

Newly evolved cytoraces of *nasuta–albomicans* complex of *Drosophila* live better than their parents as revealed by life-history trait analysis at three different temperatures

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The long-range interracial hybridization experiments between a pair of cross fertile races, *Drosophila nasuta nasuta* ($2n = 8$) and *Drosophila nasuta albomicans* ($2n = 6$) have resulted in the evolution of two new karyotypic strains called cytoraces 1 and 2, which harbour chromosomes from both their parents and differ in their karyotypic composition, mating preference and morphometric traits. In the present study, the effect of temperature on certain life-history traits such as lifetime fecundity, lifetime fertility, ovariole number and longevity of virgin and mated males and females was tested in the parents and two cytoraces. The results revealed that in most of the assessments, the newly evolved cytoraces showed maximum fitness, suggesting that the newly evolved cytoraces survive and live better than their parental races.

Keywords: Cytoraces, *Drosophila nasuta–albomicans* complex, fitness, life-history traits, temperature regimes.

THE interesting features of *nasuta* subgroup of *Drosophila* include little morphological differentiation despite their distribution over a wide territory, the ability to intercross in the laboratory, often producing fertile offspring and substantial chromosomal evolution make this subgroup as one of the potent systems to study the genetics of speciation in *Drosophila*¹. *D. nasuta nasuta* ($2n = 8$) and *D. n. albomicans* ($2n = 6$) are a pair of sibling allopatric chromosomal races of the *nasuta* subgroup of *Drosophila* which have been extensively studied^{2–5}. Interracial hybridization between these followed by the maintenance of hybrid populations for over 20 generations has resulted in the emergence of two new karyotypic strains called cytoraces 1 and 2. Cytorace 1 is the product of interracial hybridization between the males of *D. n. nasuta* and females of *D. n. albomicans* with $2n = 7$ in males ($2^n2^aY^n3^nX3^a4^n4^n$) and $2n = 6$ in females ($2^n2^aX3^a4^n4^n$). Cytorace 2 is the outcome of interracial hybridization between females of *D. n. nasuta* and males of *D.*

n. albomicans, where both males and females of cytorace 2 have $2n = 6$ ($2^n2^aX3^aX3^a/Y3^a4^n4^n$; superscript ‘*n*’ and ‘*a*’ represent the chromosomes of *D. n. nasuta* and *D. n. albomicans* respectively). Each of these cytoraces is the recombined genome of the parental races and has chromosomes of both the parents and differs in their karyotypic composition⁵.

These newly created cytoraces along with their parental races constitute a new assemblage, the *nasuta–albomicans* complex of *Drosophila*⁶. These cytoraces are considered as the members of the hybrid zone of *Drosophila* with ‘allo-sympatric’ populations under laboratory conditions⁷. Earlier studies on cytogenetic differentiation^{5,6}, mating preference⁸, body size and fitness^{9,10} have shown significant differences between parental races and cytoraces. Ramachandra and Ranganath¹¹ have also shown the extent of ecogenetic divergence and competitive ability among these races as to their ability to exploit different types of food resources. Here we report their performance in certain life-history traits exposed to different temperatures, which indicates that the newly evolved cytoraces live better than the parental races.

Materials and methods

Experimental strains

(a) *Drosophila nasuta nasuta* (Coorg, India) (N), (b) *Drosophila nasuta albomicans* (Okinawa strain, Texas collection, USA, 3045.11) (A), (c) Cytorace 1 (C1)⁵, (d) Cytorace 2 (C2)⁵.

These cytoraces are evolved under laboratory conditions by interracial hybridization between *D. n. nasuta* and *D. n. albomicans*⁵. It took approximately 20–40 generations for each cytorace to stabilize its karyotype and to breed true. Each of these cytoraces was the product of hybrid recombination and selection. At the time of the present experiment, cytoraces 1 and 2 were passing through 350 generations. Stocks were cultured at three different temperature regimes, i.e. standard culture temperature ($22 \pm 1^\circ\text{C}$),

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constant lower temperature ($18 \pm 0.5^\circ\text{C}$) and fluctuating room temperature (ranging from 18 to 28°C) for two generations in an uncrowded culture medium, in order to get the stabilized stocks with particular temperature. Further, the offspring from these stocks was used to assess the following fitness parameters.

Lifetime fecundity assay

For the assessment of lifetime fecundity, the method of Buck *et al.*¹² was followed, with slight modification. Thirty virgin females and males were isolated and sexed separately for two days and an unmated male and a virgin female were mated in $4'' \times 1''$ culture vials. Thirty such replicates were maintained at three different experimental temperatures for all the four above-mentioned strains. After two days, flies from all the replicates were transferred to fresh food vials, supplemented with yeast grains. Likewise, once in two days, flies from each replicate were transferred successively to the next set of vials. Immediately after each transfer, the vials were checked for the number of eggs laid and were counted under a stereomicroscope till egg-laying stopped. The mean number of eggs laid by 30 females at three different experimental temperatures was recorded.

Counting of ovariole number

Thirty virgin female flies of the four strains under study reared at three different experimental temperature regimes were collected from uncrowded culture conditions and were aged for five days. These flies were anaesthetized and dissected for left ovary in saline and the bundles of ovarioles were separated by a fine needle and counted under a stereomicroscope¹³. The mean number of ovarioles of all the four strains at the three different temperatures was calculated.

Lifetime fertility assay

Fertility of a mating is the proportion of eggs that produce offspring. The same set of vials (30 replicates) that were used to assess lifetime fecundity at three different experimental temperatures was used for this experiment after counting the eggs laid. The number of flies that emerged from 30 replicates at three different experimental temperatures was recorded and mean lifetime fertility was calculated.

Longevity assay

Virgin flies: Longevity was assessed using the modified protocol of Luckinbill and Clare¹⁴. Thirty virgin females and unmated males were isolated from all the four strains

reared at three different temperature regimes and aged for two days separately. Virgin female and male flies of all the four strains in 30 replicates for all the three temperatures were maintained separately in standard wheat cream agar medium. Once in two days the flies were transferred to fresh vial seeded with yeast grains, likewise a series of changes were made till the flies were alive. Each fly was observed everyday from the day of emergence to record the lifespan at three different temperatures selected for this study.

Mated flies: Virgin females and males of *D. n. nasuta*, *D. n. albomicans*, cytoraces 1 and 2 were aged for five days at three different temperatures, separately from the day of emergence. On the sixth day, a male and a female fly were placed in fresh food vials seeded with yeast grains and were allowed to mate. Once in two days, each pair was transferred to fresh vials. Likewise, a series of changes were made once in two days till the flies were alive. For each experiment, 30 replicates were assessed and each fly was observed from the day of emergence to record the lifespan.

Statistical analysis

One-way ANOVA was performed by considering strain as a factor and fitness traits, namely fecundity, fertility, ovariole number and longevity as dependent variables. Duncan's multiple range test (DMRT) was performed to ascertain the differences. All analyses were performed using the statistical presentation system software package (SPSS, 2001) 10.0 for MS Windows.

Results

Lifetime fecundity

Table 1 presents mean lifetime fecundity in four races assessed at three different temperatures, indicating that *D. n. nasuta* and cytorace 2 had the lowest and the highest lifetime fecundity respectively. Although ANOVA revealed significant differences among the four races at 22°C , the difference between the parental races and cytorace 1 was not significant by DMRT. At 18°C temperature, except for the differences between *D. n. nasuta* and *D. n. albomicans*, all the other comparisons were significant. Analysis revealed that at fluctuating room temperature, flies of *D. n. nasuta* had significantly lower fecundity than cytoraces 1 and 2. In addition, *D. n. nasuta* showed differences in the mean lifetime fecundity between 18°C and 22°C and also 18°C with fluctuating room temperature regimes, while the other three races showed significant differences among all the comparisons at all the three temperature regimes (Table 1). *D. n. nasuta* and *D. n. albomicans* had increased fecundity at 18°C , while cytoraces 1 and 2 had increased fecundity at 22°C . However,

Table 1. Mean lifetime fecundity in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes (values are mean \pm SE of 30 replicates) along with statistical analysis

Race	Mean lifetime fecundity			Analysis of variance*	Duncan's multiple range test**
	Standard culture temperature ($22 \pm 1^\circ\text{C}$)	Constant lower temperature ($18 \pm 0.5^\circ\text{C}$)	Fluctuating room temperature ($18\text{--}28^\circ\text{C}$)		
<i>Drosophila nasuta nasuta</i> (N)	184.90 ± 10.46	194.65 ± 7.69	181.80 ± 9.69	$F = 4.910$ d.f. = 2, 87 $P < 0.01$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	199.75 ± 10.63	208.65 ± 12.38	194.50 ± 10.29	$F = 6.196$ d.f. = 2, 87 $P < 0.02$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	255.95 ± 11.91	219.10 ± 12.75	200.60 ± 12.47	$F = 3.874$ d.f. = 2, 87 $P < 0.01$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	256.80 ± 5.59	243.45 ± 9.35	219.90 ± 8.76	$F = 4.483$ d.f. = 2, 87 $P < 0.01$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 3.051$ d.f. = 3, 116 $P < 0.034$	$F = 3.665$ d.f. = 3, 116 $P < 0.016$	$F = 2.329$ d.f. = 3, 116 $P > 0.082$		
Duncan's multiple range test**	N/C2, A/C2	N/C1, N/C2, A/C1, A/C2	N/C1, N/C2		

*Denotes race-wise comparison. **Denotes temperature-wise comparison.

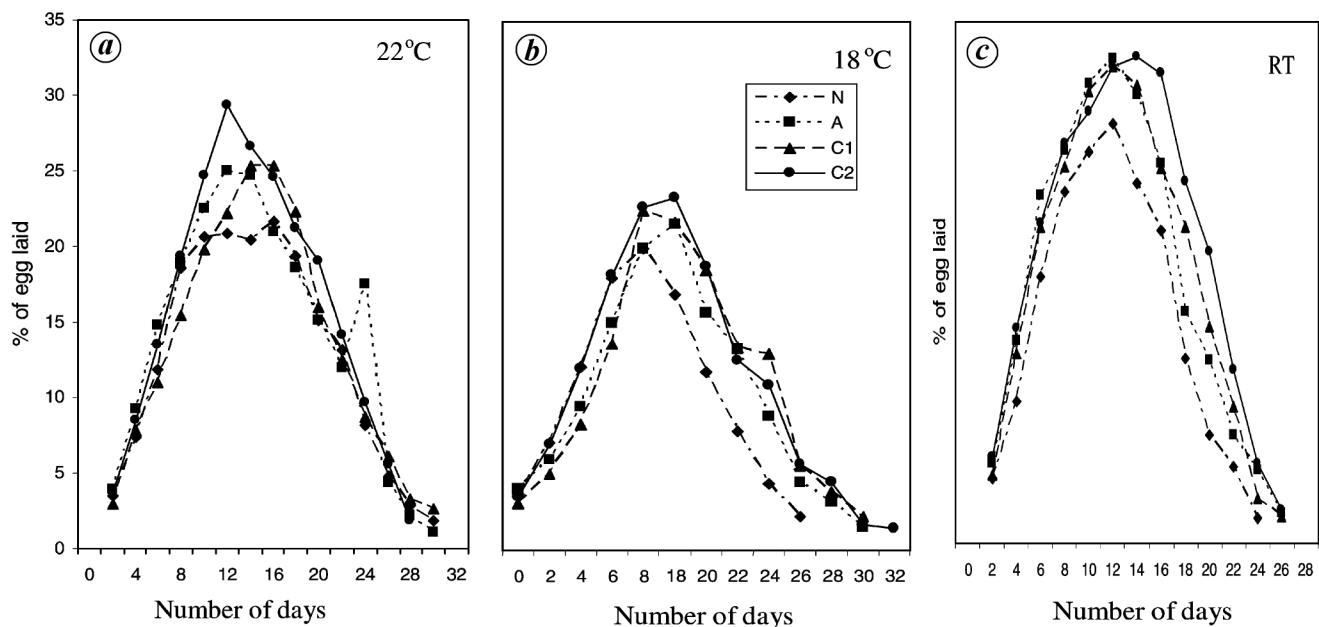


Figure 1 a–c. Pattern of egg-laying capacity in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperatures (N, *D. n. nasuta*; A, *D. n. albomicans*; C1, Cytorace 1; C2, Cytorace 2).

all the four races showed decreased fecundity at fluctuating room temperature.

Figure 1 a–c illustrates the egg-laying capacity in the four races at three different temperatures. Data revealed that fecundity was observed up to 30–34 days in all the four races at 22°C and 18°C regimes, while at fluctuating room temperature, egg-laying was seen up to 24–26 days. *D. n. nasuta* laid the maximum number of eggs on the

16th day at 22°C and 18°C ; and on the 12th day at fluctuating room temperatures. *D. n. albomicans* and cytorace 1 laid the maximum number of eggs at 22°C , 18°C and fluctuating room temperatures on the 14th, 16th and 12th day respectively. Cytorace 2 laid the maximum number of eggs on the 12th day at 22°C , and on the 14th day at 18°C , and also at fluctuating room temperature. This indicates that these four races have prolonged egg-laying capacity

Table 2. Mean ovariole number in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes (values are mean \pm SE of 30 replicates) along with statistical analysis

Race	Mean ovariole number			Analysis of variance*	Duncan's multiple range test**
	Standard culture temperature ($22 \pm 1^\circ\text{C}$)	Constant lower temperature ($18 \pm 0.5^\circ\text{C}$)	Fluctuating room temperature ($18\text{--}28^\circ\text{C}$)		
<i>D. n. nasuta</i> (N)	14.00 ± 0.35	22.33 ± 0.35	13.50 ± 0.39	$F = 7.001$ d.f. = 2, 87 $P < 0.01$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$,
<i>D. n. albomicans</i> (A)	16.63 ± 0.72	24.16 ± 0.54	14.76 ± 0.61	$F = 5.199$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$,
Cytorace 1 (C1)	16.73 ± 0.76	24.23 ± 0.60	15.03 ± 0.55	$F = 4.869$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$,
Cytorace 2 (C2)	17.63 ± 0.80	25.60 ± 0.55	16.43 ± 0.60	$F = 4.963$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$,
Analysis of variance*	$F = 5.009$ d.f. = 3, 116 $P < 0.003$	$F = 6.574$ d.f. = 3, 116 $P < 0.001$	$F = 4.783$ d.f. = 3, 116 $P < 0.004$		
Duncan's multiple range test**	N/A, N/C1, N/C2	N/A, N/C1, N/C2	N/C2, A/C2		

*Denotes race-wise comparison. **Denotes temperature-wise comparison.

at constant temperature, i.e. 22°C and 18°C , but it is not so at fluctuating room temperature.

Ovariole number

Table 2 presents the mean ovariole number in the four races of the *nasuta–albomicans* complex of *Drosophila* at three different temperatures. At 22°C , 18°C and fluctuating room temperatures, *D. n. nasuta* and cytorace 2 had the lowest and the highest number of ovarioles respectively. ANOVA revealed significant differences at the three different temperatures. Based on DMRT, *D. n. nasuta* showed significant differences at 18°C and 22°C from other races, and cytorace 2 showed significant differences from *D. n. nasuta* and *D. n. albomicans* at fluctuating room temperature. Comparison between cytoraces was nonsignificant. In inter-temperature comparisons, DMRT showed that except for the comparison between 22°C and room temperature in *D. n. nasuta*, all the other three races differed significantly in the other temperature regimes.

Lifetime fertility

Mean lifetime fertility in the four races of the *nasuta–albomicans* complex of *Drosophila* at three different temperatures (Table 3) revealed that cytorace 2 and *D. n. nasuta* had the highest and lowest lifetime fertility respectively. DMRT revealed that at 22°C , *D. n. nasuta* had decreased fertility than the other three races, whereas in both the experimental temperatures, except *D. n. nasuta* and cytorace 2, all the other comparisons were nonsig-

nificant. In race-wise comparisons at three different temperatures (Table 3), *D. n. nasuta* showed decreased fertility at 22°C , while the other three races showed decreased fertility at 18°C . In DMRT, except *D. n. albomicans* and cytorace 1, the other two races showed significant differences among the three different temperatures. In the case of *D. n. albomicans* and cytorace 1, except for the comparisons between 22°C and fluctuating room temperature, all other comparisons showed significant differences.

Figure 2 a–c illustrates the pattern of lifetime fertility in the four races at three different temperature regimes. Data revealed that at 22°C , maximum number of flies of *D. n. nasuta*, *D. n. albomicans* and cytorace 1 emerged on the 14th day, while cytorace 2 had maximum number of flies on the 12th day. At 18°C , maximum number of flies of all the four races emerged on the 14th day. At fluctuating room temperature, *D. n. nasuta* and *D. n. albomicans* had maximum number of progeny on the 10th day, while cytoraces 1 and 2 had maximum progeny on the 12th and 14th day respectively.

Longevity of virgin flies

Females showed increased mean longevity values than males in all the four races (Table 4). At 22°C and 18°C regimes, mean lifespan of the males and females of cytorace 2 and *D. n. nasuta* had the highest and lowest values respectively. Based on DMRT at 22°C , both males and females of *D. n. nasuta* had decreased longevity than the other races. In addition, males of *D. n. albomicans* also differed significantly from those of cytorace 2 in longevity. Mean longevity of virgins in the four races at

Table 3. Mean lifetime fertility in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes (values are mean \pm SE of 30 replicates) along with statistical analysis

Race	Mean lifetime fertility			Analysis of variance*	Duncan's multiple range test**
	Standard culture temperature ($22 \pm 1^\circ\text{C}$)	Constant lower temperature ($18 \pm 0.5^\circ\text{C}$)	Fluctuating room temperature ($18\text{--}28^\circ\text{C}$)		
<i>D. n. nasuta</i> (N)	113.83 ± 4.36	126.40 ± 8.66	145.90 ± 7.96	$F = 6.100$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	151.30 ± 6.45	136.60 ± 9.25	154.90 ± 9.25	$F = 9.288$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	164.16 ± 6.78	137.60 ± 9.22	159.70 ± 9.25	$F = 5.371$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	166.50 ± 7.52	153.90 ± 7.45	171.50 ± 8.12	$F = 4.409$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 14.519$ d.f. = 3, 116 $P < 0.001$	$F = 1.717$ d.f. = 3, 116 $P > 0.171$	$F = 1.529$ d.f. = 3, 116 $P > 0.214$		
Duncan's multiple range test**	N/A, N/C1, N/C2	N/C2	N/C2		

*Denotes race-wise comparison. **Denotes temperature-wise comparison.

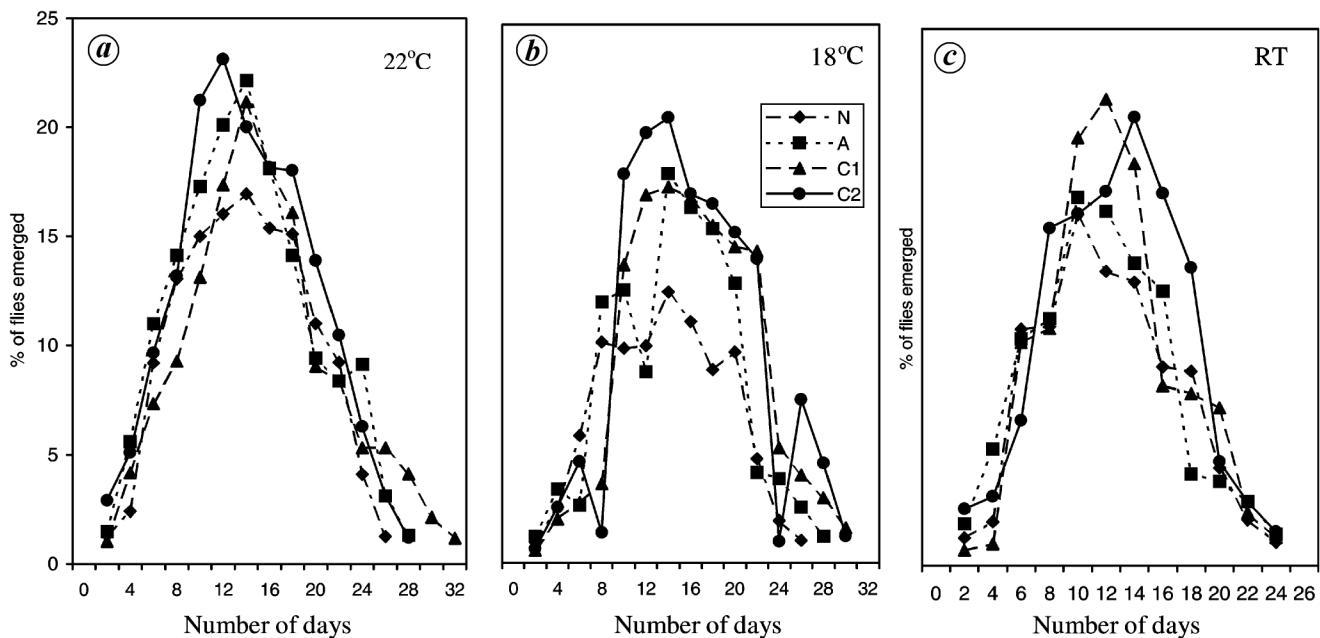


Figure 2a–c. Pattern of lifetime fertility in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperatures.

fluctuating room temperature revealed that the males of *D. n. nasuta* and cytorace 1 had minimum and maximum longevity respectively, while virgin females of all the four races did not show significant difference in longevity (Table 4).

Males and females of all the four races had the highest longevity at 18°C and lowest longevity at fluctuating room temperature. DMRT revealed significant differences in males and females of all the four races among all

the three temperature regimes, except between 22°C and fluctuating room temperature in males and females of *D. n. nasuta* and males of *D. n. albomicans*.

Longevity of mated flies

Mean longevity of mated males and females of the four races of the *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes is given in Table 5.

Table 4. Mean longevity (in days) of unmated males and virgin females in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes (values are mean \pm SE of 30 replicates) along with statistical analysis

Race	Standard culture temperature ($22 \pm 1^\circ\text{C}$)	Constant lower temperature ($18 \pm 0.5^\circ\text{C}$)	Fluctuating room temperature ($18\text{--}28^\circ\text{C}$)	Analysis of variance*	Duncan's multiple range test**
Mean longevity of unmated males					
<i>D. n. nasuta</i> (N)	48.33 ± 1.41	67.70 ± 1.42	46.36 ± 1.63	$F = 6.320$ d.f. = 2, 87 $P < 0.020$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	53.96 ± 1.49	73.86 ± 1.50	48.20 ± 1.42	$F = 7.553$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	57.20 ± 1.78	77.20 ± 1.78	49.80 ± 1.25	$F = 7.331$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	61.66 ± 1.76	81.00 ± 1.84	48.33 ± 1.83	$F = 6.240$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 11.968$ d.f. = 3, 116 $P < 0.001$	$F = 11.673$ d.f. = 3, 116 $P < 0.001$	$F = 0.821$ d.f. = 3, 116 $P > 0.485$		
Duncan's multiple range test**	N/A, N/C1, N/C2, A/C2	N/A, N/C1, N/C2, A/C2	N/C1		
Longevity of virgin females					
<i>D. n. nasuta</i> (N)	55.53 ± 1.36	75.20 ± 1.44	50.26 ± 1.87	$F = 4.266$ d.f. = 2, 87 $P < 0.011$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	63.76 ± 1.29	83.40 ± 1.24	49.80 ± 1.87	$F = 5.621$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	64.06 ± 1.53	83.06 ± 2.04	50.20 ± 1.64	$F = 4.472$ d.f. = 2, 87 $P < 0.020$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	64.86 ± 1.50	84.66 ± 1.58	51.60 ± 1.72	$F = 5.001$ d.f. = 2, 87 $P < 0.002$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 9.380$ d.f. = 3, 116 $P < 0.001$	$F = 7.211$ d.f. = 3, 116 $P < 0.001$	$F = 0.201$ d.f. = 3, 116 $P > 0.896$		
Duncan's multiple range test**	N/A, N/C1, N/C2	N/A, N/C1, N/C2	Non significant		

*Denotes race-wise comparison. **Denotes temperature-wise comparison.

Females of all the four races had increased longevity than males. DMRT revealed significant differences between males of *D. n. nasuta* and those of *D. n. albomicans*, cytoraces 1 and 2. Also differences were significant between *D. n. albomicans* and cytorace 2 at 22°C . Females of *D. n. nasuta* had significant differences with cytoraces 1 and 2. At 18°C , males and females of *D. n. nasuta* had significantly reduced longevity than the other three races. At fluctuating room temperature, the difference in longevity was significant between males of *D. n. nasuta* and cytorace 2, while the longevity of *D. n. nasuta* females had significant differences with the females of all the other three races.

Temperature-wise comparison revealed that the mean longevity increased at 18°C and decreased at fluctuating room temperature. DMRT indicated that the longevity of males and females of *D. n. nasuta* and *D. n. albomicans*

showed significant differences in all the comparisons, except between 22°C and fluctuating room temperature. Cytoraces 1 and 2 had significant differences among the three different temperatures.

Discussion

Andrewartha and Birch¹⁵ have recognized some important components of the environment of an animal. They are the presence of other animals, food and weather conditions. Environmental effects are themselves not heritable, but susceptibility to environmental effects is potentially heritable and thus provides a basis for evolution of environmental variance. Quantitative variations in different environments have been extensively studied in the last decade using *Drosophila*^{16–21}. Different studies in *Drosophila*

Table 5. Mean longevity (in days) of mated flies in four members of *nasuta–albomicans* complex of *Drosophila* at three different temperature regimes (values are mean \pm SE of 30 replicates) along with statistical analysis

Race	Standard culture temperature ($22 \pm 1^\circ\text{C}$)	Constant lower temperature ($18 \pm 0.5^\circ\text{C}$)	Fluctuating room temperature ($18\text{--}28^\circ\text{C}$)	Analysis of variance*	Duncan's multiple range test**
Mean longevity of mated males					
<i>D. n. nasuta</i> (N)	40.00 ± 1.24	60.76 ± 1.26	40.36 ± 1.48	$F = 4.214$ d.f. = 2, 87 $P < 0.034$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	45.76 ± 1.50	66.96 ± 1.30	42.13 ± 1.69	$F = 6.311$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	48.73 ± 2.20	66.93 ± 1.65	42.60 ± 1.66	$F = 4.440$ d.f. = 2, 87 $P < 0.002$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	50.70 ± 2.25	70.23 ± 2.83	45.86 ± 1.69	$F = 9.211$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 6.323$ d.f. = 3, 116 $P < 0.001$	$F = 5.238$ d.f. = 3, 116 $P < 0.002$	$F = 1.812$ d.f. = 3, 116 $P > 0.149$		
Duncan's multiple range test**	N/A, N/C1, N/C2, A/C2	N/A, N/C1, N/C2	N/C2		
Mean longevity of mated females					
<i>D. n. nasuta</i> (N)	44.93 ± 1.51	63.83 ± 1.54	41.86 ± 1.40	$F = 2.315$ d.f. = 2, 87 $P < 0.011$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
<i>D. n. albomicans</i> (A)	49.56 ± 2.13	68.23 ± 2.01	46.80 ± 1.65	$F = 5.243$ d.f. = 2, 87 $P < 0.001$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$
Cytorace 1 (C1)	51.06 ± 2.10	68.20 ± 1.85	46.36 ± 1.74	$F = 3.997$ d.f. = 2, 87 $P < 0.021$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Cytorace 2 (C2)	51.76 ± 2.44	71.76 ± 2.43	46.03 ± 1.51	$F = 7.331$ d.f. = 2, 87 $P < 0.002$	$18^\circ\text{C}/22^\circ\text{C}$, $18^\circ\text{C}/\text{RT}$, $22^\circ\text{C}/\text{RT}$
Analysis of variance*	$F = 2.192$ d.f. = 3, 116 $P > 0.093$	$F = 2.852$ d.f. = 3, 116 $P < 0.040$	$F = 2.082$ d.f. = 3, 116 $P < 0.006$		
Duncan's multiple range test**	N/C1, N/C2	N/A, N/C1, N/C2	N/A, N/C1, N/C2		

*Denotes race-wise comparison. **Denotes temperature-wise comparison.

phila using the effect of temperature at different levels have suggested that temperature plays an important role in the life-history of *Drosophila* and has a significant influence on fitness^{22–29}.

Fecundity is the major determining factor of female fitness^{29,30}, which is a composite measure of the consequences of a number of reproductive events in both sexes. The egg-laying capacity is one of the suitable parameters to compare the performance of different strains of *Drosophila*^{31,32}. In the present study, the newly evolved cytoraces 1 and 2 lay more eggs than the parental races (*D. n. nasuta* and *D. n. albomicans*), suggesting better fitness of the cytoraces. In addition, cytoraces 1 and 2, as well as parental races have maximum fecundity at 22°C and 18°C respectively, but all these four races produce less eggs at fluctuating room temperature. This indicates that the

parental races and the newly evolved cytoraces adapted differently at different temperatures.

Ovariole number is correlated with female reproductive success through a simple relationship between the number of ovarioles and the rate at which the female produces eggs. There are reports on the assessment of growth, temperature and reaction norms for various morphometrical traits, including ovariole numbers in equatorial and temperate populations of *Drosophila*^{28,33,34}. In the present study, the parental races have significantly decreased ovarioles than the cytoraces at all the three temperatures. Another interesting observation is that all the four races have maximum ovarioles at 18°C and less ovarioles at fluctuating room temperature.

Fertility as an important component of fitness measured in terms of productivity has been extensively studied

in different species of *Drosophila*³⁴. In the present study, cytoraces are more fertile than the parental races. In addition, all the four races have increased and decreased fertility at fluctuating room temperature and at 18°C respectively. This feature is in contrast to lifetime fecundity and ovariole number, wherein, lifetime fecundity and ovariole number are decreased at room temperature, but lifetime fertility is increased at room temperature. This indicates that fitness of these races varies with different temperatures, suggesting the trade-off between fecundity and ovarioles when compared with fertility in these four races.

For all the three traits, cytoraces have exhibited high performance than parental races indicating that evolution has favoured the newly evolved cytoraces to adapt better. Based on chromosomal, mitochondrial DNA and mating preference studies, *D. n. nasuta* is considered as the ancestor race and *D. n. albomicans* as the derived race^{1,8}. These races are the parents of the newly evolved cytoraces. Thus, one can surmise that fecundity, ovariole number and fertility are the important life-history traits to assess the direction of evolution in the *nasuta* subgroup of *immigrans* species group of *Drosophila*, as well as in the newly evolved *nasuta–albomicans* complex of *Drosophila*.

Quantitative aspects of lifespan and its correlates are well categorized in *Drosophila*^{35–37}. There are reports that females of *Drosophila* had significantly increased lifespan than males, with a few exceptions^{36,38}. Chippindale *et al.*³⁸ suggested that because of their distinctive roles in reproduction, females and males are selected towards different optimal phenotypes. The present observations revealed that the virgin females and males of all the four races have shown increased lifespan than that of the mated males and females, which is in agreement with the earlier reports³⁹. Cytoraces, which are recently derived have achieved greater lifespan than their ancestral races (*D. n. nasuta* and *D. n. albomicans*).

Variations of lifespan within natural populations are partly attributable to both genetic and environmental effects³⁷. It has been reported that at least two types of environmental stress factors, extreme temperature and poor nutrition, consistently increase the phenotypic plasticity⁴⁰. The flies of the four races under study have experienced increased longevity at lower temperature (18°C) and decreased longevity at fluctuating room temperature. This suggests that the newly evolved cytoraces live longer than the ancestral races with better fitness. Therefore, the effect of temperature is an important facet which determines longevity.

Buck *et al.*⁴¹ have reported that long-lived strains of *Drosophila* with reduced fitness and extension of longevity involve costs as well as benefits. Similarly, negative correlation is found between longevity and productivity in *D. ananassae*²⁵. In contrast, in the present investigation, the cytoraces in general are smaller in size and live longer with better fitness than their parents. These cyto-

races are unique products of interracing hybridization and evolution, and are evolved due to the recombinational speciation and transgressive segregation¹⁰. One can surmise that fitness variations reflect the action of evolutionary processes in the evolution of these newly evolved races. Therefore, we put forth that the newly evolved cytoraces live better and longer than their parents. This observation is yet another important evidence to quantify subtle evolutionary divergence among the members of the *nasuta–albomicans* complex of *Drosophila*.

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