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Correlation Between Mating Propensity and Productivity in Few Species of *Drosophila* Exposed to Light and Dark Cycle under Laboratory Environments

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Abstract: Survival and continuity of an organism has to be tuned with its internal as well as external environment neither of which is ever static. Consequently, the organism and its constituent cells incessantly adjust their physiological milieu to remain in harmony with the dynamic environment. The adjustments involve long- term evolutionary adaptations as well as short - term responses to sudden changes. The sudden changes in the environment are stressful to cells and since the nature of changes experienced by organs are enormously varied. An integrative perspective on molecular mechanisms of stress resistance requires understanding of these mechanisms not just *in vitro* or in the model organism in the research laboratory, but in the populations and species in natural communities and ecosystems. The present study aimed to address "does environmental stress reduce or enhance genetic variation?" in terms of fitness and the extent of adaptive correlation in few species of *Drosophila*. Interestingly, the observation reveals that either reared at any stress condition, the increase in copulation duration as resulted in increase productivity and vice-versa in all the four different species of the present study and exist a strong positive correlation between mating propensity (copulation duration) and productivity (fertility). Exposure to dark regime has created a stressful environment which has been noticed by the depletion of the reproductive success of the individuals in the different species of *Drosophila*.

Key words: *Drosophila* · Dark - light regimes · Courtship duration · Copulation duration · Fecundity and Fertility.

INTRODUCTION

Over the last few decades *Drosophila* has developed as a model for the study of adaptation to environmental stress responses and much progress has been achieved. All organisms are strongly affected by their surrounding environment and the environmental factors play an important part in shaping ecology and evolution of biological systems. Environmental stress is especially important at many levels of biological organization. In this context environmental stress is recognized as an environmental factor causing a change in a biological system, which is potentially injurious and which has some fitness consequences. To study and gain the further understanding of nature and consequences of environmental stress from an ecological and evolutionary perspective and to investigate the role of stress response mechanisms [1]. Environmental stress plays an important role in the maintenance of genetic variation [2] and in evolution [3].

Physiological adjustments of metabolism and membrane composition also respond to stress and can play a role of stress tolerance [4]. The physiological changes in turn affect life history and fitness traits such as fecundity, longevity and stress resistance. Many organisms live in variable environment, which pose substantial challenges to survival and reproduction. In response to environmental variation organisms must adopt, disperse to more favorable localities or face extinction. Understanding mechanism by which animals respond to environmental variation has taken on new urgency, due to increasing effects of change on natural

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systems. There are many - documented example of shifts in species ranges gene frequencies, changes in mating and migratory behavior [5].

Population genetics is a fundamental issue in evolutionary biology. *Drosophila* is ecologically a rather highly specialized, but closely - knit group which offers valuable opportunities for studies on organism - environment relation. A better understanding of how different species are affected by current climates and why they sometimes respond differently to climate change is necessary for predicting future effects of climate change [6].

The success of a population depends on its adaptations to climatic conditions [7]. Fundamental features of living world depend on the structure of fitness variation [8]. Variation in life - history characters of organisms has always been evoked particular interest among evolutionary geneticists. Many of these characters are important components of fitness, such that a directional change in a character will lead to an increase in total fitness. Any genetic variant that confers such an advantage should be rapidly swept to fixation of selection [9].

The evolution of fitness is always with reference to definite environmental condition since it differs or varies in different situations. A minimal requirement of adaptive phenotypic plasticity is that the phenotype and the environment interact to enhance individual fitness that is the phenotypes induce by a particular set of environmental conditions results in a fitness gain [10]. A substantial progress has been made in documenting and understanding the phenomena regarding fitness components and their adaptations *Drosophila* has been used as a representative system by population geneticists to understand the genetic basis of ecological differentiation at the level of populations and species [11].

The fitness of a genotype is defined as "the average number of progeny left by the carrier of that genotype relative to the number of progeny left by the carriers or other genotypes" [12]. The success of a population depends on its adaptation to climatic conditions [7]. Andrewartha and Birch [13] have recognized some important components of the environment and their effects. These effects are themselves not heritable, but the susceptability to environmental effects is potentially heritable and thus provides a basis for evolution of environmental variance. For ectothermic organism like *Drosophila* temperature and light are the most important factors of the environment. Circadian clocks are ubiquitous and are found in organisms ranging from bacteria to mammals. This ubiquity of occurrence implies adaptive significance, but to date there has been no rigorous empirical evidence to support this. It is believed that an organism possessing circadian clocks gains fitness advantages in two ways: a) Synchronizing its behavioural and physiological processes to cylcic environmental factors (Extrinsic adaptive value) b) Co-coordinating its internal metabolic processes (Intrinsic adaptive value) [14]. Circadian co-ordination of life functions is believed to contribute to organism fitness. In view of this, the experiment was set up to analyze fitness of four species of *Drosophila* and their relations with the stress applied in the form of variable light and dark cycle.

MATERIALS AND METHODS

The *Drosophila* stocks assessed were *Drosophila melanogaster, Drosophila ananassae, Drosophila nasuta and Drosophila albomicans*. *Drosophila melanogaster*, a cosmopolitan and domestic species belongs to the *melanogaster* species complex of the *melanogaster* subgroup of the *melanogaster* species group of the subgenus sophophora have been extensively studied for the mating activity [15-16]. *D. melanogaster*, a cosmopolitan and domestic species is most widespread geographically in distribution. It is also genetically most variable compared to other species [17]. *Drosophila ananassae*, a cosmopolitan and domestic species, belongs to the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group. This species occupies a unique status in the whole of the genus *Drosophila*, owing to certain peculiarities in its genetic behaviour [18]. *Drosophila nasuta and D. nasuta albomicans* are a pair of sibling allopatric races of *immigrans* species group of *Drosophila* extensively studied [19-27]. The stocks were obtained from the *Drosophila* stock centre, Mysore, India.

The fly stocks were cultured in standard wheat cream agar medium in uncrowded culture condition at 22±1°C (rearing temperature) and a relative humidity of 70%. The parental stocks used for the present assays were reared at three different light - dark regimes (constant light - LL, constant dark- DD and 12 hours light - dark LD) for about 8 to 10 generations to breed stabilize stocks. The progeny from these stabilized stocks were used to assess the mating propensity (courtship duration and copulation duration), productivity (fecundity and fertility) and longevity.

Assessment of Mating Propensity: Mating propensity was recorded accordingly with slight modification [28-29]. About 100 replicates of both males and females were

observed for the mating activity exposed to variable light - dark regimes. From each species virgin flies (males and females) were collected on the day of eclosion, anaesthetized with ether to facilitate sorting of the sexes and stored in food vials. Flies were aged for seven days in food vials for sexual maturity. The males and females were then placed in an empty vial (measuring the length of about 9.3cm and width of about 2.1cm) and observed for the courtship duration (The time taken by a male to mount on female) and copulation duration (Time from mounting to detaching). The mating activity was observed for 60 minutes. The pairing of flies from the time of mounting to detaching was recorded. The pairs which do not mate within a stipulated time of 60 minutes were discarded.

Assessment of Productivity: The same set of flies which were used for the observation of mating propensity and copulation duration were used to assess the rate of productivity (Fecundity and fertility) and longevity.

Life Time Fecundity: The life time fecundity is defined as the number of eggs laid by an individual during its lifetime [30]. For the assessment of lifetime fecundity, the method of Buck *et al.* [31] was used with slight modifications. After recording the mating propensity the individual mated males and females were placed in a separate vials for recording the number of eggs laid by a female. Likewise once in two days each replicate was transferred successively to the next set of fresh food vials. Immediately after each transfer, the vials were checked for the number of eggs laid and were counted under stereomicroscope till egg laying is stopped. The mean number of eggs laid by these pairmated females were recorded for hundred replicates.

Lifetime Fertility: The lifetime fertility was conducted accordingly to protocol of Singh *et al.* [32]. Fertility of a given mating means the relative proportion among the newly produced offspring attributed to that mating. The same set of vials that were used to assess life time fecundity was used for this experiment after counting of the eggs laid. The number of flies emerged from 100 replicates were recorded for the total lifetime fertility. Percent of survival was obtained after the assessments of lifetime fecundity and lifetime fertility, which is the percentage of the flies, emerged out from the total number of eggs laid.

Longevity of Mated Males and Females: Longevity was assessed using the modified protocol of Luckinbill and Clare [33]. Simultaneously along with the lifetime fecundity and fertility the same set of flies were continued to assess the longevity. Each vial was observed daily from day of emergence to record the lifespan.

Statistical Analysis: The analysis of variance (ANOVA) and Duncan multiple range test (DMRT) were used to record the divergence among different species subjected to variable exposures of light and temperature. To compile the data the programme used was statistical presentation system software (SPSS) 10.0 for MS windows.

RESULTS

Observation of Mating Propensity: Table 1 reveals the mean courtship duration assessed in four different species of *Drosophila* to variable light-dark exposure. The courtship duration is minimum at LD exposure and is maximum at DD exposure in all the four species of the present study. *D. melanogaster* and *D. albomicans* has taken maximum and minimum time to mount on female respectively. The analysis of variance reveals significant differences among all the four species of the present study. Except between *Drosophila nasuta* and *D. albomicans* all the other comparisons are significant as per DMRT. According to DMRT, the order of ranking of courtship duration is as follows *D.albomicans* > *D. nasuta* > *D. ananassae* > *D.melanogaster*.

Table 1: Mean courtship duration in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	Courtship Duration		
Light regimesSpecies	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	12.59±0.28	13.22±0.36	11.51±0.14
D. ananassae (D.a)	9.23±0.18	9.45±0.20	10.21±0.21
D.nasuta (D.n)	13.79±0.21	15.39±0.37	12.71±0.23
D. albomicans (D.al)	13.94±0.20	15.67±0.44	13.17±0.51
Analysis of Variance (ANOVA)	F=2.292; d.f= 3,396; P<0.001	F=2.567; d.f= 3,396; P<0.008	F=3.651; d.f= 3,396; P<0.001
Duncan's Multiple Range Test (DMRT)	m/a, m/n, m/al, a/n, a/al	m/a, m/n, m/al, a/n, a/al	m/a, m/n, m/al, a/n, n/al

Table 2: Mean copulation duration in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	Copulation Duration		
Light regimesSpecies	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	4.99±0.23	4.89±0.19	5.02±0.20
D. ananassae (D.a)	4.84±0.09	3.96±0.01	4.92±0.29
D.nasuta (D.n)	5.26±0.36	5.12±0.39	5.74±0.38
D. albomicans (D.al)	6.38±0.35	5.29±0.42	6.94±0.43
Analysis of Variance (ANOVA)	F=2.951; d.f= 3,396; P<0.079	F=3.233; d.f= 3,396; P<0.010	F=3.118; d.f= 3,396; P<0.001
Duncan's Multiple Range Test (DMRT)	m/a, a/al	a/m, a/n, a/al	m/s, a/al

Table 3: Mean lifetime fecundity in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	lifetime fecundity		
Light regimes Species	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	139.25±7.37	122.47±12.34	143.63±9.49
D. ananassae (D.a)	104.60±8.76	98.30±10.75	134.29±6.42
D.nasuta (D.n)	144.26±5.39	128.33±9.25	152.00±8.36
D. albomicans	146.32±10.63	138.17±8.77	153.68±9.43
Analysis of Variance (ANOVA)	F=3.051; d.f= 3,396; P<0.001	F=3.456; d.f= 3,396; P<0.006	F=2.872; d.f= 3,396; P<0.013
Duncan's Multiple Range Test (DMRT)	m/a, m/n, m/al, a/n, a/al	m/a, m/n, m/al, a/n, a/al,	m/a, m/n, a/n, a/al

Table 4: Mean lifetime fertility in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	Lifetime fertility		
Light regimes Species	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	131.75±7.89	116.68±10.10	135.45±7.28
D. ananassae (D.a)	86.20±9.34	74.04±8.36	122.00±9.45
D.nasuta (D.n)	127.05±10.44	106.00±6.34	134.83±8.85
D.albomicans(D.al)	129.71±9.72	113.25±10.93	138.02±7.36
Analysis of Variance (ANOVA)	F=5.291; d.f= 3,396; P<0.002	F=4.083; d.f= 3,396; P<0.006	F=3.859; d.f= 3,396; P<0.040
Duncan's Multiple Range Test (DMRT)	m/a, m/n, m/al, a/n, a/al	m/a, m/n, m/al, a/n, a/al,	m/a, m/n, m/al, a/n, a/al, n/al

The mean copulation duration of four different species of *Drosophila* represented in Table 2. which provides the information that the copulation duration is maximum at LD and minimum at DD. Of the four species, *D.ananassae* varies significantly with less, while *D.albomicans* has taken significantly longer copulation duration than the other species. The analysis of variance reveals that the differences among all the species are significant in both LL and LD exposure. While, in DD except *D. ananassae*, the differences is negligible among the other three species. As per DMRT the order of ranking is *D.albomicans > D. nasuta > D.melanogaster >D. ananassae*.

Productivity

Lifetime Fecundity: Table 3. depicts the mean fecundity of four different species of *Drosophila* exposed to different light regimes. The data reveals the minimum fecundity at DD and maximum number of egg production at LD. According to the ANOVA the differences in eggb production among all the species are significant. While the differences is negligible between *D. albomicans* and *D. nasuta* in both LL and LD. According to DMRT the range of egg production follows *D.albomicans > D. nasuta > D.melanogaster >D. ananassae.*

Lifetime Fertility: The mean lifetime fertility of the four species of Drosophila is provided in Table 4. The number of flies emerged is lowest and highest in DD and LD respectively. *D.ananassae* has shown to differ significantly with the other three species, but the differences among *D.melanogaster, D. albomicans* and *D.nasuta* is not much. In all the three experimental light exposures. The order of ranking according to DMRT is *D.albomicans > D. nasuta > D.melanogaster >D. ananassae.*

Longevity: Table 5. represents the mean fertility among the four different species of *Drosophila* at three different light regimes. The mean longevity has shown to increase and decrease at DD and LD in all the four species of the present study. Even though the flies of different species

Table 5a: Mean longevity of males in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	Longevity (males)		
Light regimes Species	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	43.25±1.37	41.47±1.29	47.36±1.50
D. ananassae (D.a)	37.27±1.23	32.02±1.04	42.12±1.45
D.nasuta (D.n)	41.00±1.24	33.83±1.16	45.28±1.56
D.albomicans(D.al)	47.73±2.20	45.55±1.53	49.53±1.21
Analysis of Variance (ANOVA)	F=2.291; d.f= 3,396; P<0.025	F=2.082; d.f= 3,396; P<0.010	F=3.769; d.f= 3,396; P<0.040
Duncan's Multiple Range Test (DMRT)	m/a, m/n, m/al, a/n, a/s, n/al	m/a, m/n, m/al, a/n, a/al, n/al	m/a, m/n, m/al, a/n, a/al, n/al

Table 5b: Mean longevity of females in four different species of *Drosophila* exposed to three different light regimes (values are mean ±100 replicates) along with statistical analysis.

Traits	Lifetime fertility (females)		
Light regimesSpecies	Constant light (LL)	Constant dark (DD)	12 hrs Light/ Dark(LD)
D.melanogaster (D.m)	48.25±1.48	41.85±1.30	48.36±1.73
D. ananassae (D.a)	40.20±1.46	37.16±1.21	43.10±1.46
D.nasuta (D.n)	44.80±1.50	38.23±1.20	46.62±1.67
D. albomicans (D.al)	50.74±2.25	45.92±1.60	53.12±2.32
Analysis of Variance (ANOVA)	F=2.351; d.f= 3,396; P<0.005	F=1.812; d.f= 3,396; P<0.004	F=4.238; d.f= 3,396; P<0.002
Duncan's Multiple Range Test (DMRT)	m/a, m/n, m/al, a/n, a/al, n/al	m/a, m/n, m/al, a/n, a/s, n/al	m/a, m/n, m/al, a/n, a/s, n/al

Table 6: Correlation between mating propensity vs productivity (fertility) of females in four different species of *Drosophila* (LL:LD)

	Mating Propensity			
Fertility	*D.melanogaster*	*D. ananassae*	*D.nasuta*	*D. albomicans*
D.melanogaster	.001	0.051	0.023	0.020
D. ananassae		.001	0.06	0.091
D.nasuta			.001	.020
D. albomicans				.001

Mating propensity and fertility are positively correlated at 5% level

are reared at variable light exposure, it is found that that the females of all the four species are long lived than males. The order of ranking according to DMRT is *D.albomicans* > *D. nasuta* > *D.melanogaster* >D. ananassae.

Mating Propensity vs Productivity (Fertility): Table 6. provides the correlation between mating propensity and fertility at 5% significant level. The information from the data reveals that the increase duration of copulation has led to increased fertility, while decrease in copulation duration found to decrease the fertility. Thus, a strong positive correlation do exist between copulation duration versus productivity (fertility).

DISCUSSION

Experimental and natural variations is the mechanism that underlie stress resistance have clear, consistent and predictable consequences for ecological and evolutionary attributes. One might logically expect the following to be true: species that have robust mechanism for stress tolerance are able to exploit more stressful environments or persist longer in them than species with greater stress tolerance exhibit more robust mechanisms than species with lesser intolerance [34]. The natural populations are constantly exposed to challenging environmental variation to maintain the cellular homeostasis and high performance across environments. The stress response and heat shock proteins are important for this buffering in relation to stress resistance and adaptation to the environment under some conditions [35].

In *Drosophila*, successful mating depends upon male activity and female receptivity because usually the female is the discriminating partner in the mating act. Courtship time (mating speed), the time from the beginning of the courtship to copulation is a good estimate of sexual activity in males and sexual receptivity in females. The courtship behaviour of *Drosophila* enables conspecifics to distinguish non-conspecifics and enables males to distinguish females, including the physiological readiness of the female to copulate [36]. Mating activity is correlated with fitness in many species of *Drosophila* [37].

Courtship duration has increased in DD exposure with decreased copulation duration and vice versa in LD, but it is intermediate in constant light (LL) exposure. Eventhough the duration of courtship activity is maximum and minimum in DD and LD exposure respectively and as a reverse the minimum time of copulation activity is observed in DD and maximum at LD in all the four species of *Drosophila* of the present study. Thus it is evident that the courtship duration and copulation duration are negatively correlated.

There is considerable variation in copulation duration among *Drosophila* species [38], but causal factors influencing variation in copulation duration have been described for some species. These factors are complex and depend on the form of sperm precedence, female mating status and oviposition patterns, size of males and age of males [39-41]. In general, longer the copulation duration the higher reproductive success have been achieved and the present results also opines the same pattern.

Fecundity, the number of egg laid by an individual is the major determinig factor of female fitness [42]. The egg laying capacity is one of the suitable parameter to compare the performance of different strains of *Drosophila* [20-21, 26]. Fecundity is a composite measure of consequences of a number of reproductive events in both sexes including courtship and copulation [43]. All the four species have produced more number of eggs in LD exposure but production of egg is inversely proportional incase of DD regime. Egg laying potentiality is an important attribute, which determines to certain extent the reproductive success of a population dermined increasingly at LD.

The fertility is an important component of fitness, has been extensively studied in different strains of *Drosophila* [26, 27, 44]. Even in life time fertility study also all the four species have shown maximum fertility to LD exposure and minimum at DD exposure. This observation depicts that high fertility is shown in the regular rhythm of 12 hrs light and 12hrs dark.

The quantitative aspects of lifespan and its correlates are well categorized in *Drosophila* [45]. In all the four species of the present analysis interestingly the females had significantly greater longevity than males, which is similar to the results of Viera *et al.* [46]. Variation in lifespan within natural population is partly attributable to both genetic and environmental effects [47]. Even the lifespan is also seen to increase in LD exposure and it is quite lesser in DD exposure. The copulation duration has not effected the lifespan in the present analysis. *D. albomicans* with delayed copulation duration has successfully achieved increased longevity, while the *D. ananassae* with minimum copulation duration has lesser longevity.

To surmise, the significant differences exerted in the four species of the present study shows divergence to some extent across a broad complex of traits associated with survival and reproduction at variable light/ dark exposures. Courtship duration has increased in DD with the decreased copulation duration and vice versa in LD, but intermediate in LL. The mean courtship duration is significantly highest and lowest in *D. albomicans* and *D. ananassae* respectively and it is contradictory incase the copulation duration. Interestingly, the reproductive success (fecundity, fertility and longevity) is significantly high in *D. albomicans* than *D. ananassae* at all the experimental light exposures. The present investigation reveals that *D. albomicans* have explored significantly more values for mating activity, productivity and longevity even when exposed to different light regimes. However, the differences between *D. albomicans* and *D.nasuta* are insignificant. Whereas, the differences do exist between *D.ananassa*e and *D.melanogaster*. Differences in photoperiod may also have contributed to the selection response as fitness traits may be affected by photoperiod [48]. Trade-offs resulting from pleiotropy may constrain evolutionary change within both field and laboratory environments [49, 50].

Interestingly, the present observation reveals that either reared at any stress condition, the increase in copulation duration as resulted in increase productivity and vice-versa in all the four different species of the present study and exist a strong positive correlation between mating propensity (copulation duration) and productivity (fertility). It is also important to note that, rearing of the flies at appropriate dark - light exposure is equally important with suitable temperature regimes. The present study as an impact on the reproductive success of the flies exposed to variable light regimes (as a factor of stress). The responses clearly involve developmental plasticity and the acclimation of life history traits in adults to their prevailing environment. Thus the present study represents that, DD regime seems that it has created a stressful environment which has been noticed by the depletion of the reproductive success of the individuals in the different species of *Drosophila*.

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