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Racial divergence of a rare laboratory evolved centromeric fission Cytorace of *nasuta-albomicans* complex of *Drosophila*

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Fissioncytorace-1, a member of the *nasuta-albomicans* complex of *Drosophila* is an evolutionary product of centric fission, which had occurred in the chromosome X3 of Cytorace 1, a hydridization product of *Drosophila nasuta nasuta* male (2n=8) and *Drosophila nasuta albomicans* female (2n=6). Cytorace 1 (males 2n=7; females 2n=6) has inherited this chromosome from its *D. n. albomicans* parent. The chromosome X3 of *D. n. albomicans* is a derivative of a centric fusion between the acrocentric chromosome 3 and the chromosome X of *D. n. nasuta*. The Fissioncytorace-1 has crossed 200 generations from the time of its evolution in the laboratory environment. When this centromeric fission race was subjected to some of the morphophenotypic and fitness assessment to find its overall population fitness showed, increased body size, sternopleural bristle, ovarioles, lifetime fecundity and fertility with reduced interspecific competitive ability and hatching success when compared with its parent (Cytorace 1). These results suggest that the hybrid races must have encountered an early event of recombinational raciation during their evolution in the laboratory environment, which is a unique observation in animal system illustrating the increase in the tempo of evolution after the event of hybridization.

Keywords: Centric fission, Fissioncytorace-1, Recombinational raciation

Chromosome reshuffling due to inversions, translocations, fusions, fissions, heterochromatin variations and other chromosomal changes occurring as transient events in natural populations play an important role in karyotype evolution and in speciation. Centric fission is a process of misdivision of the centromere through transverse breakage[1,2]. It has been defined as the splitting of one of the functional centromere of a metacentric or sub-metacentric chromosome to produce two derivative stable chromosomes, each containing a fully functional centromere. Centric fission has been reported in plants, grasshoppers, birds, root voles, primates and humans[3-8]. Although centric fission is known to play a role in karyotype evolution and speciation, the molecular mechanism underlying it is little understood.

Drosophila nasuta nasuta and *Drosophila nasuta albomicans* are morphologically indistinguishable members of the *nasuta* subgroup of the *immigrans* species group of *Drosophila*. They are allopatric in distribution[9]. The diploid chromosome number of *D. n. nasuta* and *D. n. albomicans* is 2n=8 and 2n=6 respectively. The karyotype of *D. n. nasuta* consists of a pair of metacentrics, representing chromosome 2, a pair of acrocentric chromosomes 3, an acrocentric X, a submetacentric Y and a pair of dot chromosomes. *D. n. albomicans* has two pairs of metacentrics, the smaller one represents chromosome 2. The larger metacentric, believed to be a product of centric fusion between the autosome 3 and the sex chromosomes of a *nasuta*-like parent, is referred to as the X3 and Y3 chromosomes[10]. These karyotypically different sibling forms are cross hybridizable in the laboratory environment and the hybrid progeny produced can be maintained for many generations. The resultant recombinant populations, each with new and different karyotypic combinations are called Cytoraces[11]. Sixteen such Cytoraces have been evolved differing by the contribution of *D. n. nasuta* and of *D. n. albomicans* chromosomes[12]. The assemblage containing *D. n. nasuta*, *D. n. albomicans* and the 16 Cytoraces is referred to as the '*nasuta-albomicans*' complex (NAC) of *Drosophila*[12]. The occurrence of a rare event of centric fission in a laboratory population of *Drosophila*, called Cytorace 1 (males 2n=7: $2^a2^n\ 3^n\ Y^n\ X3^a\ 4^n4^{n;}$ females 2n=6: $2^a2^n\ X3^aX3^a\ 4^n4^n$), which is a hybridization product of

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D. n. nasuta (males) and *D. n. albomicans* (females), has been reported[13]. Within the karyotype of Cytorace 1 there is no freely available X-chromosome and it always existed as a part of the $X3^a$ chromosome. This centric fission has occurred in the metacentric X3 chromosome of *D. n. albomicans*, which is phylogenetically a product of three centric fusions. The derivative race of this centric fission event is known as the Fissioncytorace-1 with a diploid number of 2n=8, both in males ($2^a2^n\ 3^n3^n$ Xsm $Y^n\ 4^n4^n$) and females ($2^a2^n\ 3^n3^n$ XsmXsm 4^n4^n), wherein the Xsm indicates the new submetacentric X-chromosome[13]. The uniqueness of this fission race is the presence of a submetacentric X-chromosome (Xsm), while such a chromosome is absent in its parents namely Cytorace 1 and also in its grandparents namely *D. n. nasuta* and *D. n. albomicans*.

In the light of the above, the present investigation has been undertaken to evaluate the parameters of morphometric, meristic, population fitness and competitive ability of Fissioncytorace-1 in comparison with its parents and grandparents to understand the role of centric fission, if any, in the population differentiation/ racial divergence of Fissioncytorace-1.

Materials and Methods

Drosophila stocks

(i) *D. n. nasuta* (2n=8) Coorg, South India

(ii) *D. n. albomicans* (2n=6) Okinawa, University of Texas collections, 3045.11

(iii) Cytorace 1 (males, 2n=7; females, 2n=6) produced by interracial hybridization between males of *D. n. nasuta*, Coorg strain and females of *D. n. albomicans*, Okinawa strain[11]. Cytorace 1 has crossed 600 generations since their evolution in the laboratory environment.

(iv) Fissioncytorace-1 (2n=8) produced by the rare occurrence of a centric fission in a subpopulation of Cytorace 1[13]. The Fissioncytorace-1 has crossed 200 generations since their evolution way back in 1999.

(v) *D.melanogaster white* eye mutant (Drosophila Stock Centre, University of Mysore, Mysore).

For the present experiments, fly stocks were built up by serial transfer of adults to fresh culture bottles once in every 3 days and the larvae were allowed to develop under uniform conditions of quantity of food media, temperature and relative humidity. From these cultures, well-fed adults were used to collect synchronized eggs by modified Delcour technique[14]. Equal numbers (100) of synchronized eggs thus collected were placed in each culture bottle and were allowed to develop under uniform conditions of temperature, space, amount of food and humidity. Unmated males and virgin females from these cultures were isolated within 4 h of their eclosion and transferred to fresh media vials. These adults were used for the assessment after aging them for 5-8 days. All the stocks and the experimental cultures were maintained on standard wheat cream agar medium seeded with yeast at 22º ± 1ºC and 70-80% RH.

At the time of the present investigation, the Fissioncytorace-1 had already crossed 200 generations. The morphophenotypic and the fitness traits were assessed following the descriptions of earlier workers[15].

Wing length and wing width—For both of these parameters, 30 flies were measured separately from 8 days old males and females. Each fly was anaesthetized separately using ether and left wing was dissected under stereomicroscope and mounted on slides with DPX. The wing length was measured from the humeral cross vein to the tip of the wing, while wing width was measured exactly from the middle of the wing vertically by using calibrated ocular micrometer at 4X magnification under a microscope.

Sternopleural bristle number—Adult males and females were etherized and the sternopleural bristles present on the lateral side of the flies on a triangular shaped sternum were recorded under a stereomicroscope.

Counting of ovariole numbers—Virgin female flies (30) were collected from uncrowded culture conditions and aged for 5 days. Then, each fly was anaesthetized and dissected out the left ovariole in saline. The bundles of ovariole were separated by a fine needle and counted under stereomicroscope.

Lifetime fecundity assay—For the assessment of lifetime fecundity, 30 pairs of virgin females and males were isolated and sexed them separately for two days and then pair mating was done. After 2 days, they were transferred to fresh food media vials supplemented with yeast grains. Likewise daily, each replicate was transferred successively to the next set of vials. If a male died before the female, a similar aged male replaced him no later than the following day[16]. Female egg production was counted every 24 h, under a stereomicroscope till the egg laying was stopped. Thus, the mean number of eggs laid by these

pair mated females was recorded.

Lifetime fertility assay—The same set of vials, which were used to assess lifetime fecundity of a single female, was also used in this assessment. The number of flies emerged from each replicate were recorded for the total lifetime fertility. Hatching success was calculated by dividing mean values of lifetime fecundity by mean values of lifetime fertility.

Inter-genotypic competitive ability assessment—*D. n. nasuta, D. n. albomicans*, Cytorace 1 and Fissioncytorace-1 were allowed to compete independently against a common *white* eye mutant strain of *D. melanogaster*. Mixed cultures were established with 24 flies (12 males+12 females) of *D. melanogaster* and 24 flies (12 males+12 females) of one of the four experimental strains. Each set of mixed cultures was maintained in four replicates. The cultures were maintained at 22º±1ºC by adopting the serial transfer technique of Ayala[17]. The adult flies were introduced into ¼ pint (125 ml) milk bottles containing equal amount of cream of wheat agar medium seeded with yeast. Every 7 days, they were etherized, counted, and transferred to fresh media bottles. When new flies began to emerge in the bottles where adult flies had deposited eggs, the newly emerged flies were etherized, counted, and added to the bottles with the older flies. This number was taken as the productivity of the race in question. Population size of a race for a particular week was defined by the total number of the newborn flies plus the survivors from the previous week. These weekly assessments were expressed in terms of the average of productivity and population size[14]. After 4 weeks each bottle was discarded. The adult ovipositing flies were thus always in a single bottle, while other bottles contained flies at different preadult stages. Each experiment was conducted till one of the competing races completely eliminates the mutant strain of *D. Melanogaster*. Two facets of competitive ability, population size and productivity were calculated.

Statistical analysis—Data obtained from the assessments of the present study were individually subjected to one-way Analysis of variance (ANOVA) followed by Dunken's Multiple Range test (DMRT) to analyze the significance of differences.

Results

Body size—Quantitative genetic traits, namely, wing length, wing width and sternopleural bristle number assessed in *D. n. nasuta* (males), *D. n. albomicans* (females), Cytorace 1 and Fissioncytorace-1 flies are shown in Table 1. Data revealed that the females of all the races under study have increased mean wing length and width than the males. Fissioncyotrace-1 showed a larger body size than its parent, Cytorace 1, but lesser than its grandparents (*D. n. nasuta* ♂ × *D. n. albomicans* ♀). ANOVA revealed significant differences ($P < 0.001$) in both wing length and width among all males, females and between males, females of the races under study. DMR test revealed that except for the comparison between the mean values of wing width of the females of Fissioncytorace –1 and Cytorace 1, all other comparisons were significant at 5% level.

Fissioncytorace-1 and its grandparents had the highest and lowest number of sternopleural bristles respectively. ANOVA revealed significant difference ($P < 0.001$) for sternopleural bristle number among

Table 1—Mean wing length (mm), wing width (mm) and sternopleural bristle number of virgin flies of *D.n.nasuta* (males) and *D.n. albomicans* (females), males and females of Cytorace 1 and Fissioncytorace-1 of the *nasuta-albomicans* complex of *Drosophila*
[Values are mean ± SE of 30 replicates]

Races	Wing length		Wing width		Sternopleural bristle number	
	Males	Females	Males	Females	Males	Females
D.n.nasuta (♂) × *D.n.albomicans* (♀)	86.03 ± 0.13^{a}	92.26 ± 0.17^{b}	46.13 ± 0.11^{a}	47.36 ± 0.11^{b}	13.17 ± 0.13^{a}	13.03 ± 0.14^{b}
Cytorace 1	82.03 ± 0.13^{c}	88.36 ± 0.13^{d}	41.46 ± 0.18^{c}	46.03 ± 0.13^{d}	14.02 ± 0.14^{c}	14.16 ± 0.15^{d}
Fissioncytorace-1	83.76 ± 0.12^{e}	90.30 ± 0.17^{f}	44.10 ± 0.12^{e}	46.30 ± 0.12^{f}	14.96 ± 0.14^{e}	15.73 ± 0.15^{f}
ANOVA	For both males and females: F= 697.51; DF= 5, 174; $P < 0.001$		For both males and females: F= 246.24; DF= 5, 174; $P < 0.001$		For both males and females: F= 50.52; DF= 5, 174; $P < 0.001$	
DMRT	The comparison of the mean values between a/c, a/e, c/e, b/d, b/f, d/f, a/b, c/d and e/f are significant at 0.05 level		The comparison of the mean values between a/c, a/e, c/e, b/d, b/f, a/b, c/d and e/f are significant at 0.05 level		The comparison of the mean values between a/c, a/e, c/e, b/d, b/f, d/f and e/f are significant at 0.05 level	

the races under study. Based on DMR test, the comparison among the mean values of sternopleural bristle number of males and females showed significant differences at 5% level, except for the comparisons between the males and females of the parents and grandparents of Fissioncytorace-1.

Life history traits

The mean ovariole number, lifetime fecundity, lifetime fertility and hatching success in the females of three races of the NAC of *Drosophila* are given in Fig. 1. Data revealed that Fissioncytorace-1 had the highest number of ovarioles. Lifetime fecundity and fertility was higher in Fissioncytorace-1 than its parents, Cytorace 1 but the hatching success (in %) was more in Cytorace 1 than in Fissioncytorace-1. *D. n. albomicans* had the highest lifetime fecundity, lifetime fertility and hatching success. ANOVA revealed significant differences for fecundity, fertility and ovariole number among the races studied with $P < 0.001$. Based on DMR test, the comparison of the mean values of ovariole number of all the females showed significant differences at 5% level, except for the comparison between the females of *D. n. albomicans* and Cytorace 1. DMR test for fecundity and fertility revealed that the comparison of the mean values of all the females showed significant differences at 5% level, except for the comparison between Cytorace 1 and Fissioncytorace-1.

Competitive ability—The dynamics of interspecific competition (mean population size of four replicates) between the four experimental races are given in Fig. 2 (a-d). In each of the mixed cultures, the *white* eye mutant strain of *D.melanogaster* was eliminated. The parental races and the evolved races exhibited competitive superiority over *D. melanogaster* strain, but the time taken to achieve this was strikingly different. The grandparental race, *D. n. nasuta* and *D. n. albomicans* competitively eliminated *D. melanogaster* following 40 and 44 weeks respectively. On the other hand, the parental race, Cytorace 1 achieved the same result in 52 weeks, followed by Fissioncytorace-1 at 57 weeks. The mutant strain of *D. melanogaster* survived for a longer period in mixed culture with either Cytorace 1 or Fissioncytorace-1, than in the mixed culture with *D. n. nasuta* or *D. n. albomicans*. The mean values of the two components of competitive ability i.e., productivity and population size, are given in Table 2. ANOVA revealed that the four races exhibited

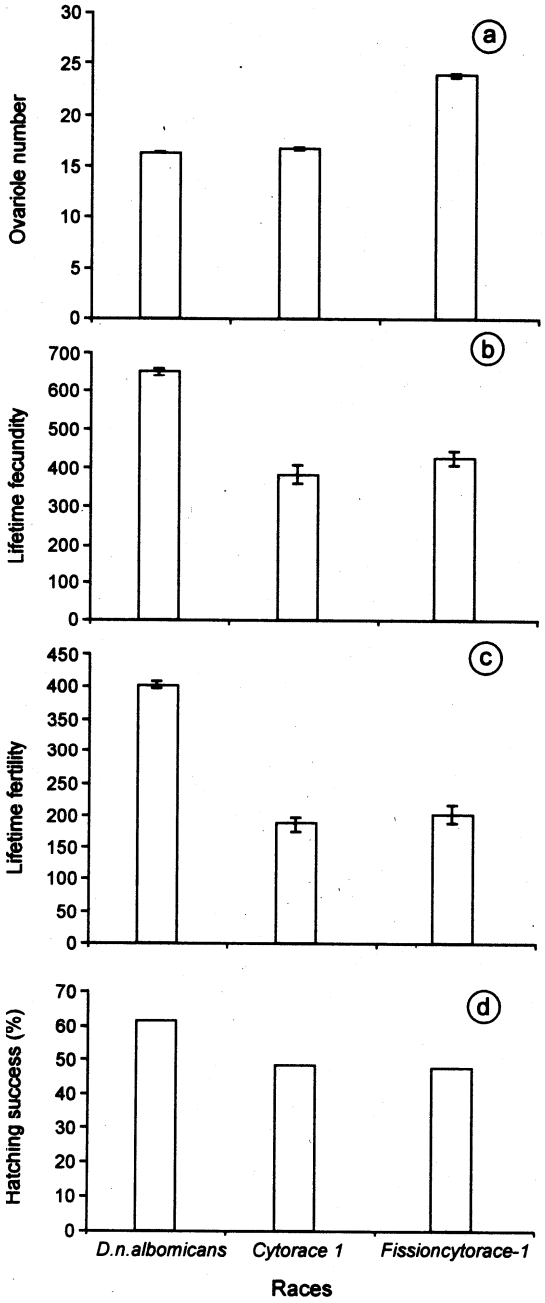

Fig. 1—Mean of (a) ovariole number, (b) lifetime fecundity, (c) lifetime fertility and (d) hatching success of the female flies of *D.n.albomicans*, Cytorace 1 and Fissioncytorace-1 of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 30 replicates)

statistically significant differences. The gene pool that maintains a larger population size may be said to be performing better than the one having a smaller population size. Of the four races under study, the parental races had significantly higher values for the two parameters of competitive ability than the

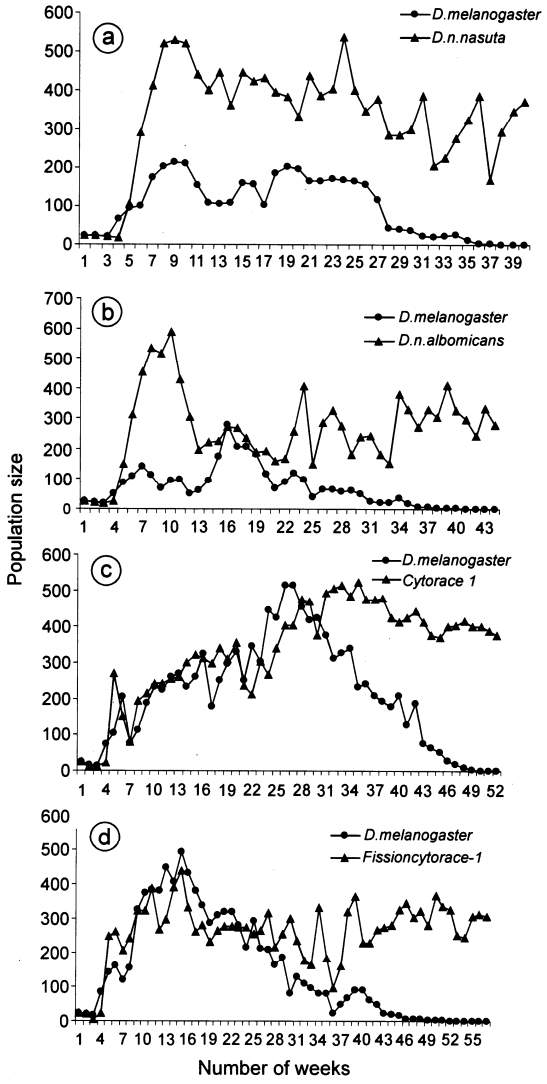

Fig 2—Population dynamics of interspecific competition (mean of four replicates) between (a) *D.n.nasuta* and *white* eye mutant of *D.melanogaster*, (b) *D.n.albomicans* and *white* eye mutant of *D.melanogaster*, (c) Cytorace 1 and *white* eye mutant of *D. melanogaster* and (d) Fissioncytorace-1 and *white* eye mutant of *D.melanogaster*

newly evolved Cytoraces. The ranking is, *D. n. nasuta* > *D. n. albomicans* > Cytorace 1> Fissioncytorace-1.

Discussion

Although chromosome number and karyotypes remain to be the basic feature of a species, there are other observable and measurable phenotypic trait and fitness parameters that highlight the genetic variation in a population in an evolutionary response to selection. The likelihood of establishing a new hybrid lineage depends largely on its fitness in parental and/or divergent habitats. In this regard, various investigators have contributed towards the understanding of measurements of population fitness and its components in natural and experimental populations of *Drosophila*[11,14,15,18-21].

Table 2—Competitive ability of four members of the *nasuta-albomicans* complex of *Drosophila* during interspecific competition with *white* eye mutant strain of *D. melanogaster*
[Values are mean ± SE of four replicates]

Strains	Parameters	
	Productivity	Population size
D. n. nasuta	235.39 ± 16.62[a]	331.61± 22.14[a]
D. n. albomicans	187.81 ± 13.07[b]	266.03 ± 19.43[b]
Cytorace 1	234.17 ± 14.86[c]	332.50 ± 18.71[c]
Fissioncytorace-1	181.63 ± 8.64[d]	261.18 ± 11.92[d]
ANOVA	F = 4.914; df = 3, 189; $P < 0.05$.	F = 5.031; df = 3, 189; $P < 0.05$.
DMRT	The difference between a/b, b/c, c/d, and a/d are significant at 5% level	The difference between a/b, b/c, c/d, and a/d are significant at 5% level

Body size is the most easily observable and measurable phenotypic trait, that is closely linked with life history traits and has been widely used in the studies of quantitative genetics. In *Drosophila* wing length is an excellent index of body size[22]. Other morphological traits such as wing width, thorax length, and face width and front leg length have also been used as an index of body size[23]. Several strains of *D.melanogaster* and *D.simulans*, exhibit genetic latitudinal clines, with a larger size under cold climate[24]. Large phenotypic variations of wing and thorax length in wild living flies in response to the instability of natural environmental conditions during their development have been reported[25]. Variations among the laboratory-reared flies tend to be smaller when compared to the natural population. In the present investigation, the females were larger than the males, which could be a general finding in *Drosophila*. The mean values of body size of Cytorace 1 and Fissioncytorace-1 are lesser than *D. n. nasuta* and *D. n. albomicans*, indicating that the newly evolved laboratory races are evolved with smaller body size.

Sternopleural bristle number in natural and laboratory populations of *Drosophila* has been observed as a quantitative trait[26,27]. A number of studies have demonstrated the association of sternopleural bristles with several components of fitness, which indicates that sternopleural bristle number is an adaptive trait[28-30]. The females of Fissioncytorace-1 have significantly higher number of sternopleural bristle than the males. In the parents and the grandparents, both the males and females are

almost similar in sternopleural bristle number. This suggests that the newly evolved Fissioncytorace-1 has gained more number of sternopleural bristles during its evolution.

Estimation of fitness is the first step towards understanding the adaptive evolution of a population[31]. Ovariole number is an anatomical trait determined during pupation for which a polygenic basis is known in various species of *D. melanogaster* complex[32]. Female reproductive success and ovariole number is often correlated through a simple relationship between the number of ovarioles and the rate at which the female reproduces eggs[33]. The newly evolved Fissioncytorace-1, which has crossed 200 generations, has greater number of ovarioles than its parents and grandparents. The least number of ovarioles was seen in *D. n. albomicans* indicating the significant divergence of Fissioncytorace-1 from its parents and grandparents. Female fitness is mainly determined by its egg laying capacity[34]. In the present study, Cytorace 1 and Fissioncytorace-1 have lower fecundity than *D. n. albomicans*, however Fissioncytorace-1 lay more number of eggs than Cytorace 1, suggesting a better fitness than its parents. These findings indicate that Fissioncytorace-1 is a unique recombinant centric fission product exhibiting its evolutionary independence with a higher fitness.

Fertility, the newly produced offsprings from a particular mating pair is an important component of fitness measured in terms of productivity has been extensively studied in *Drosophila.* Fertility and body size are known to be independent in *D. melanogaster*[35]. Fissioncytorace-1 showed better fertility than its parents but the overall highest fertility was shown by *D. n. albomicans*. Even though Fissioncytorace-1 showed higher fecundity and fertility, the hatching success was more in the case of its parents.

Interspecific competitive fitness is an important attribute in any population and one, which will determine its success in a sympatric association of different species. Population fitness can be assessed by evaluating the inter-genotypic competitive ability of particular strains either with strains of a different species or with strains of the same species[17,18,36,37]. Zimmering[38] has demonstrated that a mutant strain of *Drosophila* can be used as an interspecific competitor to determine the relative fitness of different species or strains of the same species. The inter-genotypic competitive fitness of the newly evolved Fissioncytorace-1 has been assessed in comparison to its parents and grandparents. The productivity of the four races also differs significantly. Fissioncytorace-1 had the lowest value for population size and productivity. Cytorace 1 displayed a better fitness than Fissioncytorace-1. These different degrees of competitive fitness suggest divergence between the Fissioncytorace-1 and its parents. These morphologically similar, karyotypically different yet closely related races have revealed detectable differences in their adaptive phenotypes. Therefore Fissioncytorace-1 is a unique race, evolved through centric fission, having smaller size and better fitness. No race is the best for all the components of fitness and also the fitness hierarchy is reversed when one considers different parameters, which could be an evolutionary strategy to have a diversity of adaptive phenotypes of the species so as to promote coexistence of different species with variable levels of divergence for components of fitness.

Taking together the metric, meristic, female fitness and competitive ability, Fissioncytorace-1 has remarkably diverged from its parents by exhibiting larger body size, increased bristle number, better fitness in ovariole number, fecundity and fertility with reduced hatching success and interspecific competitive ability.

Hybrid recombination preceded by interracial hybridization has led to the evolution of Cytoraces in the laboratory environment. These Cytoraces are the representatives of novel genetic variations and an admixture of the parental genomes following chromosome recombination. Some of the Cytoraces despite of sharing same chromosome number, do not exhibit similarities in their body size, reproductive fitness and competitive ability. Thus, the rapid divergence recorded in the chromosomes, karyotypes, body size, bristle number, fitness traits, and competitive ability is suggestive of an early event of recombinational raciation in their evolution in the laboratory environment which is a rare observation in animal system illustrating the increase in the tempo of evolution following hybridization. Evolution of the *nasuta-albomicans* complex is a large scale evolutionary experimentation, which generates a lot of interests among the evolutionary biologists, as the members of this complex are passing through varied stages of divergence that offers a rare and a unique opportunity to study the multidimentional facets of raciation.

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References

1 Rivera H & Cantu J M, Centric fission consequences in man, *Ann Genet*, 29 (1986) 223.

2 Perry J, Slater H R & Choo K H A, Centric fission-simple and complex mechanisms, *Chromosomes Res*, 12 (2004) 627.

3 Darlington C D, Misdivision and the genetics of the centromere, *J Genet*, 37 (1939) 341.

4 Southern D I, Stable telomeric chromosomes produced following centric misdivision in *Myrmeleotettix maculate* (Thunb.), *Chromosoma*, 26 (1969) 140.

5 Takagi N & Sasaki M, A phylogenetic study of bird karyotypes, *Chromosoma*, 46 (1974) 91.

6 Fredga K & Bergstrom U, Chromosome polymorphism in the root vole (*Microtus oeconomus*), *Hereditas*, 66 (1970) 145.

7 Egozcue J, A possible case of centric fission in a primate, *Experientia*, 27 (1971) 969.

8 Sinha A K, Nora J J & Pathak S, Isochromosomes arising from a human "C"-autosome, *Human Hered*, 21 (1971) 231.

9 Ranganath H A & Ramachandra N B, Chromosomal basis of raciation in *Drosophila*: A study with *Drosophila nasuta* and *Drosophila albomicana*, *Proc Indian Acad Sci* (*Anim Sci*), 96 (1987) 451.

10 Ranganath H A & Hagele K, Karyotypic orthoselection in *Drosophila*, *Naturwissenschaften*, 68 (1981) 527.

11 Ramachandra N B & Ranganath H A, The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana* IV. Hybridization and Karyotype repatterning, *Chromosoma*, 93 (1986) 243.

12 Ramachandra N B & Ranganath H A, Evolution of the *nasuta-albomicans* complex of *Drosophila*, *Curr Sci*, 71 (1996) 515.

13 Tanuja M T, Ramachandra N B & Ranganath H A, Evolution of a new chromosomal lineage in a laboratory population of *Drosophila* through centric fission, *J Biosci*, 24 (1999) 421.

14 Ramachandra N B & Ranganath H A, Estimation of population fitness of parental races (*Drosophila nasuta nasuta*, *Drosophila nasuta albomicana*) and of the newly evolved Cytoraces (I and II) - the products of parental interracial hybridization, *Genome*, 30 (1988) 58.

15 Harini B P & Ramachandra N B, Evolutionary experimentation through hybridization under laboratory condition in *Drosophila*: Evidence for Recombinational Speciation, *BMC Evolutionary Biology*, 3 9(2003) 1.

16 Friberg U & Arnqvist G, Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females, *J Evol Biol*, 16 (2003) 797.

17 Ayala F J, Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii*, *Genetics*, 51 (1965) 527.

18 Ramachandra N B & Ranganath H A, Estimation of population fitness in two strains of *Drosophila nasuta albomicana* with and without supernumerary chromosomes, *Indian J Exp Bio*, 24 (1986) 137.

19 Yamazaki T, Measurement of fitness and its component in laboratory strains of *Drosophila melanogaster*, *Genetics*, 108 (1984) 201.

20 Yamazaki T & Matsuo Y, Genetic analysis of natural populations of *Drosophila melanogaster* in Japan. III. Genetic variability of inducing factors of amylase and fitness, *Genetics*, 108 (1984) 223.

21 Mackay T F C, A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*, *Genetical Research*, 47 (1986) 59.

22 Sokoloff A, Morphological variation in natural and experimental populations of *Drosophila pseudobscura* and *Drosophila persimilis*, *Evolution*, 20 (1966) 49.

23 Ruiz A M, Santos A, Barbadilla J E, Quezada-Diaz E, Hassan & Fontdevila A, The evolutionary history of *Drosophila buzzatii* XVIII. Genetic variation for body size in a natural population, *Genetics*, 128 (1991) 739.

24 Capy P, Pla E & David J R, Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *Drosophila simulans* I. Geographic variations**,** *Genet Sel Evol*, 25(1993) 517.

25 Moreteau B, Capy P, Alonso-Moraga A, Munoz-Serrano A, Stockel J & David J R, Genetic characterization of geographic populations using morphometrical traits in *Drosophila melanogaster*: isogroups versus isofemale lines, *Genetica*, 96 (1995) 207.

26 Kitagawa O, Wakahama K, Fuyama Y, Shimada E, Takanashi M, Hatsumi M, Uwabo & Mita Y, Genetic studies of *Drosophila nasuta* subgroup, with notes on distribution and morphology, *Jpn J Genet*, 57 (1982) 113-141.

27 Shrimpton A E & Robertson A, The isolation of polygenic character controlling bristle score in *D. melanogaster*. II. Distribution of third chromosome bristle effects within chromosome sections, *Genetics*, 118 (1988) 445.

28 Sherief A K & Skibinski O F, Stabilizing selection on three chaeta characters in *Drosophila melanogaster*, *Heredity*, 60 (1988) 427.

29 Singh B N & Mathew S, Greater fertility of *Drosophila ananassae* flies possessing high number of sternopleural bristles, *Curr Sci*, 72 (1997) 112.

30 Harini B P & Ramachandra N B, Racial divergence in sternopleural bristles among the parental races and the newly evolved Cytorace 1 and Cytorace 2 of the *nasuta-albomicans* complex of *Drosophila*, *Curr Sci*, 76 (1999) 1017.

31 Ramachandra N B, *Contributions to population cytogenetics of Drosophila: Studies on interracial hybridization and B-chromosomes*, Ph.D. Thesis, University of Mysore, Mysore, 1987.

32 Stammer W T, Polak M, Wolf L L & Barker J S, Reproductive characteristics of the flower-breeding *Drosophila hibisci* Bock (*Drosophilidae*) in eastern Australia: Within – population genetic determinants of ovariole number, *Genetika*, 37 (2001) 66.

33 Chakir M, David J R, Pla E & Capy P, Genetic basis of some morphological differences between temperate and equatorial populations of *Drosophila melanogaster*, *Experentia*, 51 (1995) 744.

34 Sisodia S & Singh B N, Effect of exposure to short-term heat stress on survival and fecundity in *Drosophila ananassae*, *Can J Zool*, 84 (2006) 895.

35 Harini B P & Ramachandra N B, Does evolution reduces the body size? A study *in the four members of newly evolved nasuta-albomicans complex of Drosophila*, *Genetica*, 105 (1999) 1.

36 Futuyama D J, Variation in genetic response to interspecific competition in laboratory populations of *Drosophila*, *Am*

Nat, 104 (1970) 239.
37 Goodman D, Competitive hierarchies in laboratory *Drosophila*, *Evolution*, 33 (1979) 207.
38 Zimmering S, Competition between *Drosophila pseudoobscura* and *Drosophila melanogaster* in population cages, *Am Nat*, 82 (1948) 326.