) × AA♂♂ (from 10 strains) — AA homozygotes

BB♀♀ (from 10 strains) × BB♂♂ (from 10 strains) — BB homozygotes

From these crosses, 4 types of eggs and adults were obtained, the formers were to be tested for hatchability, developmental rate and viability and the latters to be tested for longevity and fecundity. Throughout the experiment temperature was kept at 25°C.

Experimental Results

1. Artificial population studies

Four population cages, C-2, C-1, C-5 and C-3 were initiated with about 90% A and 10% B, 70% A and 30% B, 50% A and 50% B, 30% A and 70% B, respectively.

Fig. 1 shows the changes observed in these populations. All 4 populations reached equilibria at about 60% A and 40% B after 3~4 months regardless of the initial frequencies of the chromosome types. Accordingly it is evident that balanced chromosomal polymorphism was established in the populations. Relative adaptive values (*W*) and selection coefficients (*s* and *t*) of the karyotypes were calculated according to the method adopted by Wright and Dobzhansky (1946) and modified somewhat by Yoshida (unpub.). The values obtained were as follows:

	Genotype	W		
C-2	AA	0.50	<i>s</i> =0.50	$\hat{q}_A=0.60$
	AB	1.00	<i>t</i> =0.75	
	BB	0.25		

C-1	AA	0.73	$s=0.27$	$\hat{q}_A=0.57$
	AB	1.00	$t=0.36$	
	BB	0.64		
C-5	AA	0.78	$s=0.22$	$\hat{q}_A=0.61$
	AB	1.00	$t=0.35$	
	BB	0.65		
C-3	AA	0.67	$s=0.33$	$\hat{q}_A=0.62$
	AB	1.00	$t=0.54$	
	BB	0.46		

2. Analysis of heterosis

In these experiments, the physiological traits seemingly related to heterosis were examined, making the cultural conditions rather unfavorable approximating cage conditions. For the fecundity and the longevity experiments, mass matings of 35

flies of each sex were made in 2.5×12 cm vials with ordinary medium (lightly yeasted cornmeal-molasses-agar medium). Since the females were allowed to lay eggs for 24 hours, the medium became crowded with the larvae. Adult flies emerged from these vials were used.

Fig. 1. Changes observed in frequencies of *In3L-A* in artificial populations containing *In3L-A* and *-B* arrangements.

(a) Fecundity: 10 newly-hatched females of one chromosomal type, and 20 males of the same type were placed together in a separate vial with ordinary medium. Eight bottles for each chromosomal type, in this manner, were set up and stored in a constant temperature room at 25°C . Every day after transference of the flies to new vials, eggs laid on the medium were counted. Only for first 2 weeks, during which high fecundity lasted, records were continually taken. The results were summarized in Table 1. Heterosis was seen in AB heterozygote showing the highest

number of eggs per 10 females per day, while BA heterozygote showed the lower fecundity than AB, although no less than homozygotes.

Table 1. Egg production at 25°C

Type	No. of eggs/10 females/day	Relative fecundity
AA	57.7±6.2	0.893
AB	68.8±9.2	1.000
BA	44.2±6.2	0.642
BB	44.7±7.2	0.650

Significant differences: AB>BB, AB>BA

Table 2. Mean longevity and relative longevity of different genotypes at 25°C

Type	Mean longevity in days	Relative longevity
AA	28.12±0.78	0.919
AB	30.64±0.82	1.000
BA	33.92±0.92	1.107
BB	23.72±0.42	0.774

Significant differences: BA>AB>AA>BB

(b) Longevity of adults: 20 females and 20 males of newly emerged flies from crowded larval conditions to be tested were placed in the regular culture bottles with ordinary medium. Eight bottles for each karyotype were set up at the same time. Everyday the survivors of each bottle were transferred to a fresh bottle and the number of deaths occurring for one day was recorded. Heterosis is obvious in this trait. The results showing that both heterozygotes AB and BA have significantly greater mean longevity than both homozygotes are given in Table 2.

(c) Hatchability of eggs: The parent flies (30 females and 30 males) aged about 5~7 days, having high egg laying capacity, were introduced into separate vials with the test medium for hatchability and allowed to lay eggs for 2 hours. After the parent flies were discarded, eggs laid on food were counted under a binocular microscope. About 100 eggs per vial randomly selected were left to be examined for hatchability and others were removed. The vials were stored at 25°C for 40 hours. Thereafter the newly hatched larvae and unhatched eggs in each vial were counted. Eight bottles for each type were run at the same time and the results of this experiment were summarized in Table 3. The percentage of hatched larvae in both heterozygotes was significantly higher than that of BB homozygote, but not significantly so than that of AA homozygote.

Table 3. Hatchability of eggs at 25°C

Genotype of eggs	Eggs tested	Eggs hatched	Percent hatched	Relative hatchability
AA	819	532	65.0	0.888
AB	756	555	73.4	1.000
BA	786	528	67.2	0.915
BB	820	498	60.7	0.829

Significant differences: AB > BB, BA > BB

Table 4. Viabilities and relative viabilities of different genotypes at 25°C

Type	No. of 1st inst. larvae	No. of adults emerged	Percent emerged	Relative viability
AA	250	202	80.8	0.948
AB	250	213	85.2	1.000
BA	250	205	82.0	0.962
BB	250	153	61.2	0.718

Significant differences: AB > BB, BA > BB, AA > BB

(d) Pre-adult viability: In the same way as the hatchability experiment, females were allowed to oviposit for 2 hours on sugar-agar medium in separate vials. After these vials were kept for 24 hours at 25°C, newly hatched first instars were collected and 50 of them belonging to one karyotype were placed on the food (4 cc of the 5% boiled yeast-sugar-agar medium) which was not sufficient to support 50 larvae, approximating the crowded condition in a cage to be compared later. Five bottles for each type were started simultaneously. Emerged flies from these vials were counted daily. The results are given in Table 4. From the percentage of eclosion BB homozygote showed significantly lower viability than the other three types, among which no significant differences could be detected.

(e) Rate of development: The experimental procedure was the same as before except for 2 nutritional conditions, namely, 20% boiled-yeast medium as a favorable one and 5% boiled-yeast medium as poor one. About 100 eggs among those laid during 2 hours were left on each medium. Four bottles for each condition for one type were set up at the same time. Every 2 hours new pupae were counted. Table 5 gives the mean hours elapsed before pupation for each karyotype. In both conditions the heterozygotes AB and BA developed faster than the homozygotes, showing obvious heterosis. In 20% yeast no differences could be found between the homozygotes as well as between the heterozygotes, while in 5% yeast significant differences were

obtained in the direction that AA was faster than BB, and AB was faster than BA.

Table 5. Rate of development: Duration of larval life in hours at 25°C

Type	20% yeast	5% yeast	Relative rate of development (in 5% yeast)
AA	109.74±0.20	180.16±1.32	0.852
AB	107.89±0.20	153.64±2.61	1.000
BA	108.58±0.21	163.96±1.33	0.937
BB	110.02±0.19	184.00±2.23	0.835

Significant differences: AB>AA, AB>BB, BA>AA, BA>BB in 20% yeast,
AB>BA>AA>BB in 5% yeast

Table 6. Relative adaptive values of genotypes determined from life cycle components of flies raised from unfavorable condition at 25°C

Life cycle components	AA	AB	BA	BB
Viability	.948	1	.962	.718
Longevity	.919	1	1.107	.774
Fecundity	.893	1	.642	.650
Rate of development	.852	1	.937	.835
Egg hatchability	.888	1	.915	.829
Net W	.553	1	.587	.250
Net W'	.603		1	.273
W (from cage experiments)	.69		1	.55

3. Estimation of adaptive values

An attempt was made by Wallace (1948) and Moos (1955) in *D. pseudoobscura*, and Spiess *et al.* (1956) in *D. persimilis* to estimate the combined effects of the above examined traits. Spiess wrote, "Granted that there are other factors in the fly life cycle which have yet to be tested, it is interest to compare the net fitness of the three karyotypes obtained by the methods above with adaptive values calculated from population cage data."

A similar approach was performed in *D. ananassae*. The relative adaptive values calculated according to the method worked out by Wallace (1948) were summarized in Table 6. The values in the third line from the bottom show the net adaptive values (W) estimated combining the five main factors studied. The final net values (W') were estimated on the basis of chromosomal frequencies in the experimental popu-

lations at equilibrium with 60% A and 40% B, in which 20% of the heterozygotes may be considered as derived from BB mothers. By using the calculated frequencies of AB and BA, the total adaptive value of the mixture of AB and BA was calculated. By putting this as 1 the values of AA and BB were adjusted which are the final values (W').

By comparing these final adaptive values (net W') with those estimated by population cage experiments (W), it can be said that the value for BB of net W' is underestimated, while the value of AA turns out to agree fairly well.

Conclusion

In the experimental populations with terminal inversion, *In3L*, balanced chromosomal polymorphism was established. Hence it is likely that the inversion, notwithstanding it had been believed not to suppress crossing over, plays the role to prevent the breakdown of gene blocks tied together, acting as a super-gene. As a matter of fact, from the other points it seems that this terminal inversion might suppress crossing over after all (Moriwaki 1958). Therefore the present result in artificial populations seems to give another evidence in support of the above statement by Moriwaki.

The experimental populations always came to an equilibrium with about 60% A and 40% B, regardless of the initial frequencies. However the relative adaptive values, from cage experiments, of three karyotypes regarding the inversion (two homozygotes and one heterozygote) are not necessarily the same in every population. Although the reason why these values vary among cages is still unknown, it seems that the difference could be caused by some differences of internal environments rather than external ones. Further discussion will be seen elsewhere.

Moreover, the net adaptive values estimated by measuring life cycle components were considerably close to those from the cage experiments, although the value for BB was relatively discrepant. These results, in general, suggest that most of the components measured in these experiments should be the factors which contribute to the adaptive values of karyotypes. BB homozygote which had the lowest adaptive value as compared with the values of AA and AB in the cage experiments was always most inferior with regard to all life cycle components examined, while AB heterozygote was most superior regarding the all components, showing the highest adaptive value.

Summary

1. Artificial populations containing reciprocal arrangements, A and B, of the third chromosome due to a terminal inversion, *In3L*, of *Drosophila ananassae* were studied. All populations reached equilibria at about 60% A and 40% B after 3~4 months.

2. Heterosis regarding the karyotypes, AA, BB and AB, was expressed in longe-

vity, viability, hatchability, fecundity and rate of development.

3. AA was always superior to BB in longevity, viability and rate of development.

4. Some differences between AB and BA heterozygotes raised by reciprocal crosses were observed in fecundity, longevity and rate of development in poor medium with only 5% boiled yeast.

5. Comparing net adaptive values estimated by measuring life cycle components with those from population cage experiments, a close coincidence was ascertained for AB as well as for AA, although an underestimation was found for BB homozygote.

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