BMC Evolutionary Biology

BioMed Central

Research article

Evolutionary experimentation through hybridization under laboratory condition in *Drosophila*: Evidence for Recombinational Speciation

Ballagere P Harini and Nallur B Ramachandra*

Address: Drosophila stock centre, Department of Studies in Zoology University of Mysore Manasagangotri Mysore – 570 006, India

Email: Ballagere P Harini - bpharini@hotmail.com; Nallur B Ramachandra* - rnallur@sancharnet.in

* Corresponding author

Published: 01 October 2003

BMC Evolutionary Biology 2003, **3**:20

Received: 02 July 2003

Accepted: 01 October 2003

This article is available from: http://www.biomedcentral.com/1471-2148/3/20

Abstract

Background: *Drosophila nasuta nasuta* (2n = 8) and *Drosophila nasuta albomicans* (2n = 6) are a pair of sibling allopatric chromosomal cross-fertile races of the *nasuta* subgroup of *immigrans* species group of *Drosophila*. Interracial hybridization between these two races has given rise to new karyotypic strains called Cytorace 1 and Cytorace 2 (first phase). Further hybridization between Thailand strain of *D. n. albomicans* and *D. n. nasuta* of Coorg strain has resulted in the evolution of two more Cytoraces, namely Cytorace 3 and Cytorace 4 (second phase). The third phase Cytoraces (Cytorace 5 to Cytorace 16) have evolved through interracial hybridization among first, second phase Cytoraces along with parental races. Each of these Cytoraces is composed of recombined genomes of the parental races. Here, we have made an attempt to systematically assess the impact of hybridization on karyotypes, morphometric and life history traits in all 16 Cytoraces.

Results: The results reveal that in most cases, the newly evolved Cytoraces, with different chromosome constitutions, exhibit decreased body size, better fitness and live longer than their parents. Particularly, Cytorace 5, 6 and 8 have evolved with very much higher range values of quantitative traits than the parents and other Cytoraces, which suggests the role of transgressive segregation in the evolution of these Cytoraces.

Conclusion: Thus, the rapid divergence recorded in the chromosomes, karyotypes, body size and fitness traits of Cytoraces exhibit the early event of recombinational raciation / speciation in the evolution of the Cytoraces under laboratory conditions.

Background

One of the important aspects of studying evolution is to understand how new species are formed and their uniqueness maintained. Dobzhansky et. al [1] treat hybridization as an 'evolutionary catalyst' which, may lead to perfection of isolating mechanisms, be a source for the origin of a new species or increase the quantum of genetic variability. Templeton [2] had also felt that hybridization experiments provide the best tool for distinguishing the genetics of speciation and the genetics of species differences. Speciation genetics concentrates on populations or races that have been recently separated from each other and has not yet attained the status of species. More advanced the stage of speciation of two diverging populations; the more difficult it becomes to delineate the genetic event that has set the process into motion. Thus, it may not be possible to understand the process of speciation by looking at the finished products [3].

During last two decades, the *nasuta* subgroup of the *immigrans* species of *Drosophila* has attracted the attention of taxonomists, cytogenetists, biochemists, molecular biologists and evolutionary biologists. The *nasuta* subgroup has certain evolutionary peculiarities that make this subgroup a potent system to study the genetics of speciation in *Drosophila* [4]. *D. nasuta* was first described from Seychelles islands [5]. Later, morphologically similar forms were reported in India, Sri Lanka, Madagascar and in coastal regions of Africa [6–8]. *D. albomicans* was described from Paroe, Formosa [9]. More recently, it was reported from several Japanese islands, Malaysia, Thailand and Taiwan [6–8]. Even though the morphological description of *D. albomicans* was almost identical to that of *D. nasuta*, Wilson et al. [10] considered them as allopatric sibling species. Though the *D. nasuta* (2n = 8) and *D. albomicans* (2n = 6) differ in their chromosome number, based on their open genetic systems, cross fertility, similarities and differences in karyotypes, they have been treated as chromosomal races and called *D. n. nasuta* and *D. n. albomicans* [11,12].

The cytological distinctness of these two races has been extensively studied [12–17,8]. In brief, *D. n. nasuta* with 2n = 8 has a pair of metacentrics representing chromosome 2, two pairs of acrocentric chromosome 3 and chromosome X (an acrocentric X, a submetacentric Y in males) and a pair of dot chromosomes. Whereas *D. n. albomicans* has 2n = 6 with two pairs of metacentrics, one of which represents chromosome 2, while the other represents X3, X3 in females and X3, Y3 in males; and a pair of long dots (Chromosome 4). Under laboratory conditions they are cross-fertile. In nature no hybrids were found. They are isolated from each other by more than 3000 miles and are allopatrically distributed. Ranganath and Hagele [18], while discussing the karyotypic evolution in the *nasuta* subgroup have demonstrated that the karyotype of *D. n. albomicans* is the recent product of karyotypic orthoselection involving successive centric fusions. In view of this karyotypic phylogeny, *D. n. nasuta* is ancestral to *D. n. albomicans*.

Interracial hybridization between these two races followed by the maintenance of hybrid populations for over 20 generations in the laboratory has resulted in the evolution of new karyotypic strains called Cytoraces [16]. These Cytoraces were constructed in three phases. In the first phase, Cytorace 1 and Cytorace 2 were formed through hybridizing *D. n. nasuta* (Coorg strain) and *D. n. albomicans* (Okinawa strain) [16]. The second phase yielded two more karyotypic strains called Cytorace 3 and Cytorace 4 from the hybridization between *D. n. nasuta* of Coorg strain and *D. n. albomicans* of Thailand strain [19]. The third phase interracial hybridization among *D. n. nasuta*, *D. n. albomicans*, Cytorace 1, Cytorace 2, Cytorace 3 and Cytorace 4 has resulted in the creation of 12 new stabilized karyotypic strains, named from Cytorace 5 to Cytorace 16 (Table 1) [17,20]. Each of these Cytoraces is composed of recombined genomes of the parental races, contain chromosomes of both parents, and differ in their karyotypic composition. These newly created Cytoraces along with their parental races constitute a new assemblage the "*nasuta-albomicans* complex of *Drosophila*" [17]. Tanuja et al [21] have suggested a new terminology called "allo-sympatric" for these members of the *nasuta-albomicans* complex, because, each race is passing through a phase of racial differentiation in 'genetic isolation' through physical as opposed to behavioral barriers to interbreeding while inhabiting the same area and more importantly common set of environmental (cultures in the laboratory) conditions. They also suggested considering this complex as an 'artificial hybrid zone' in the environs of laboratory. Earlier studies on cytogenetic differentiation [16,17], mating preference [22], sternopleural bristles number [23], body size [24], body weight [25] and abdominal bristles number [26] of parental races namely *D. n. nasuta* and *D. n. albomicans* as well as Cytorace 1 and Cytorace 2 have shown significant differences between parental races and Cytoraces.

The impact of hybridization on karyotypes, morphometric and fitness traits has not been studied systematically in all these 16 Cytoraces together. In the present study, we measured the body size, body weight and certain life history traits in all 18 members of the *nasuta-albomicans* complex of *Drosophila*. The rapid divergence recorded in the chromosomes, karyotypes, body size and fitness traits of Cytoraces exhibits the early event of recombinational raciation / speciation during the evolution of these Cytoraces under laboratory condition.

Results

Chromosomes of Cytoraces

The comparative account of *D. n. nasuta*, *D. n. albomicans*, F1 and Cytoraces chromosomes were presented in our earlier publications (Table 1) [16,19,17,20]. One of the advantages in the karyotypic analysis of these races and hybrids is that the chromosomes of *D. n. nasuta* and *D. n. albomicans* can easily identified based on their size and heterochromatin content. Chromosome 2 of *D. n. nasuta* was larger in size than *D. n. albomicans* with more of pericentric heterochromatin. While chromosome 3 and X of *D. n. albomicans* were the product of centric fusion forming metacentrics X3 and Y3 chromosomes. On the other hand, chromosome 4 of *D. n. albomicans* was longer than *D. n. nasuta* with more of heterochromatin content. Cytoraces were the karyotypically stabilized hybrid forms having different chromosome composition with 2n = 6, 2n = 7 and 2n = 8 (Table 1). Some of the Cytoraces such as Cytorace 1, Cytorace 4, Cytorace 5, Cytorace 6,

Table 1: Karyotypic composition of 16 Cytoraces along with their parental crosses and the contribution of parental chromosomes in the evolution of the *nasuta-albomicans* complex of *Drosophila*. The superscripts 'n' and 'a' indicate the chromosomes derived from '*D. n. nasuta*' and ' *D. n. albomicans*' parents respectively. O = Okinawan and T = Thailand strains of *D. n. albomicans*. (Roman numbers in bracket denote the Cytorace groups based on number of chromosomes contributed by *D. n. nasuta* and *D. n. albomicans*).

Parents and the cross	Races	Karyotypes	Chromosomes of	
			nasuta	*albomicans*
	D. n. nasuta (N)	♂ - 2n = 8 - $2^n2^n X^n Y^n 3^n 3^n 4^n 4^n$ ♀ - 2n = 8 - $2^n 2^n X^n X^n 3^n 3^n 4^n 4^n$	16	0
	D.n. albomi cans (A)	♂ - 2n = 6 - $2^a2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^a 2^a X3^a X3^a 4^a 4^a$	0	12
N♂ X A(O)♀	Cytorace 1 (C 1)	♂ - 2n = 7 - $2^n 2^a X3^a Y^n 3^n 4^n 4^n$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^n 4^n$	8	5 (I)
A(O)♂ X N♀	Cytorace 2 (C 2)	♂ - 2n = 6 - $2^n 2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	2	10 (II)
N♂ X A(T)♀	Cytorace 3 (C 3)	♂ - 2n = 8 - $2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a4^a$	10	6 (III)
A(T)♂ X N♀	Cytorace 4 (C 4)	♂ - 2n = 7 - $2^n 2^a Y3^a X^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a 4^a$	8	7 (IV)
C1♂ X A(T)♀	Cytorace 5 (C 5)	♂ - 2n = 7 - $2^n 2^a X3^a Y^n 3^n4^a4^a$ ♀ - 2n = 6-$2^n2^a X3^a X3^a4^a4^a$	4	9 (V)
C4♂ X C1♀	Cytorace 6 (C 6)	♂ - 2n = 7 - $2^n 2^a Y3^a X^n 3^n 4^n 4^n$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$	12	3 (VI)
C1♂ X C2♀	Cytorace 7 (C 7)	♂ - 2n = 7 - $2^n 2^a X3^a Y^n 3^n4^a4^a$ ♀ - 2n = 6 -$2^n2^a X3^a X3^a4^a4^a$	4	9 (V)
C1♂ X C4♀	Cytorace 8 (C 8)	♂ - 2n = 7 - $2^n 2^a X3^a Y^n 3^n 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	4	9 (V)
C2♂ X N♀	Cytorace 9 (C 9)	♂ - 2n = 6 - $2^n 2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	2	10 (II)
C3♂ X N♀	Cytorace 10 (C 10)	♂ - 2n = 8 - $2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a 4^a$	10	6 (III)
C2♂ X A♀	Cytorace 11 (C 11)	♂ - 2n = 6 - $2^n 2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	2	10 (II)
A♂ X C1♀	Cytorace 12 (C 12)	♂ - 2n = 6 - $2^n 2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	2	10 (II)
A♂ X C2♀	Cytorace 13 (C 13)	♂ - 2n = 6 - $2^n 2^a X3^a Y3^a 4^a 4^a$ ♀ - 2n = 6 - $2^n 2^a X3^a X3^a 4^a 4^a$	2	10 (II)
C4♂ X C3♀	Cytorace 14 (C 14)	♂ - 2n = 7 - $2^n 2^a Y3^a X^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a 4^a$	8	7 (IV)
C3♂ X C4♀	Cytorace 15 (C 15)	♂ - 2n = 8 - $2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a 4^a$	10	6 (III)
N ♂ X C3♀	Cytorace 16 (C 16)	♂ - 2n = 8 - $2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a$ ♀ - 2n = 8 - $2^n 2^a X^n X^n 3^n 3^n 4^a 4^a$	10	6 (III)

Cytorace 7, Cytorace 8 and Cytorace 14 had unequal diploid number in males (2n = 7), and equal diploid number in females (2n = 6 or 2n = 8), even then, they breed true along with a few sterile anueploids. Both males and females of the remaining 9 Cytoraces, Cytorace 2, Cytorace 9, Cytorace 11, Cytorace 12 and Cytorace 13 had 2n = 6, while Cytorace 3, Cytorace 10, Cytorace 15, and Cytorace 16 had 2n = 8. The chromosomes of these Cytoraces were the introgressed genomes between the parental races and they are not the same as their parents. Although some of these Cytoraces had same chromosome number and chromosome morphology, they are numbered as different Cytorace since they were derived from different parental genomes.

Based on the relative contributions of *D. n. nasuta* and *D. n. albomicans* chromosomes, these 16 Cytoraces are categorized into six types (Table 1). Of which, Cytorace 6 (belonging to group VI) is with more number of chromosomes which have the morphology like that of *D. n. nasuta* chromosomes (12N+ 3A), while Cytorace 2, Cytorace 9, Cytorace 11, Cytorace 12 and Cytorace 13 (belonging to group II) are with more number of chromosomes which have the morphology like that of *D. n. albomicans* chromosomes (10A+2N). The dot chromosomes in all the Cytoraces were in homozygous condition, wherein only Cytorace I and Cytorace 6 have *D. n. nasuta* dot chromosomes, while all other Cytoraces evolved with *D. n. albomicans* dot chromosomes. Except in the males of Cytoraces who have stabilized with 2n = 7, the sex and chromosome 3 were evolved in homozygous condition. It has been reported that the chromosome 2 is always in polymorphic nature with more frequency of heterozygous combination. Taking all the chromosomes of all the 16 Cytoraces together, it has been observed that chromosomes of *D. n. albomicans* were more favored (123 chromosomes) than *D. n. nasuta* (98 chromosomes) during the evolution of these Cytoraces. However, all these chromosomes of the karyotypically stabilized Cytoraces were the introgressed chromosomes by recombination during the evolution of these Cytoraces.

Body size

The females of all 18 members of the *nasuta -albomicans* complex (NAC) of *Drosophila* have increased mean wing length (Table 2) and width (Table 3) than males. The analysis of variance test has revealed significant differences in males, females and both males and females with $P < 0.001$. Based on Dunken's multiple range test (DMRT), *D. n. nasuta*, *D. n. albomicans* and the products of first phase of interracial hybridization (Cytorace 1 and Cytorace 2) have increased wing length (Fig. 1) than recently evolved second and third phase Cytoraces, except in males, wherein Cytorace 12 had higher mean values than Cytoraces 1, however, the difference is insignificant. In contrast to this, the mean values of wing width in

Table 2: Mean wing length of 18 members of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 30 flies) along with statistical analysis

		Mean wing length in		
Sl. No.	Races	Males	Females	Both males and females together
1	*D. n. nasuta* (N)	233.16 ± 1.51	252.50 ± 1.59	242.83 ± 1.55
2	*D. n. albomicans* (A)	237.58 ± 1.71	259.50 ± 1.76	248.54 ± 1.73
3	Cytorace 1 (C1)	222.41 ± 1.14	243.75 ± 2.57	233.08 ± 1.85
4	Cytorace 2 (C2)	232.50 ± 1.88	255.25 ± 1.64	243.79 ± 1.76
5	Cytorace 3 (C3)	219.30 ± 1.13	230.06 ± 2.80	224.30 ± 1.96
6	Cytorace 4 (C4)	212.00 ± 1.07	226.20 ± 2.26	219.18 ± 1.66
7	Cytorace 5 (C5)	208.16 ± 1.45	225.90 ± 1.81	217.03 ± 1.63
8	Cytorace 6 (C6)	206.30 ± 1.31	229.23 ± 2.78	217.76 ± 2.04
9	Cytorace 7 (C7)	215.10 ± 1.38	234.06 ± 2.38	221.50 ± 1.88
10	Cytorace 8 (C8)	197.03 ± 1.21	227.25 ± 2.29	215.55 ± 1.75
11	Cytorace 9 (C9)	213.00 ± 1.03	233.60 ± 1.52	220.08 ± 1.27
12	Cytorace 10 (C10)	220.15 ± 2.34	227.11 ± 2.87	226.87 ± 2.60
13	Cytorace 11 (C11)	212.70 ± 1.16	235.76 ± 2.42	219.90 ± 1.79
14	Cytorace 12 (C12)	224.16 ± 2.76	238.30 ± 3.58	229.96 ± 3.17
15	Cytorace 13 (C13)	219.58 ± 2.56	233.73 ± 2.31	228.92 ± 2.43
16	Cytorace 14 (C14)	211.13 ± 1.31	233.68 ± 2.58	222.43 ± 1.94
17	Cytorace 15 (C15)	221.21 ± 1.94	235.90 ± 1.11	227.45 ± 1.52
18	Cytorace 16 (C16)	213.03 ± 1.14	227.06 ± 2.64	224.46 ± 1.89
Analysis of variance		F = 33.219 d.f. = 17, 522 P < 0.001	F = 19.290 d.f. = 17, 522 P < 0.001	F = 39.354 d.f = 33, 1044 P < 0.001

Table 3: Mean wing width of 18 members of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 30 flies) along with statistical analysis

		Mean wing width in		
Sl. No.	Races	Males	Females	Both males and females together
1	*D. n. nasuta* (N)	88.08 ± 0.94	100.90 ± 0.78	94.49 ± 0.86
2	*D. n. albomicans* (A)	91.83 ± 1.59	107.16 ± 1.20	99.49 ± 1.39
3	Cytorace 1 (C1)	86.33 ± 1.03	97.00 ± 1.61	91.66 ± 1.38
4	Cytorace 2 (C2)	87.66 ± 0.80	98.50 ± 1.06	93.08 ± 0.93
5	Cytorace 3 (C3)	97.85 ± 1.60	101.60 ± 1.30	99.72 ± 1.45
6	Cytorace 4 (C4)	91.68 ± 1.15	96.86 ± 1.21	94.27 ± 1.18
7	Cytorace 5 (C5)	88.98 ± 0.90	94.73 ± 1.03	91.85 ± 0.96
8	Cytorace 6 (C6)	86.11 ± 0.94	91.03 ± 0.86	88.57 ± 0.90
9	Cytorace 7 (C7)	96.51 ± 1.67	101.41 ± 1.63	98.96 ± 1.65
10	Cytorace 8 (C8)	82.50 ± 0.98	88.35 ± 1.00	85.42 ± 0.99
11	Cytorace 9 (C9)	94.81 ± 1.18	100.83 ± 1.07	97.82 ± 1.12
12	Cytorace 10 (C10)	97.96 ± 1.26	101.88 ± 1.30	99.92 ± 1.28
13	Cytorace 11 (C11)	92.03 ± 1.13	100.83 ± 1.07	96.56 ± 1.10
14	Cytorace 12 (C12)	102.68 ± 1.00	107.81 ± 1.21	105.24 ± 1.10
15	Cytorace 13 (C13)	102.50 ± 1.62	107.58 ± 1.35	105.04 ± 1.48
16	Cytorace 14 (C14)	89.75 ± 1.29	95.75 ± 1.67	92.75 ± 1.48
17	Cytorace 15 (C15)	99.21 ± 1.39	106.11 ± 1.26	102.66 ± 1.32
18	Cytorace 16 (C16)	95.25 ± 1.33	100.95 ± 1.74	98.10 ± 1.53
Analysis of variance		F = 21.775 d.f. = 17, 522 P < 0.001	F = 17.602 d.f. = 17, 522 P < 0.001	F = 30.151 d.f.= 33, 1044 P < 0.001

males, females, and both males and females together majority of the Cytoraces had outside the range of parental races (Fig. 2) indicating that the wing width varies significantly among the Cytoraces.

The analysis of variance test (Table 4) has revealed significant differences among the 18 members for genitalia length ($P < 0.001$) and genitalia width ($P < 0.002$). *D. n. albomicans* and Cytorace 8 had the highest and the lowest mean genitalia length and width, respectively (Table 4). Among the Cytoraces, Cytorace 16 has increased genitalia length (Fig. 3a) and Cytorace 2 has increased genitalia width (Fig. 3b) (which is also equivalent to *D. n. albomicans*) than rest of the Cytoraces. It has been observed that none of the other Cytoraces have increased size of genitalia than *D. n. albomicans*, however, most of the Cytoraces exhibited their genitalia size outside the range of *D. n. nasuta* parents.

The mean body weight of 2 days old flies (Table 5) in 18 members of the NAC of *Drosophila* revealed that females show increased body weight than males. The males of Cytorace 10 and Cytorace 8 had lesser and greater body weight respectively, while in females and both males and females together, *D. n. nasuta* and Cytorace 8 has decreased and increased body weight respectively, however, the difference between the values of *D. n. nasuta* and Cytorace 10 is insignificant (Fig. 4). Most of the Cytoraces have increased body weight than parental races. These results are contrasting with the other body size traits indicating that body weight trait is independent of body size traits.

Life history traits

The mean lifetime fecundity, lifetime fertility, ovariole numbers and hatching success in the 18 members of the NAC complex of *Drosophila* (Table 6) revealed that *D. n. nasuta*, has decreased lifetime fecundity, lifetime fertility and hatching success than *D. n. albomicans* as well as most of the Cytoraces. While Cytorace 12 in fecundity and Cytorace 13 in fertility as well as hatching success (Table 6) have reduced values than all other Cytoraces. On the other hand, Cytorace 8 had the highest lifetime fecundity and lifetime fertility, while Cytorace 6 and *D. n. albomicans* had the highest ovariole number and hatching success respectively (5a-c). The mean lifetime fecundity of the parental races is lower than Cytoraces, however, in Cytoraces, the mean lifetime fecundity extends outside the range of parental races. These observations suggest that, the newly evolved Cytoraces of the third phase have also exhibited the lowest as well as the highest fecundity, indicating the extent of divergence. The results of decreased ovariole number in *D. n. nasuta* and *D. n. albomicans* and also first phase Cytoraces (Cytorace 1 and Cytorace 2) suggest that recently evolved Cytoraces are having increased ovariole number than ancestral races (Fig. 5b). The lifetime fertility of all the 16 Cytoraces also extends outside the range of parental races. The percent of hatching success (which was measured by dividing fertility by fecundity values) across the lineages ranges from 60 and 75% with the exception of Cytorace 13 (36%).

The mean longevity of unmated males and virgin females (Table 7) in the 18 members of the NAC complex of *Drosophila* revealed that the virgin females (55.53 to 83.63 days) of all these races lived longer than males (48.33 to 68.60 days). Both mated and virgin flies of *D. n. nasuta* and *D. n. albomicans* had the reduced longevity than most of the 16 newly evolved Cytoraces. Of these two, *D. n. albomicans* lived longer than *D. n. nasuta*. Among the Cytoraces, in both mated and virgin flies, Cytorace 8 lived longer (63.53 to 83.63 days) than other Cytoraces, whereas males of Cytorace 12 (47.16 days) and females of Cytorace 1 (51.06 days) showed the reduced longevity than all other Cytoraces. These results suggest that, *D. n. nasuta* which is ancestor to all these races based on chromosomal evolution has decreased life span than the newly evolved Cytoraces (Fig. 6a,6b,6c,6d). In addition, the results revealed that virgin flies live longer than mated flies in the *nasuta-albomicans* complex of *Drosophila* (Fig. 6e). It is clear that all the Cytoraces live longer than parental races indicating that the hybridization followed by recombination played a major role in this process of raciation / speciation.

Correlation analysis

Correlation analysis based on the Euclidean distance dissimilarity in 18 members of the NAC complex of *Drosophila* is compiled in Table 8 - Additional file: 1. The traits used for male, female and both male and female together analysis were 7 (wing length and width, genitalia length and width, body weight, longevity in unmated and mated males), 8 (wing length and width, body weight, lifetime fecundity, ovariole numbers, lifetime fertility, longevity in virgin and mated females) and 5 (wing length and width, body weight, longevity in virgin and mated flies) respectively. The Euclidean distance between two values is the arithmetic difference. The minimum value is 0, and it has no upper limit. Based on this, Cytorace 8 had the maximum distance from *D. n. nasuta* and *D. n. albomicans* than other Cytoraces. Figure 7 illustrates the Cytoraces, which deviate with higher and lower range of Euclidean distance from the parental range. Interestingly, some of the 3rd phase Cytoraces have higher range of Euclidean distance with *D. n. nasuta*, *D. n. albomicans*, Cytorace 2 and Cytoraces 1. In particular, Cytorace 5, Cytorace 6 and Cytorace 8 had maximum divergence with *D. n. nasuta* and *D. n. albomicans*. While some of the 3rd phase and 2nd phase Cytoraces had minimum divergence among themselves

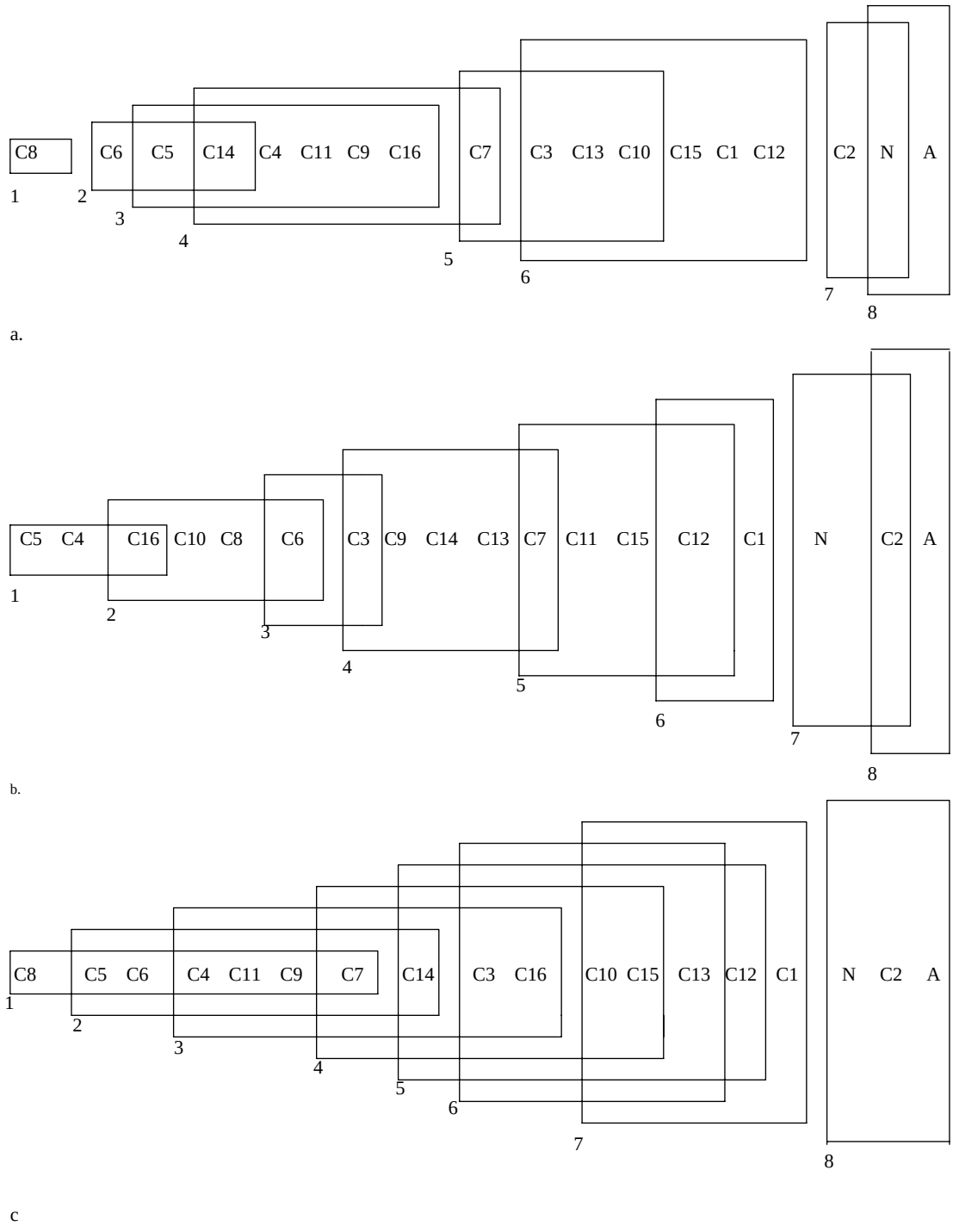


Figure 1
Based on the Duncan's multiple range test (DMR), 18 members of the *nasuta-albomicans* complex of *Drosophila* are constructed into clusters and named in the hierarchical form from the lowest to the highest mean wing length. The members belonging to each cluster have insignificant differences. In males, (Fig. 1a), females (Fig. 1b) and both males and females together (Fig. 1c), 8 clusters are made. In males, cluster 1 (C8) with only one member forms an independent cluster, while N, A and C2 emerged as independent (Fig. 1c) as well as overlapping clusters (Fig. 1a and 1b) from the other clusters. All others are with overlapping clusters.

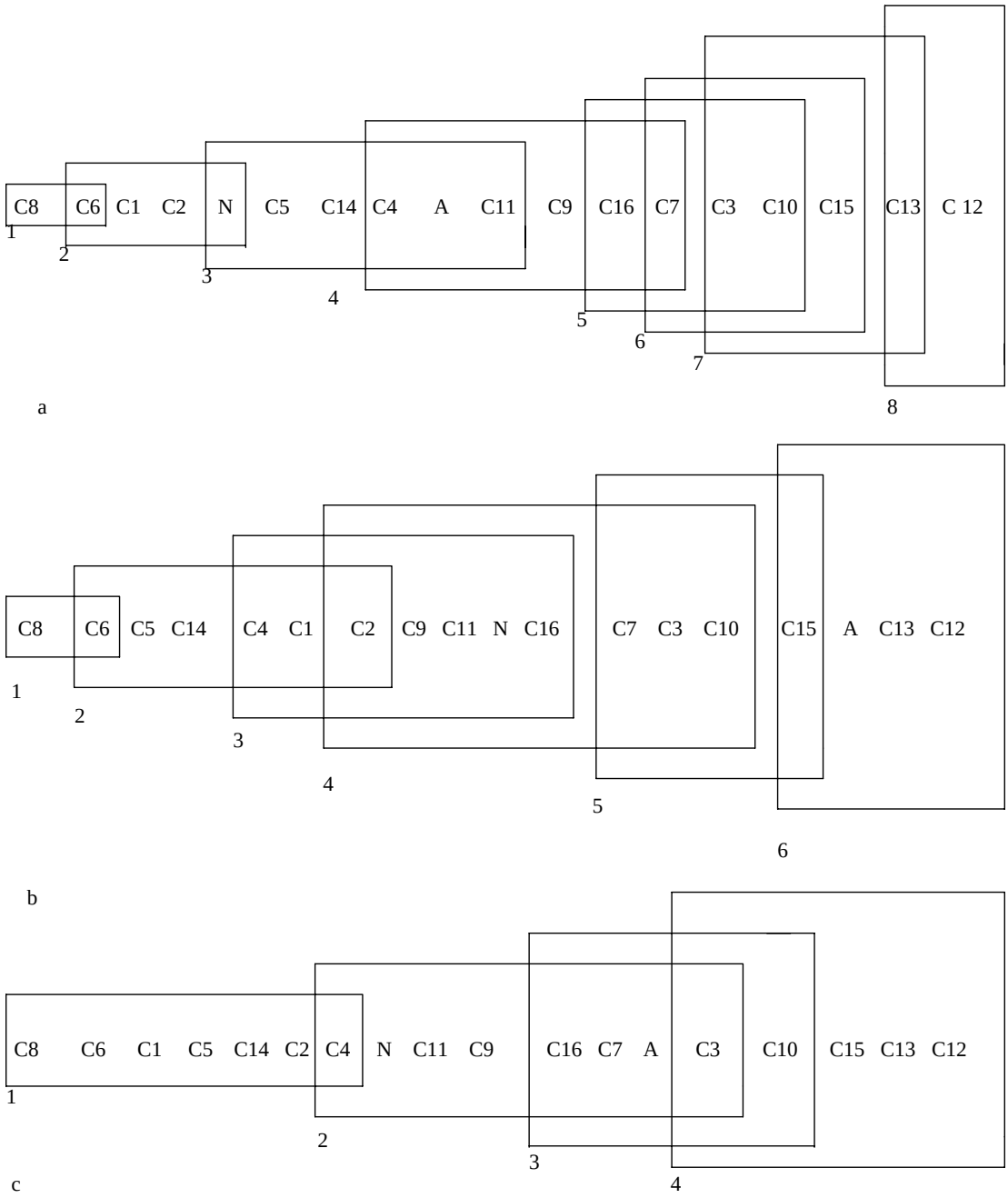


Figure 2
Based on the DMR test, 18 members of the *nasuta-albomicans* complex of *Drosophila* are constructed into clusters and named in the hierarchical form from the lowest to the highest mean wing width. The members belonging to each cluster have insignificant differences. In males, (Fig. 2a), females (Fig. 2b) and both males and females together (Fig. 2c), 8, 6, and 4 clusters are made respectively. All the members are with overlapping clusters.

Table 4: Mean genitalia length and width in males (n = 50) of 18 members of the *nasuta-albomicans* complex of *Drosophila* with statistical analysis

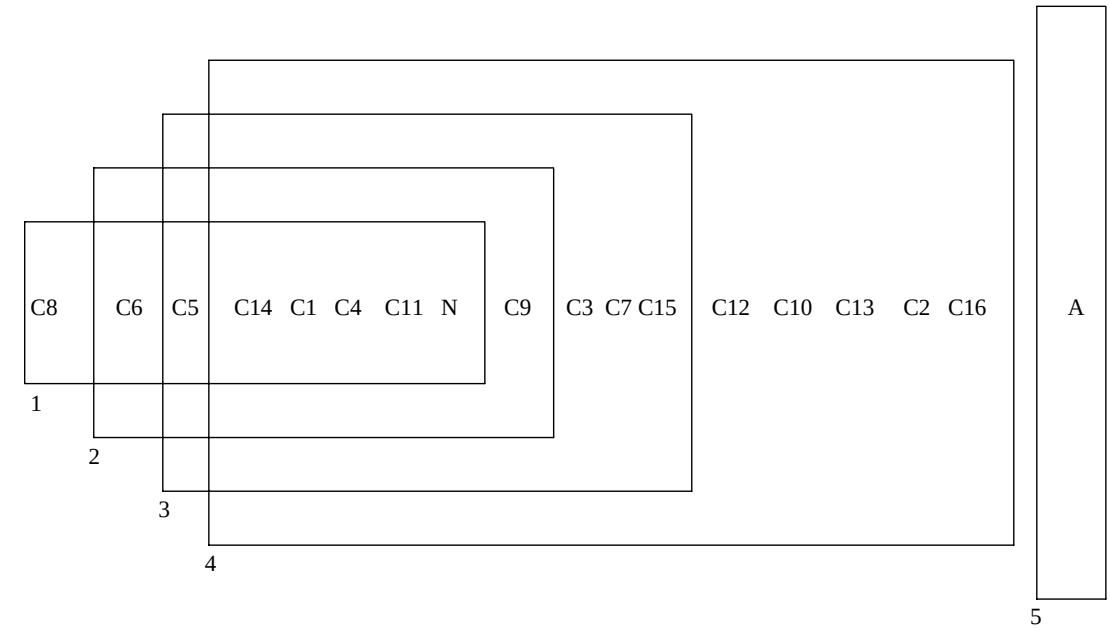
Sl. No.	Races	Genitalia length	Genitalia width
1	D. n. nasuta (N)	18.33 ± 0.05	12.38 ± 0.05
2	*D. n. albomicans* (A)	18.67 ± 0.04	12.44 ± 0.05
3	Cytorace 1 (C1)	18.29 ± 0.04	12.30 ± 0.05
4	Cytorace 2 (C2)	18.44 ± 0.06	12.44 ± 0.05
5	Cytorace 3 (C3)	18.39 ± 0.05	12.43 ± 0.05
6	Cytorace 4 (C4)	18.33 ± 0.05	12.33 ± 0.04
7	Cytorace 5 (C5)	18.22 ± 0.04	12.27 ± 0.04
8	Cytorace 6 (C6)	18.18 ± 0.04	12.20 ± 0.04
9	Cytorace 7 (C7)	18.39 ± 0.06	12.35 ± 0.04
10	Cytorace 8 (C8)	18.16 ± 0.03	12.17 ± 0.03
11	Cytorace 9 (C9)	18.34 ± 0.05	12.33 ± 0.05
12	Cytorace 10 (C10)	18.41 ± 0.05	12.40 ± 0.06
13	Cytorace 11 (C11)	18.33 ± 0.05	12.31 ± 0.04
14	Cytorace 12 (C12)	18.41 ± 0.06	12.43 ± 0.05
15	Cytorace 13 (C13)	18.43 ± 0.07	12.42 ± 0.06
16	Cytorace 14 (C14)	18.25 ± 0.05	12.30 ± 0.05
17	Cytorace 15 (C15)	18.40 ± 0.05	12.35 ± 0.04
18	Cytorace 16 (C16)	18.45 ± 0.05	12.29 ± 0.04
Analysis of variance		F = 4.252 d.f.= 17, 882 P < 0.001	F = 2.271 d.f. = 17, 882 P < 0.002

indicating the role of hybrid recombination followed by segregation of parental genes during the evolution of these Cytoraces (Fig. 7).

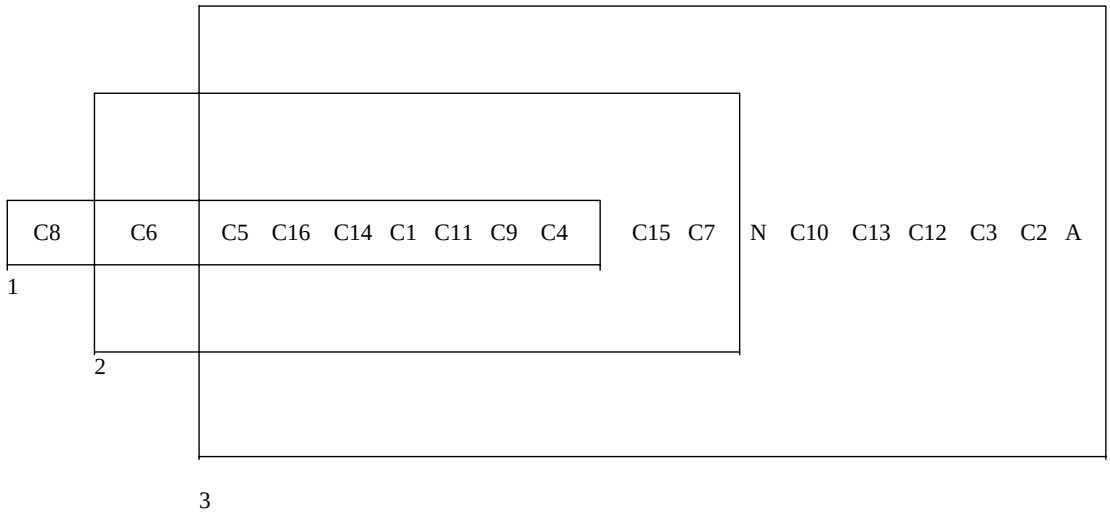
Discussion

A fundamental question in evolutionary biology is whether speciation is gradual or punctuated. This question has been difficult to evaluate critically because the evolutionary history of most plant and animal species is poorly known. However, it may be feasible to determine which modes of speciation are likely to be rapid and which ones are likely to occur gradually [27]. The most widely accepted model for diploid or homoploid hybrid speciation is the recombinational model described by Grant [28]. According to this model, the sorting of chromosomal rearrangements in later generation hybrids could, by chance, lead to the formation of new populations that are homozygous for a novel combination of rearrangements. The new hybrid population would be fertile, stable and has the same ploidy as its parents, yet would be at least partially isolated from both parental species by a chromosomal sterility barrier. Although homoploid hybrid speciation is not instantaneous, computer simulation studies suggest that it should occur rapidly. In the simulations, long periods of hybrid zone stasis are typically followed by abrupt transitions in which the new hybrid type becomes established and rapidly displaces the parental species [29]. Other factors that appear to play a critical role in recombinational speciation include: strong natural selection for most fertile or viable hybrid segregants, rapid chromosomal evolution and the availability of habitats suitable for the establishment of hybrid neospecies [30].

The feasibility of this mode of speciation has been verified experimentally via crossing studies. These studies demonstrate that fertile and viable hybrid lineages can be obtained after only a small number of generations (<10) of selfing and/or backcrossing, even if the F_1 hybrids were almost completely sterile. Furthermore, experimental crosses indicate that the synthetic hybrid lineages are often strongly reproductively isolated from the parental species [31]. Rieseberg et al [32] have demonstrated hybrid speciation accompanied by genomic reorganization in wild sunflowers. Ungerer et al [27] have also demonstrated the rapid hybrid speciation by estimating the sizes of parental species chromosomal blocks in *Helianthus anomalus*, a wild sunflower species derived via hybridization between *H. annuus* and *H. petiolaris*. Waugh O'Neill et al [32] have reported the occurrence of genome-wide undermethylation, retroviral element amplification and chromosome remodeling in the interspecific mammalian hybrids of *Macropus eugenii* with *Wallabia bicolor* could facilitate rapid karyotypic evolution. These reports clearly indicate that the rapid karyotypic evolution following hybridization can facilitate the evolution of reproductive barriers in hybrid lineages.



a



b.

Figure 3
Based on the DMR test, 18 members of the *nasuta-albomicans* complex of *Drosophila* are constructed into clusters and named in the hierarchical form from the lowest to the highest mean genitalia length (Fig. 3a) and genitalia width (Fig. 3b). The members belonging to each cluster have insignificant differences. In mean genitalia length, of the 5 clusters, *albomicans* formed an independent cluster (cluster 5). In the mean genitalia width, all the three clusters are overlapping.

Table 5: Mean body weight of 18 members of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 50 flies) along with statistical analysis

Sl. No.	Races	Mean body weight in		
		Males	Females	Both males and females together
1	*D. n. nasuta (N)*	0.97 ± 0.03	1.11 ± 0.02	1.04 ± 0.02
2	*D. n. albomicans* (A)	1.03 ± 0.02	1.24 ± 0.02	1.13 ± 0.02
3	Cytorace 1 (C1)	1.05 ± 0.02	1.21 ± 0.02	1.13 ± 0.02
4	Cytorace2 (C2)	1.17 ± 0.01	1.41 ± 0.02	1.29 ± 0.01
5	Cytorace 3 (C3)	0.97 ± 0.02	1.24 ± 0.03	1.15 ± 0.02
6	Cytorace 4 (C4)	1.14 ± 0.02	1.65 ± 0.03	1.39 ± 0.02
7	Cytorace 5 (C5)	1.23 ± 0.04	1.74 ± 0.03	1.48 ± 0.03
8	Cytorace 6 (C6)	1.22 ± 0.03	1.69 ± 0.02	1.45 ± 0.02
9	Cytorace 7 (C7)	1.25 ± 0.04	1.63 ± 0.04	1.44 ± 0.04
10	Cytorace 8 (C8)	1.51 ± 0.02	1.76 ± 0.03	1.64 ± 0.02
11	Cytorace 9 (C9)	1.02 ± 0.03	1.54 ± 0.04	1.48 ± 0.03
12	Cytorace 10 (C10)	0.94 ± 0.03	1.31 ± 0.03	1.12 ± 0.03
13	Cytorace 11 (C11)	1.06 ± 0.02	1.52 ± 0.02	1.29 ± 0.02
14	Cytorace12 (C12)	1.12 ± 0.02	1.14 ± 0.02	1.13 ± 0.02
15	Cytorace13 (C13)	1.11 ± 0.05	1.67 ± 0.03	1.39 ± 0.04
16	Cytorace 14 (C14)	1.13 ± 0.02	1.66 ± 0.03	1.39 ± 0.02
17	Cytorace 15 (C15)	0.99 ± 0.03	1.36 ± 0.03	1.17 ± 0.03
18	Cytorace 16 (C16)	0.96 ± 0.09	1.37 ± 0.03	1.16 ± 0.06
Analysis of variance		F = 15.428 d.f. = 17, 882 P < 0.001	F = 38.514 d.f. = 17, 882 P < 0.001	F = 43.639 d.f. = 33,1764 P < 0.001

Chromosomes of Cytoraces

In the present study, Interracial hybridization between *D. n. nasuta* (2n = 8) and *D. n. albomicans* (2n = 6) yielded a range of new karyotypic stabilized combinations called Cytoraces. Some of the important features in the evolution of these Cytoraces are as follows: a) Chromosome 2 in the Cytoraces has not been stabilized into one combination, and more than 50% of the karyotypes in each of the Cytoraces present in heterozygous condition, which indicate that chromosome 2 is in polymorphic state. Therefore, all these Cytoraces show fixed heterozygosity for the 2nd chromosome. b) Of the 16 Cytoraces, 14 of them retained and established *D. n. albomicans* dots chromosomes in homozygous state, the remaining two Cytoraces (Cytorace 1 and Cytorace 6) retained the *D. n. nasuta* dot chromosomes suggesting that *D. n. albomicans* dots are more stable and favoured. c) Regarding the sex and chromosome 3, there is a trend of retaining more of *albomicans* X3/Y3 chromosomes in the Cytoraces than *nasuta* chromosomes. d) There is also a tendency of retaining parental chromosomes in Cytoraces depending on its male parents (10/16). These Cytoraces evolved by reshuffling of parental chromosomes (and genes) and also retaining certain chromosomes by eliminating others. Within a span of one -and- half decades, hybridization as an evolutionary stimulus has influenced the evolution of sixteen cytogenetically different Cytoraces in the laboratory. It might have taken millions of years in nature to evolve a group of such differentiating races. These Cytoraces differ in karyotypes from their parents, which is out side the range of the parental combinations due to transgressive segregation of chromosomes and recombinational events. Evolution of these new karyotypes through hybridization and recombination ranging from 20–200 generations is a unique evidence for rapid chromosomal evolution in animals particularly in *Drosophila*.

Body size

Evolutionary response to selection depends on the amount of genetic variation expressed in the population. Because of this, the effect of environmental changes on the expression of genetic variation in the quantitative traits has important evolutionary implications [33]. Body size is the central feature of any organism – physiologically, ecologically and evolutionarily [34]. 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 [35]. In the present study, investigations on the body size traits namely, wing length, and width; genitalia length and width and body weight brings out the following: a) the females of all the 18 members of the NAC complex of *Drosophila* have larger wing length and wing width than males. b) *D. n. albomicans* has increased wing length, genitalia length and genitalia width while Cytorace 12 has increased wing width. c) Cytorace 8 has greatly reduced wing length and width, genitalia length and width along with the highest body weight. d) Except males of Cytorace

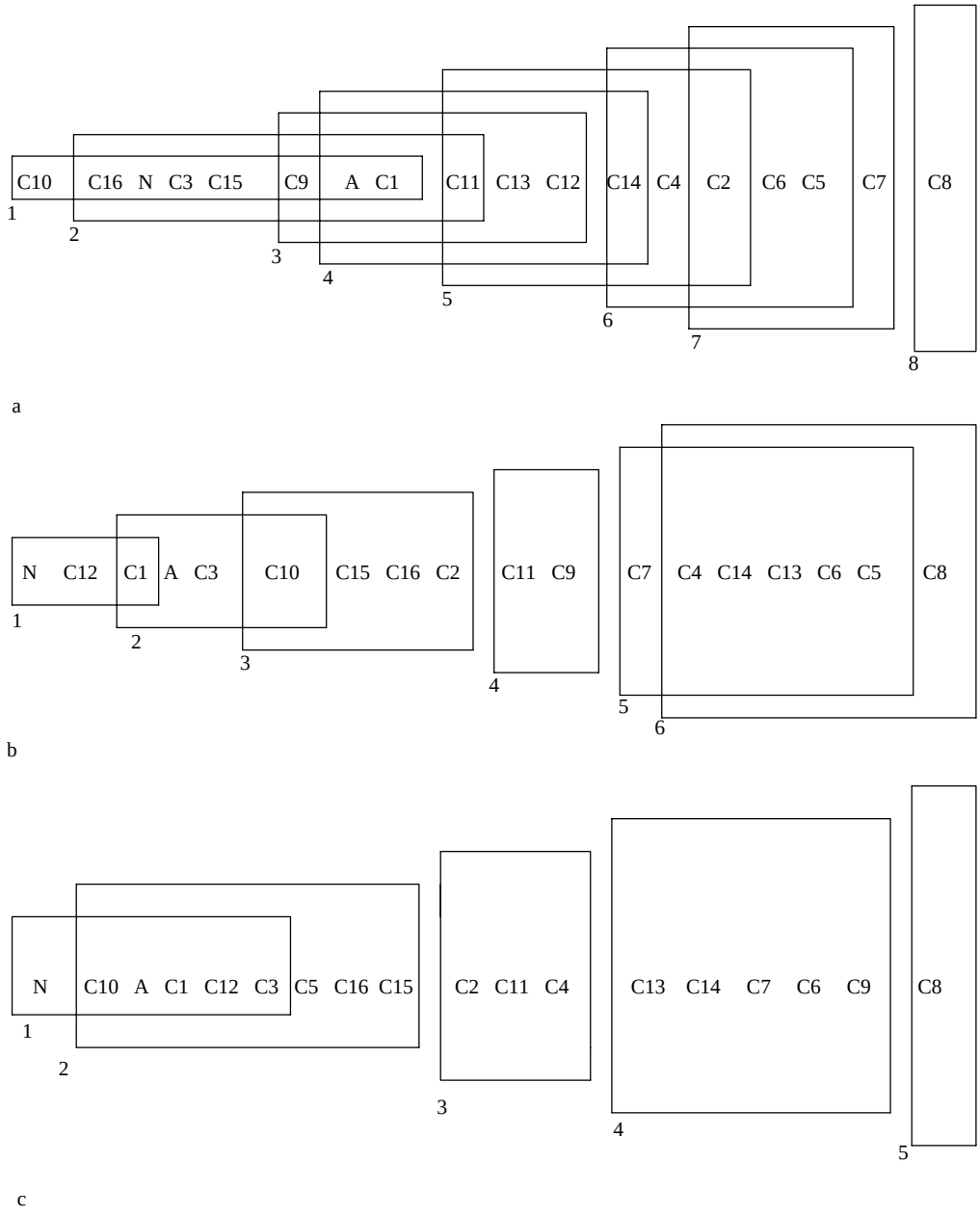


Figure 4
Based on the DMR test, 18 members of the *nasuta-albomicans* complex of *Drosophila* are constructed into clusters and named in the hierarchical form from the lowest to the highest mean body weight in 2 days old flies. The members belonging to each clusters have insignificant differences. In males, Cytorace 8 formed an independent cluster, while all others with 7 overlapping clusters (Fig. 4a). In females, six clusters are recognised, of which, cluster 1 to 3 are clustered together while cluster 4 (C11 & C9) has formed an independent cluster. In addition to this, cluster 5 and 6 are also overlapped each other and independent from the other clusters (Fig. 4b). In both males and females, 5 clusters are recognised, of which, cluster 1 and 2 are overlapped with each other, while cluster 3 (C2, C11, and C4), cluster 4 (C13, C14, C7, C6 and C9) and cluster 5 (C8) are formed as independent clusters.

Table 6: Mean life-history traits in the females of the eighteen members of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 30 replicates) along with statistical analysis

Races	Mean lifetime fecundity	Mean ovariole number	Mean lifetime fertility	Hatching success (%)
D. n. nasuta (N)	184.90 ± 10.46	14.00 ± 0.44	113.83 ± 4.36	61.56
D. n. albomicans (A)	199.75 ± 10.63	16.63 ± 0.72	151.30 ± 6.45	75.74
Cytorace 1 (C1)	255.95 ± 11.91	16.73 ± 0.76	166.50 ± 7.52	65.05
Cytorace2 (C2)	256.80 ± 5.59	17.63 ± 0.80	164.16 ± 6.78	63.92
Cytorace 3 (C3)	201.30 ± 8.58	22.26 ± 0.75	139.70 ± 10.03	69.37
Cytorace 4 (C4)	227.25 ± 5.66	22.66 ± 0.66	157.65 ± 3.81	69.37
Cytorace 5 (C5)	239.55 ± 10.78	24.06 ± 0.96	162.90 ± 5.60	68.00
Cytorace 6 (C6)	240.00 ± 10.00	24.36 ± 0.76	178.10 ± 4.14	74.21
Cytorace 7 (C7)	218.15 ± 9.48	21.96 ± 0.57	139.40 ± 5.26	63.90
Cytorace 8 (C8)	281.50 ± 12.25	22.43 ± 0.76	193.45 ± 3.70	68.72
Cytorace 9 (C9)	203.90 ± 7.98	22.56 ± 0.59	148.20 ± 7.90	72.68
Cytorace 10 (C10)	204.55 ± 10.70	21.00 ± 0.63	133.95 ± 9.06	65.48
Cytorace 11 (C11)	219.90 ± 8.19	21.93 ± 1.10	150.75 ± 5.60	68.55
Cytorace 12 (C12)	169.00 ± 9.97	19.16 ± 0.57	127.55 ± 7.69	75.47
Cytorace 13 (C13)	247.40 ± 9.97	18.73 ± 0.60	89.15 ± 7.56	36.03
Cytorace 14 (C14)	229.30 ± 8.79	24.23 ± 0.53	159.10 ± 5.91	69.38
Cytorace 15 (C15)	198.50 ± 5.69	21.80 ± 1.48	140.25 ± 7.29	70.65
Cytorace 16 (C16)	217.80 ± 11.08	21.96 ± 0.82	148.85 ± 5.35	68.34
Analysis of variance (ANOVA)	F = 9.432; d.f. = 17, 522; P < 0.001	F = 14.005; d.f = 17, 522; P < 0.001	F = 12.410; d.f.= 17, 522; P < 0.001	

10, all the other Cytoraces have shown the greater body weight than parental races. e) In most of the Cytoraces, body size and body weight are negatively correlated. If one considers only mean wing length values, one can strongly argue that the newly evolved Cytoraces are always smaller in size than their parents. When we compare wing length with body weight trait, it is clear that the Cytoraces are smaller in size with increased body weight than parental races. Such correlations cannot be achieved for the other body-sized traits. In addition to this, majority of these Cytoraces exhibit most of the morphophenotypes out side the range of parental range, which indicate that these Cytoraces are unique products of interracial hybridization followed by recombination and transgressive segregation of quantitative traits/genes.

Life history traits

Various investigators have contributed towards the understanding of measurements of population fitness and its components in natural and experimental populations of *Drosophila* [36–39]. The likelihood of establishing a new hybrid lineage depends in large part on its fitness in parental and /or divergent habitats [31]. Egg laying potentiality is an important attribute, which determines to certain extent the reproductive success of a population. Fecundity is the major determining factor of female fitness [34]. In the present study, except Cytorace 12, all other Cytoraces have higher fecundity than *D. n. nasuta* and *D. n. albomicans* parents. Therefore, one can surmise that each of these Cytoraces is the unique recombinant product exhibiting their evolutionary independence with higher fitness.

Ovariole number is an anatomical trait determined during pupation for which a polygenic basis is known in various species of the *D. melanogaster* complex [40]. Ovariole number is correlated with female reproductive success via a simple relationship between the number of ovarioles and the rate at which, the eggs have produced by the female [41]. The newly evolved second and third phase Cytoraces have greater number of ovarioles than their parents. In addition to this, the ancestor races *D. n. nasuta* emerged out as an independent member with lowest ovariole number indicating the significant divergence from all the other members of the *nasuta-albomicans* complex of *Drosophila*. This also supports the chromosomal basis of orthoselection in the *nasuta* subgroup of *Drosophila* [18,7] stating that *D. n. alboimcans* karyotype 2n = 6 is derived from *D. n. nasuta* 2n = 8 which is an ancestral race.

Fertility, the newly produced offspring from that particular mating pair is an important component of fitness, measured in terms of productivity has been extensively studied in different species of *Drosophila* [24]. Cytoraces with uneven diploid chromosome number in males and even diploid number in females (Cytorace 1, Cytorace 4, Cytorace 5, Cytorace 6, Cytorace 8 and Cytorace 14)

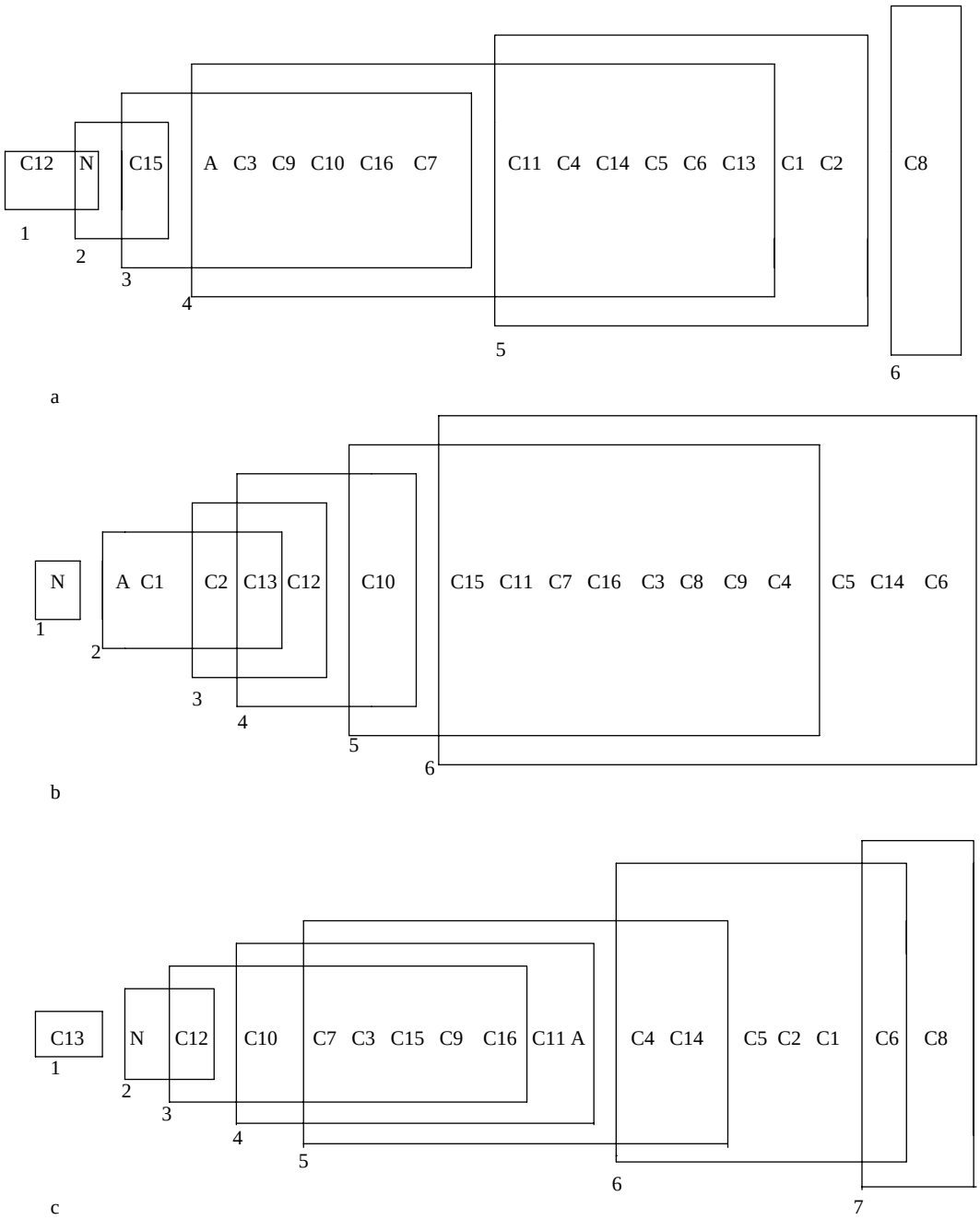


Figure 5
Based on the DMR test, 18 members of the *nasuta-albomicans* complex of *Drosophila* are constructed into clusters and named in the hierarchical form from the lowest to the highest mean lifetime fecundity (Fig. 5a), ovariole number (Fig. 5b) and lifetime fertility (Fig. 5c). The members belonging to each cluster have insignificant differences. For life time fecundity, six clusters were recognized, of which, cluster 1 to 5 are overlapped with each other, while cluster 6 (C8) is formed as independent cluster. For ovariole number, six clusters were recognised, of which, except cluster 1 (N), all the other clusters from 2 to 6 are overlapped, while cluster 1 is formed as independent cluster. For fertility, cluster 1 (C13) is formed as independent cluster, while cluster 2 to cluster 7 are overlapped with each other.

Table 7: Mean longevity in the virgin and mated flies of eighteen members of the *nasuta-albomicans* complex of *Drosophila* (values are mean ± SE of 30 replicates) along with statistical analysis

Races	*Mean longevity of virgins*		*Mean longevity of mated*	
	Males	Females	Males	Females
D. n. nasuta (N)	48.33 ± 1.41	55.53 ± 1.36	40.00 ± 1.24	44.93 ± 1.51
D. n. albomicans(A)	53.96 ± 1.49	63.76 ± 1.29	45.76 ± 1.50	49.56 ± 2.13
Cytorace 1 (C1)	57.20 ± 1.78	64.06 ± 1.53	48.73 ± 2.20	51.06 ± 2.10
Cytorace 2 (C2)	61.66 ± 1.76	64.86 ± 1.50	50.70 ± 2.25	51.76 ± 2.44
Cytorace 3 (C3)	56.83 ± 1.25	69.10 ± 1.51	50.73 ± 2.06	63.90 ± 1.53
Cytorace 4 (C4)	64.76 ± 1.75	74.30 ± 1.51	58.36 ± 1.69	68.96 ± 1.47
Cytorace 5 (C5)	65.83 ± 1.71	75.83 ± 2.67	61.36 ± 1.67	70.93 ± 2.46
Cytorace 6 (C6)	66.23 ± 1.82	69.10 ± 1.45	62.10 ± 1.69	63.26 ± 1.46
Cytorace 7 (C7)	66.46 ± 2.51	76.63 ± 1.59	60.66 ± 2.17	71.73 ± 1.58
Cytorace 8 (C8)	68.60 ± 1.83	83.63 ± 2.73	63.53 ± 1.71	78.33 ± 2.37
Cytorace 9 (C9)	63.53 ± 1.54	72.13 ± 2.24	59.46 ± 1.44	69.86 ± 2.14
Cytorace 10 (C10)	57.70 ± 1.33	69.46 ± 1.42	54.26 ± 1.22	64.30 ± 1.40
Cytorace 11 (C11)	64.56 ± 1.97	75.16 ± 1.03	60.00 ± 1.72	70.06 ± 1.78
Cytorace 12 (C12)	50.66 ± 0.95	73.43 ± 3.73	47.16 ± 1.86	71.50 ± 2.63
Cytorace 13 (C13)	63.66 ± 1.35	73.86 ± 1.87	59.26 ± 1.41	66.23 ± 1.76
Cytorace 14 (C14)	65.00 ± 1.42	74.36 ± 2.52	60.66 ± 1.19	70.96 ± 1.80
Cytorace 15 (C15)	62.66 ± 2.41	70.36 ± 1.72	57.63 ± 2.16	64.30 ± 1.73
Cytorace 16 (C16)	63.06 ± 1.32	69.53 ± 2.02	56.80 ± 1.26	64.43 ± 1.72
Analysis of variance (ANOVA)	F = 11.521 d.f.= 17, 522 P < 0.001	F = 9.808 d.f.= 17, 522 P < 0.001	F = 15.659 d.f. = 17, 522 P < 0.001	F = 23.051 d.f.= 17, 522 P < 0.001

showed more fertility than others suggesting that the hybrid combinations of chromosome 3 and X will play a role in the production of more fertile individuals. *D. n. nasuta* has less hatching success than *D. n. albomicans*. Most of the Cytoraces except Cytorace 13 have tendency to increase the hatching success like *D. n. albomicans*.

The quantitative aspect of life span is well categorized in *Drosophila* [42]. In the present study, females live longer than males in both virgins and mated flies. One of the very interesting observations is that most of the Cytoraces, which are recently evolved, have achieved greater life span than parental races, *D. n. nasuta* and *D. n. albomicans* suggesting that the hybridization has enhanced the longevity of the newly evolved Cytoraces

By considering all the fitness components, one can bring out that Cytorace 8 of the third phase is with maximum fitness and lives longer than all the other races. While *D. n. nasuta*, an ancestral to all these races experienced reduced ovariole number and lives shorter than all the other races. In contrast to this, only Cytorace 12 and Cytorace 13, evolved in third phase have the lowest fecundity and fertility. Recently, Buck et al. [43] have reported that long-lived strains of *Drosophila* with reduced fitness and extension of longevity involves costs as well as benefits, which is again contrasting to the present investigations, wherein most of the Cytoraces are smaller in size and live longer with better fitness than their parents.

Conclusions

Formation of new karyotypic combinations (Cytoraces) with different chromosome constitutions and decreased body size with better fitness is theoretically difficult, because it often requires simultaneous changes at multiple traits. One of the possible mechanisms to overcome this difficulty might be hybrid recombination which generates novel combinations of these genetic variations, Cytoraces. Therefore, the Cytoraces are the resulting set of segregants, which contain an admixture of the two parental genotypes as a result of chromosome recombination. Since the parental genotypes are quite different, segregant genotypes exhibit transgressive variation in Cytorace chromosomes, body size and fitness phenotypes that are much more extreme than those of the parents from which they arose. Although some of the Cytoraces are having same chromosome number, they do not exhibit similarities in their body size indicating that these are the rapidly evolving products of interracial hybridization. During subsequent generations parents donate contributing alleles from different genes to these hybrids in the evolution of these Cytoraces. Thus, the rapid divergence recorded in the chromosomes, karyotypes, body size and fitness traits of Cytoraces exhibit the early event of recombinational raciation / speciation in the evolution of the Cytoraces under laboratory conditions. This is a unique observation in animal system, which illustrates the power of evolution after the event of hybridization under laboratory condition. Formation of so many new and isolated karyotypic races from the same two parental species suggests that similar hybridization events might contribute to karyo-

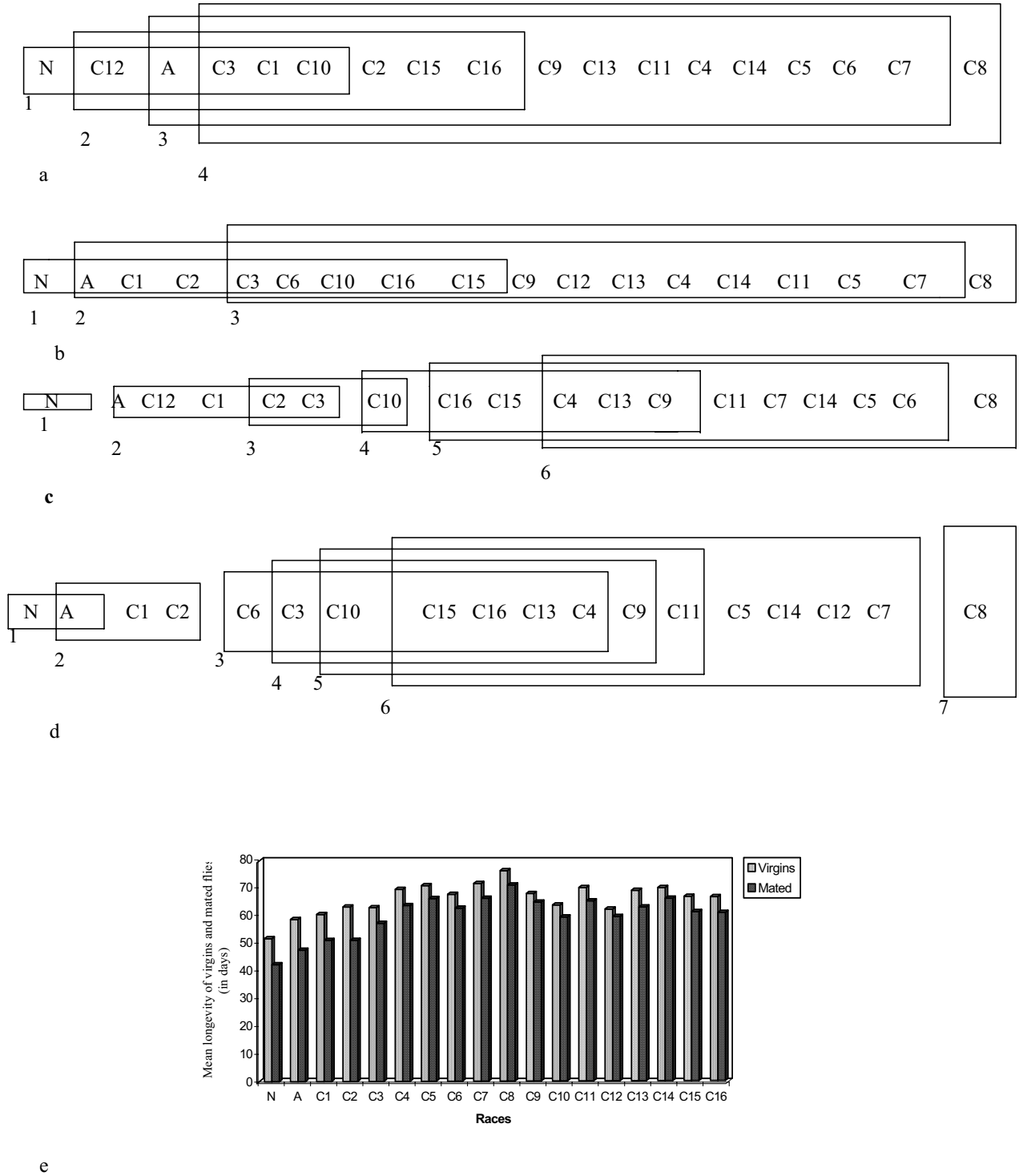


Figure 6
Based on the DMR test, 4 and 3 clusters are constructed in thehierarchical form from the lowest to the highest longevity for unmated male flies (Fig. 6a) and virgin female flies (Fig. 6b.) respectively. Similarly, 6 and 7 clusters are constructed for mated male flies (Fig. 6c) and mated female flies (Fig. 6d) respectively. The members belonging to each cluster have insignificant differences. In mated males (Fig. 6c), cluster 1(N) is formed as independent cluster, while in mated females (Fig. 6d), cluster 7 (C8) is formed as independent cluster. Virgin flies of the Cytoraces lived longer than mated Cytoraces and parental races (Fig. 6e).

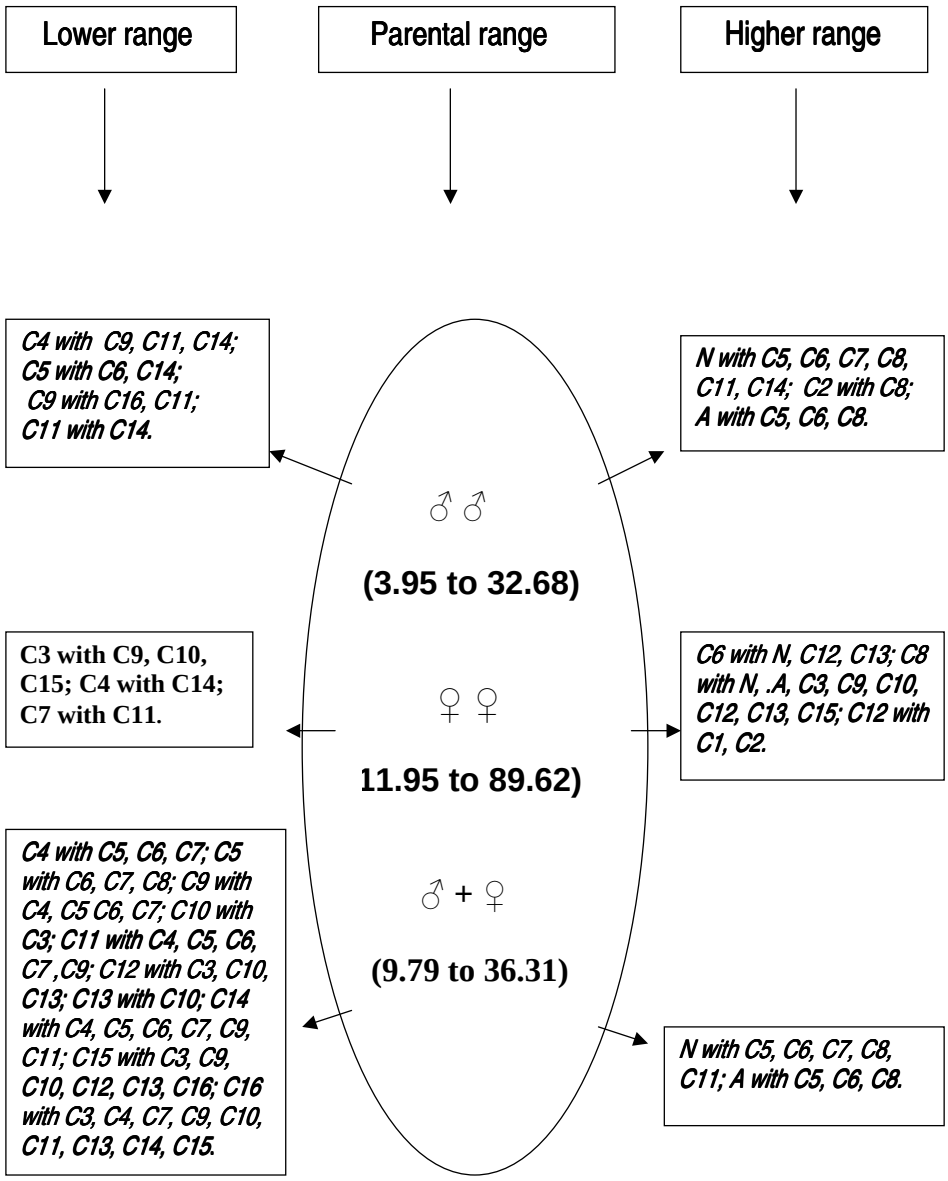


Figure 7
Evidence for recombination and transgressive segregation of quantitative traits analysed in 18 members of the *nasuta-albomicans* complex of *Drosophila*. Correlation of Euclidean distance dissimilarity (Table 8) is computed to the traits assessed separately in males (7 traits), females (8 traits) and both males and females together (5 traits). Based on these values, the members are classified into three ranges, namely parental range, higher range and lower range. Many of these newly evolved Cytoraces are more fit in the laboratory than their parental species and exhibit phenotypes that are extreme relative to either parent. The generation of extreme phenotypes through recombination in segregating hybrids is referred to as transgressive segregation.

typic evolution and speciation in nature. Therefore, the evolution of the *nasuta-albomicans* complex is very interesting which is a large scale evolutionary experimentation under laboratory condition and the members of this complex are evolving at different stages of divergence, offers a rare and unique opportunity to study the multidimensional process of raciation / speciation particularly recombination speciation.

Methods

Fly stocks

The following *Drosophila* stocks were employed in the present study:

a) *Drosophila nasuta nasuta* (Coorg, India),

b) *Drosophila nasuta albomicans* (Okinawa strain, Texas collection, USA, 3045.11),

c) Cytorace 1 and Cytorace 2 [16],

d) Cytorace 3 and Cytorace 4 [19] and

e) Cytorace 5 to Cytorace 16 [17].

The karyotypic compositions of the newly evolved 16 Cytoraces were reported elsewhere (Table 1) [17,20]. In the evolution of each of these Cytoraces, the starting population size was around 10 pairs of flies. In every generation, flies from five replicate cultures were mixed and distributed to 5 new culture bottles. At the time of the present experiment, Cytorace 1 and Cytorace 2; Cytorace 3 and Cytorace 4; and Cytorace 5 to Cytorace 16 are passing through 350, 200 and 100 generations respectively. All the above stocks were cultured in wheat cream agar media in an uncrowded culture conditions at 22 ± 1°C and were used for the following experiments.

Assessment of body size

The body size in all eighteen members of the *nasuta-albomicans* complex of *Drosophila* has been assessed by using five different body size related traits such as, wing length, wing width, genitalia length, genitalia width and body weight.

a) *Wing length and 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 ocular micrometer at 4X magnification in units of 10 μm under a microscope [6].

b) *Genitalia length and width*: To measure these, male genitalia of 30 flies were dissected out following the method of Emerald and Roy [44] with little modifications. The genitalia were mounted on the cavity slide using creosote and covered with the cover glass. These preparations were observed under microscope with an ocular micrometer and measured the length from the mid of the genitalia arch to the tip of toe vertically, and width from the mid of the left toe to the mid of the right toe horizontally at 10X magnification in units of 0.06 μm.

c) *Body weight*: To measure the body weight, two days old fifty virgin female and male flies were etherized individually and total fresh body weight was weighed using a fine balance [45].

Life history traits

Using five different traits namely, fecundity, ovariole numbers, fertility, longevity of virgin flies and longevity of mated flies have assessed the life history traits in all eighteen members of the *nasuta-albomicans* complex of *Drosophila*.

a) Lifetime Fecundity assays: For the assessment of this, the method of Buck et al. [46] was used with slight modifications. Thirty pairs of virgin females and males were isolated and sexed them separately for two days and then pair mating was made. After 2 days, they were transferred to fresh food media vials supplemented with yeast grains. Likewise once in two days, each replicate was transferred successively to the next set of vials. Immediately after each transfer, the vials were checked for the number of eggs lay by each pair and were counted under stereomicroscope till the egg laying is stopped. Thus, the mean number of eggs laid by these pair mated females was recorded.

b) Counting of Ovariole numbers: Thirty virgin female flies were collected from uncrowded culture conditions and aged for five days. Then each fly was anaesthetized and dissected the left ovarioles in saline. The bundles of ovarioles were separated by a fine needle and counted under stereomicroscope [40].

c) Lifetime Fertility assays: 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 lifetime fertility.

d) Longevity of virgin and mated flies: Longevity was assessed using the modified protocol of Luckinbill and Clare [47]. For virgin flies, single virgin female fly and male fly were transferred to fresh vials separately supplemented with yeast grains. For mated flies, virgin females

and males were aged for 5 days separately from the day of emergence. On the sixth day, a male fly and a female fly were placed in fresh media vials seeded with yeast grains and were allowed to mate for two days. Once in two days, each fly was transferred to fresh vials. Likewise a series of changes were made once in every two days till the fly was alive. For each experiment 30 replicates were assessed and each fly was observed every day from the day of emergence to record the life span.

Statistical Analysis

The analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and Euclidean absolute distance dissimilarity correlation test were used to record the racial divergence among them. To compile and calculate, the program used was statistical presentation system software 10.0 for MS Windows.

Author's contributions

BPH carried out experimental studies and drafted the manuscript. NBR conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

Additional material

Additional file 1

Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-3-20-S1.doc]

Acknowledgements

We are grateful to H.A. Ranganath, Chairman, *Drosophila* Stock Centre, Department of Studies in Zoology, for his encouragement. B.P.H. is grateful to University of Mysore for awarding research fellowship. We thank Dr Lancy D' Souza for statistical analysis. The manuscript was much improved by comments and suggestions from two anonymous reviewers and editor. This paper is dedicated to our mentor, Prof. H. A. Ranganath on the occasion of his 55th birthday.

References

1. Dobzhansky Th., Ayala FJ, Stebbins GL and Valentine JW: **Evolution.** *W.H. Freeman and Co. Surjeet Publications, New Delhi* 1976.
2. Templeton AR: **Mechanisms of speciation – A population genetic approach.** *Ann Rev Ecol Syst* 1981, **12:**23-48.
3. Zouros E: **A model for the evolution of asymmetrical male hybrid sterility and its implications for speciation.** *Evolution* 1986, **40:**1171-1184.
4. Chang H, Wang D and Ayala FJ: **On the origin of incipient reproductive isolation. The case of *Drosophila albomicans* and *D. nasuta*.** *J Mol Evo* 1989, **28:**337-348.
5. Lamb CG: **Diptera: Heteroneuridae, Ortalidae, Trypetidae, Sepsidae, Micropezidae, Drosophilidae, Geomysidae, Milichidae of Scyehelles.** *Teans Linn Soc London* 1914, **16:**307-372.
6. Kitagawa O, Wakahama K, Fuyama Y, Shimada Y, Takanashi E, Hatsumi M, Uwabo M and Mita Y: **Genetic studies of the *Drosophila nasuta* subgroup, with notes on distribution and morphology.** *Japanese J Genet* 1982, **57:**113-141.
7. Ranganath HA and Ramachandra NB: **Chromosomal basis of raciation in *Drosophila* : A study with *Drosophila nasuta* and *D. albomicans*.** *Proc Ind Acad Sci (Anim Sci)* 1987, **96:**451-459.
8. Ranganath HA: **Evolutionary biology of *Drosophila nasuta* and *Drosophila albomicans*.** *Proc Indian Natn Sci Acad (PINSA* 2002, **3:**255-272.
9. Duda O: **Die orientalischen and australischen Drosophiliden – Arten des ungarischen National Museums Zu Budapest.** *Ann Mus Nat Hung* 1914, **20:**24-59.
10. Wilson FD, Wheeler MR, Harget M and Kambysellis M: **Cytogenetic relations in the *Drosophila nasuta* subgroup of species.** *Univ Texas Publ* 1969, **6918:**207-254.
11. Nirmala SS: **Cytogenetic studies on the *Drosophilids* of Mysore State.** *Ph.D Thesis University of Mysore, Mysore*; 1973.
12. Ranganath HA, Rajasekarasetty MR and Krishnamurthy NB: **Evolutionary status of Indian *Drosophila nasuta*.** *Indian J Hered* 1974, **6:**19-25.
13. Ranganath HA and Hagele k: **The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. n. albomicans*. I. Distribution and differentiation of heterochromatin.** *Chromosoma* 1982, **85:**83-92.
14. Hagele K and Ranganath HA: **The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. n. albomicana*. II. Differences in their microchromosomes.** *Chromosoma* 1982, **85:**215-220.
15. Ramachandra NB: **Contributions to population cytogenetics of *Drosophila*: Studies on interracial hybridization and B-chromosomes.** *Ph.D Thesis University of Mysore, Mysore*; 1987.
16. Ramachandra NB, Ranganath HA and The chromosomes of two races: ***Drosophila nasuta nasuta* and *Drosophila nasuta albomicana*: IV. Hybridization karyotype repatterning.** *Chromosoma* 1986, **93:**243-248.
17. Ramachandra NB and Ranganath HA: **Evolution of *nasuta-albomicans* complex of *Drosophila*.** *Curr Sci* 1986, **71:**515-517.
18. Ranganath HA and Hagele K: **Karyotypic orhthoselection in *Drosophila*.** *Naturwissenschaften* 1981, **68:**527-528.
19. Ramachandra NB and Ranganath HA: **The chromosomes of two *Drosophila* races: *Drosophila nasuta nasuta* and *Drosophila nasuta albomicana*: V. Introgression and the evolution of new karyotypes.** *Z Zool Syst Evolut-forsh (Germany)* 1990, **28:**62-68.
20. Tanuja MT, Ramachandra NB and Ranganath HA: **Hybridization and introgression of the genomes of *Drosophila nasuta* and *Drosophila albomicans* Evolution of new karyotypes.** *Genome* 2003, **46:**605-611.
21. Tanuja MT, Ramachandra NB and Ranganath HA: **Creation of a hybrid zone in *Drosophila* with 'allo-sympatric' races.** *Cur Sci* 1998, **75:**1116-1117.
22. Ramachandra NB and Ranganath HA: **Pattern of sexual isolation between parental races (*Drosophila nasuta nasuta* and *D.n. albomicans*) and the newly evolved races (Cytoraces 1 and 2).** *Indian J Exp Biol* 1994, **32:**98-102.
23. Harini BP and Ramachandra NB: **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* 1999, **76(7):**1017-1019.
24. Harini BP and Ramachandra NB: **Does evolution reduces the body size? A study in the four members of newly evolved *nasuta-albomicans* complex of *Drosophila*.** *Genetica* 1999, **105:**1-6.
25. Harini BP and Ramachandra NB: **Racial divergence in body weight : A study in the four members of newly evolved *nasuta-albomicans* complex of *Drosophila*.** *Curr Sci* 2000, **78(3):**342-344.
26. Harini BP and Ramachandra NB: **Racial divergence in abdominal bristles among the parental races and the newly evolved Cytoraces of *nasuta-albomicans* complex of *Drosophila*.** *Indian J of Expt Bio* 2000, **38(12):**1263-1266.
27. Ungerer MC, Baird SJE, Pan J and Rieseberg LH: **Rapid hybrid speciation in wild sunflowers.** *Proc Natl Acad Sci USA* 1998, **95(20):**11757-11762.
28. Grant VA: **Plant Speciation.** *New York: Columbia University Press* 1981.
29. McCarthy EM, Asmussen MA and Anderson WW: **A theoretical assessment of recombinational speciation.** *Heredity* 1995, **74:**502-509.

30. Buerkle CA, Morris RJ, Asmussen MA and Rieseberg LH: **The likelihood of homoploid hybrid speciation.** *Heredity* 2000, **84:**441-451.
31. Rieseberg LH, Archer MA and Wayne RK: **Transgressive segregation, adaptation and speciation.** *Heredity* 1999, **83:**363-372.
32. Rieseberg LH, Van Fossen C and Desrochers A: **Hybrid speciation accompanied by genomic reorganization in wild sunflowers.** *Nature* 1995, **375:**313-316.
33. Waugh O'Neill RJ, O'Neill MJ and Graves JAM: **Undermethylation associated with retroelement activation and chromosome remodeling in an interspecific mammalian hybrid.** *Nature* 1998, **393:**68-72.
34. Imasheva AG, Bosenko DV and Bubli OA: **Variation in morphological traits of *Drosophila melanogaster* (fruit fly) under nutritional stress.** *Heredity* 1999, **82:**187-192.
35. Roff DA: **Predicting body size with life history models.** *Bio Sci* 1986, **36:**316-323.
36. Ruiz AM, Santos A, Barbadilla JE, Quezada-Diaz E, Hassan E and Fontdevila A: **The evolutionary history of *Drosophila buzzatii* XVIII. Geneitc variation for body size in a natural population.** *Genetics* 1991, **128:**739-750.
37. Ayala FJ: **Competition, coexistence and evolution.** In *Essays in evolution and genetics in honour of Th. Dobzhansky* Edited by: *Hech MK, Steere WC. Appleton-Century-Crafts, New York*; 1970:121-158.
38. Yamazaki T: **Measurement of fitness and its component of fitness and its components in sex laboratory strains of *Drosophila melanogaster*.** *Genetics* 1984, **108:**201-211.
39. Ramachandra NB and Ranganath HA: **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* 1988, **30:**58-62.
40. Stammer WT, Polak M, Wolf LL and Barker JS: **Reproductive characteristics of the flower-breeding *Drosophila hibisci* Bock (*Drosophilidae*) in eastern Australia:Within – population genetic determinants of ovariole number.** *Genetika* 2001, **37:**66-72.
41. Chakir M, David JR, Pla E and Capy P: **Genetic basis of some morphological differences between temperate and equatorial populations of *Drosophila melanogaster* .** *Experientia* 1995, **51:**744-748.
42. Wayne ML and Mackay TFC: **Quantitative genetics of ovariole number in *Drosophila melanogaster*. II, Mutational variation and genotype-environment interaction.** *Genetics* 1998, **148:**201-210.
43. Curtsinger JW, Fukui HH, Khazaeli A, Kirschner A, Pletcher S, Promislow DEL and Tartar M: **Genetic variation and aging.** *Ann Rev Genet* 1995, **29:**553-575.
44. Buck S, Vettraino J, Force AG and Arking R: **Extended longevity in *Drosophila* is consistently associated with a decrease in developmental viability.** *J Gerontol A Biol Sci Med Sci* 2000, **55:**B292-301.
45. Emerald BS and Roy JK: **Organising activities of engrailed, hedgehog, wingless and decapentaplegic in the genital discs of *Drosophila melanogaster* .** *Dev Genes Evol* 1998, **208:**504-516.
46. Watada M, Ohba S and Tobari YN: **Genetic differentiation in Japanese populations of *Drosophila simulans* and *D. melanogaster*. II Morphological variation.** *Jap J Genet* 1986, **61:**469-480.
47. Buck S, Wells RA, Dudas SP, Baker GT and Arking R: **Chromosomal localization and regulation of the longevity determinant genes in a selected strain of *D. melanogaster*.** *Heredity* 1993, **71:**11-22.
48. Luckinbill LS and Clare M: **Selection for life span in *Drosophila melanogaster* 1985.** *Heredity* 1985, **55:**9-18.