Sexual selection in search of good genes: analysis of mate choice experiments and ecophysiological stress tolerance in *Drosophila biarmipes*.

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Abstract

Female preference for male ornament is favored by sexual selection. It is not clear whether the preference is limited to male ornament only or it is actually for genes that affect fitness of the upcoming progeny. In mating systems in which males provide direct benefits of fitness to the female or their offspring, the answer seems straightforward – females should prefer to mate with males that are able to provide more resources. We tested the hypothesis whether spotted and spotless males and progeny from sexually preferred males of *Drosophila biarmipes* vary in their levels of environmental stress tolerances. Our results showed that male flies with dimorphism for wing spot varied in their mating success and stress tolerance. Data have shown significant differences in mated pairs with spotted and spotless males. Spotted and spotless males also differ significantly in ecophysiological stress tolerance. Finally, females mating with a high-quality male (spotted wing) results in offspring with high performance. We found significant effects on fecundity of mated females and egg-to-adult viability of their progeny. Our results are consistent with good genes sexual selection and suggest that mate choice could provide indirect benefits to females. To the best of our knowledge, this is the first report on the ecological significance of wing color dimorphism in a species – *Drosophila biarmipes.* **Keywords:** wing spot dimorphism, *D. biarmipes*, sexual selection, good gene.

Introduction

Evolutionary and behavioral ecologists have long been interested in, and puzzled by, mate choice. In many species, females are highly selective when it comes to mating (Darwin, 1871; Bateson, 1983; Andersson, 1994; Kokko *et al*., 2003). In some of these species, females are congruent in their mate preference for a particular male, while in other species, females are incongruent in their preference, with each preferring a different male. The least controversial models of female mate choice emerged from resource-based mating systems in which males provide resources directly to females or offspring. These resources obviously could have a profound impact on female fitness. Furthermore, researchers realized that natural selection could lead to the evolution of male indicator traits that facilitated mate choice by advertising the quality or quantity of a male's resources (Møller and Jennions, 2001). Several experimental studies on captive domestic or laboratory species reported indirect benefits (Welch *et al*., 1998; Moller and Alatalo, 1999; Petrie, 1994; Evans *et al*., 2004; Drickamer *et al*., 2000; Bluhm and Gowaty, 2004), but a demonstration that the free choices of females in nature influence offspring survival by means of a sire effect. Good genes models of sexual selection predict the evolution of female mate choice based on preferences for male traits associated with additive genetic variance in fitness (Jennions and Petrie, 2000; Iwasa *et al*., 1991; Houle and Kodrashov, 2002). The strength of the association between secondary sexual characters and offspring survival suggests that viability selection may render small but significant

increases in fitness (Moller and Alatalo, 1999), but some studies suggest otherwise (Qvarnstrom *et al*., 2006), and the subject of indirect selection on female mate choice or mating behavior remains a contentious issue in the study of sexual selection (Cameron *et al*.. 2003).

Wing melanisation patterns in insects are highly diversified and have played an important role in thermoregulation, mate choice, defense against predators and mimicry in various species of butterflies (Watt, 1968; Roland, 1982; Kingsolver, 1987; Wiernasz, 1989; Ellers and Boggs, 2002, 2003). In alpine (*Colias* species) as well as copper butterflies, wing melanisation has been associated with increased flight ability under colder environmental conditions (Watt, 1969; Roland, 1982; Ellers and Boggs, 2004; Karl *et al.*, 2009). Some studies have shown the role of wing patterns in mate recognition in butterflies (Wiernasz and Kingsolver, 1992; Jiggins *et al.*, 2001); Calypterygid damselfly (Siva-Jothy, 1999), whereas quite a few studies have shown the direct effect of wing spot on mated pairs in two *Drosophila species – D. suzukii* (Fuyama, 1979) and *D. biarmipes* (Singh and Chatterjee, 1987). However, for *Drosophila* species the ecological significance of wing spot dimorphism and sexual preference for a particular morph remains largely unknown.

Drosophila biarmipes, a warm adapted tropical species, belongs to Oriental region (eastern part of Asia), and in India, except northern region, it is found in all the parts (Markow, 2006). Based on morphological features, *D. biarmipes* was assigned to the suzukii subgroup of the melanogaster species group. In *D. biarmipes*, melanic spots always appear in roughly the same area of the wing, and the melanin pattern takes the form of an intense spot centered on the distal L-2 vein (Kopp and True, 2002). This pattern is limited to males, although very slight melanization is occasionally seen in females of this species. However, it is intriguing that this species is spread in the south to north Indian localities, which encounter seasonally varying climatic conditions. On the Indian subcontinent, $T_{ave.}$ is poorly correlated with latitude ($r = -0.54 \pm 0.30$), but seasonal variations increase with latitude. For example, seasonal variations (calculated as coefficient of variation in mean monthly temperature or humidity changes) have shown lower values in the south ($T_{cv} = 3.14\%$ and $RH_{cv} = 5.39\%$) as compared with north ($T_{cv} = 29.6\%$ and $RH_{cv} = 26.6\%$). Further, rainy and autumn seasons differ significantly in thermal as well as humidity conditions in the north. Thus, we may expect evolutionary responses to natural selection on traits related to desiccation and cold stress in subtropical populations of *D. biarmipes*.

We considered *D. biarmipes* suitable to find answers to the following questions: (i) whether there is plasticity for wing spot? (ii) whether there is any role of wing spot in mating success (mating latency, copulation duration)? (iii) do the spotted and spotless males differ in desiccation stress, corresponding rate of water loss and cold tolerance? (iv) Finally, we tested whether there is significant variation in two important components of fitness (fecundity and egg-to-adult viability) in *D. biarmipes*. Present work on impact of wing spot on mating success is interesting in several respects. (a) in *D. biarmipes*, there is no plasticity for wing spot. (b) a significant difference in mating propensity and copulation duration of spotted males and spotless males. Spotted males were desiccation resistant and have lower rates of water loss and are more tolerant towards cold stress. We have performed a quantitative genetic analysis of egg-to-adult viability and adult survival to sexual maturity. We found significant levels of differences in offspring fitness, supporting a picture of good genes sexual selection in this species.

Material and Methods

Collections and cultures

Wild *D. biarmipes* (n = 180-200 per population) were collected from four lowland localities (~ > 200 m; Rohtak, Chandigarh, Mandi and Kalka) by net sweeping method. Wild caught females

were used to initiate isofemale lines (20 lines per population). All cultures were initiated with 6-8 hour egg laying period and maintained at low density (60-70 eggs per vial of 37 × 100 mm size) on cornmeal yeast-agar medium at 21°C. All experiments were initiated soon after collections and performed with G_1 and G_2 (Generations 1 and 2) in order to avoid possible effects of laboratory adaptation. All assays were performed on 7 day old flies (sexed soon after eclosion). For each isofemale line as well as wild caught individuals of both the species, we first analyzed wing spot, and this was followed by mating latency (ML), copulation duration (CD), and desiccation resistance and rate of water loss. Males of *D. biarmipes* show wing spot dimorphism, whereas females lack wing spot.

For investigating developmental plastic effects of wing spot, 25 to 30 pairs of each isofemale line were allowed to lay eggs at 21ºC in 20 replicate vials. Five such vials were then transferred to each of 19º, 21º, 25º, and 28ºC growth temperatures for *D. biarmipes*. Thus, we checked plastic effects for wing spot area at these growth temperatures.

Since ANOVA showed non-significant F-values between wing morphs isolated from different populations, the data were pooled for spotted males. Likewise, data on spotless males were also pooled. For each trait, 10 isofemale lines per population were used and 10 randomly chosen individuals per strain were investigated. Flies were sexed soon after eclosion and were maintained as virgins on cornmeal medium seeded with live yeast. All flies were 6 to 8 days post-eclosion at the start of desiccation stress assays. For measuring desiccation stress, ten virgin males of each morph were isolated in a dry plastic vial, which contained 2 g of silica gel at the bottom and were covered with a disc of foam piece. Such vials with foam plugs were placed in a desiccation chamber (Secador electronic desiccator cabinet), which maintains 1-2% relative humidity. Mortality due to desiccation stress was inspected every hour until half the flies (LT_{50}) died, and thereafter observations were made every half an hour. Desiccation survival curves were drawn as a function of time of desiccation stress.

Rate of water loss (mg hr^{-1}) due to short-term desiccation (8 hr) was estimated in groups of five flies. Both before and after desiccation, flies were weighed on a Sartorius microbalance and water loss/hr was calculated as: (initial body weight – body weight after 8 hour desiccation stress)/ initial body weight × 8. Total body water (%) was estimated as the difference between wet and dry weight / initial wet weight × 100.

To evaluate cold hardiness or chill coma recovery, spotted and spotless males were placed in groups of ten each in 10 ml glass vials, which were submerged in a 10% glycol solution cooled to 0ºC. The vials were removed after varying duration of stress (1 – 10 hours) and recovery time was scored. The flies were considered recovered when they were able to stand up on their legs.

Mating propensity experiments on laboratory reared virgin females and males (spotted and spotless) were performed. In each mating chamber, 10 virgin females and 5 virgin males, spotted as well as spotless, were placed and observations on 10 such pairs were made for 60 minutes under female choice condition. For all the observed matings, % mated pairs (MP); mating latency (ML; the time from introduction of flies to initiation of copulation time); copulation duration (CD; from initiation to detachment of mated pairs); were recorded. In this way, matings were observed for 10 lines × 10 replicates each.

Fecundity of mated females and egg-to-adult viability of progeny

For estimating fecundity, each mated pair was aspirated and placed in an oviposition chamber for 24 hours and thereafter the male was removed. The eggs laid on the food placed at the replaceable bottom plate of the oviposition chamber were counted daily. The flies were transferred to fresh food vials every day, and the number of eggs laid during 24 hours by each female was recorded.

This was followed for 15 successive days (7^{th} to 31^{st}) as this period coincided with maximum egg production, and the data were shown as daily fecundity.

Given that each individual replicate (n = 20 lines × 10 replicates, for each of spotted and non-spotted male) contains a single offspring, we were able to measure egg-to-adult viability by monitoring adult emergence from the eggs. Egg-to-adult viability can be affected by mortality during embryonic, larval, or pupal stages and their transitions. Emergence of adults occurred over the period of two weeks. On emergence, flies undergo a period of maturation feeding, during which males mature their testes and begin to produce sperm and females mature their ovaries and develop eggs. We adult survival to sexual maturity.

Statistical analyses

Means (n = 10 lines × 10 replicates × 10 individuals) along with standard deviation (SD) for all traits were used for illustrations and tabular data. Possible variations between homozygous spotted and spotless males and significant levels of other assays were checked on the basis of ANOVA. The Statistica package (Statsoft Inc., Release 5.0, Tulsa, OK, USA) was used for statistical calculations as well as illustrations.

Table 1. Analysis of nested ANCOVA (body mass as a covariate) to compare the ecophysiological traits for wing color morphs in D. biarmipes (20 lines × 10 replicates each; lines were nested into morphs). df – degree of freedom; MS – means square; ns = nonsignificant, $^{**}P < 0.01$; $^{***}P < 0.001$; Percent data were arcsine transformed for ANCOVA.

Traits		Morphs	Lines	Error
	df	1	38	359
1. Wing Melanisation Area (%)	MS	126.48	1.34	2.64
	F	23.84***	0.41ns	
2. Cuticular lipid mass (µg cm $^{-2}$)	MS	3.31	2.24	12.10
	F	0.52 ns	1.07 ns	
3. Desiccation resistance (hours)	MS	1275.90	244.53	1.37
	F	945.17***	48.43***	
4. Rate of Water loss (%)	MS	91189.54	329.14	7.37
	F	5931.35***	49.20**	
5. Cold Survival (%)	MS	49912.01	387.21	5.21
	F	9150.04***	29.44**	

Results

We found lack of plastic effects due to three growth temperature (20°, 25°, and 30°C) for spotted and spotless wing males. Thus, the data across growth temperature were pooled for three sets of traits (body melanisation, stress related traits, and mating propensity), and results for ANCOVA on various traits between spotted and spotless males are given in Table 1. Cuticular lipid content did not vary between the two males ($F = 0.52$ ns; Table 1). Spotted and spotless males evidenced significant differences in desiccation survival curves, *i.e.*, LT_{100} values are 17.12 and 6.40 hours, respectively (Figure 1B). Spotted males exhibits significant lower rates of water loss as compared with spotless males. Since changes in body size may impact desiccation resistance, we checked vial effects on body size due to possible differences either in nutrition or density. ANOVA showed no variation due to within as well as between vial effects for populations and morphs (data not shown). Thus, the

observed differences in desiccation resistance in *D. biarmipes* cannot be attributed to body size. Interestingly, this warm adapted species lacks thermal selection effects for body size in geographical populations as well as between morphs. Further, spotted males showed significantly higher desiccation resistance as well as cold stress survival (Figure 1A, B), higher mated pair frequency, longer copulation duration, and higher fecundity per day (Figure 2). In contrast, the light morph is characterized by higher values of cuticular water loss, percent mortality, as well as longer recovery time due to cold stress (Figure 1B), and a much longer mating latency (Figure 2B). For all the traits, differences were highly significant ($p < 0.001$) on the basis of F- values from ANCOVA analysis.

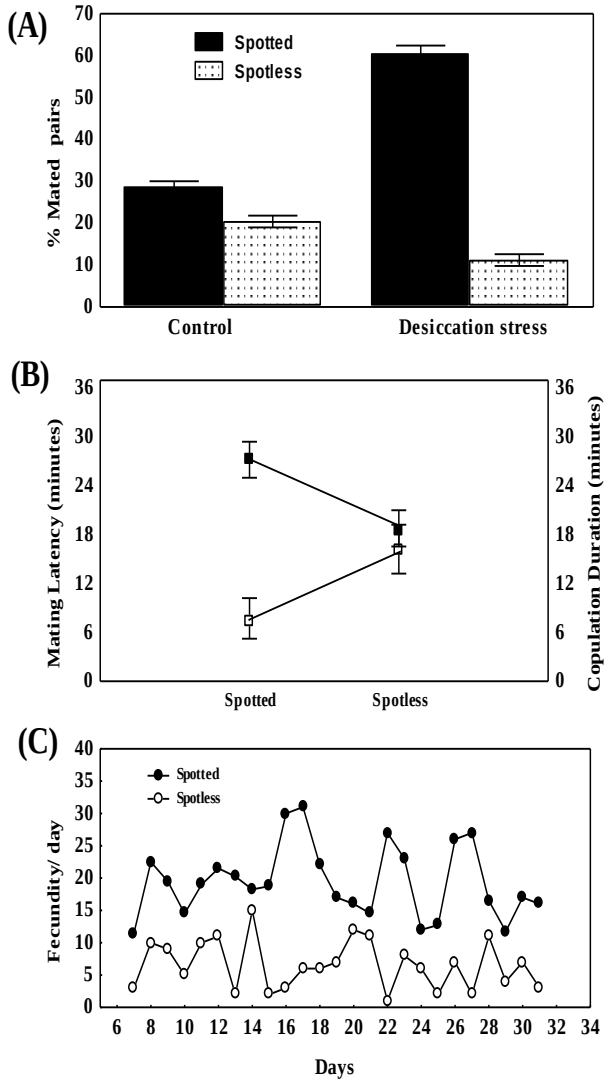

Figure 1. Comparison of fitness traits in *D. biarmipes.* (A) Percent mated pairs under control and desiccating conditions, (B) mating latency and copulation duration, (C) fecundity of spotted and spotless males (means ± s.e.m. of 20 isofemale lines grown at 21°C).

In order to test for mating preferences of spotted and spotless males with females in *D. biarmipes*, we attempted no choice mating experiments, and the data on observed matings are given in Figure 2. For all types of matings spotted males showed higher percent mated pairs (MP) as compared to spotless males (Figure 2A; Table 2). Based on male-choice mating experiments, we tested mating propensity of spotted and spotless wing males under control *vs.* stress conditions (due to desiccation or cold) and the data are shown in Table 2 and Figure 2A. As we found no obvious differences under desiccation and cold stress, we used data from desiccation stress for analysis. Mating preferences or propensity were estimated on the basis of two components of mating process (mating latency and copulation duration), and significant differences were found under control and desiccation conditions (Table 2). Finally there are significant differences in daily fecundity for all possible matings under control conditions ($F = 7897.36$, $p \leq 0.001$; Table 3; Figure 1C) in agreement with sexual selection for fitness benefits. Egg-to-adult viability exhibited significant differences in offspring from both types of matings. Progeny from spotted males had significantly higher egg- adult as compared spotless male progeny ($F = 3279.14$, $p \leq 0.001$; Table 3). Genetic correlations between fecundity and egg-to-adult viability were not significantly different ($p = 0.12$ ns) suggesting that these components of fitness are likely to be independent.

Table 2. Data on the basis of no choice method for frequency of mated pairs (MP), mean ± S.E. for mating latency (ML), copulation duration (CD) and fecundity under control and stressful conditions for *D. biarmipes.* For each experiment, there were twenty replicates. *** p < 0.001, ns = nonsignificant.

Experiment		Observed Mated pairs	MP (%)	ML (min) m ± SE	CD (min) m ± SE	Fecundity m ± SE
Contingency χ^2		-----	***	***	***	***
Control	1.	♀ X S ♂	28.66	5.40 ± 1.75	29.40 ± 2.10	24.10 ± 2.33
	2.	♀ X SL ♂	19.50	15.10 ± 2.16	17.54 ± 2.43	16.40 ± 2.50
Contingency χ^2		-----	***	***	***	***
Desiccation stress	1.	♀ X S ♂	61.58	6.37 ± 1.15	30.21 ± 2.00	23.15 ± 2.42
	2.	♀ X SL ♂	11.15	10.21 ± 2.04	15.00 ± 1.70	17.00 ± 2.61
Contingency χ^2		-----	***	***	***	***

Table 3. Results of nested ANOVA for fecundity and egg to adult viability (E- A Viability) of progeny of females mated to spotted and spotless males of *D. biarmipes*. ns = nonsignificant, **P < 0.001.

		Fecundity		E- A Viability	
Source	df	MS	*F*	MS	*F*
Morph	1	24527.21	7897.36***	30198.41	3279.14***
Lines	38	109.71	32.26**	121.98	14.97**
Error	360	3.23		9.14	

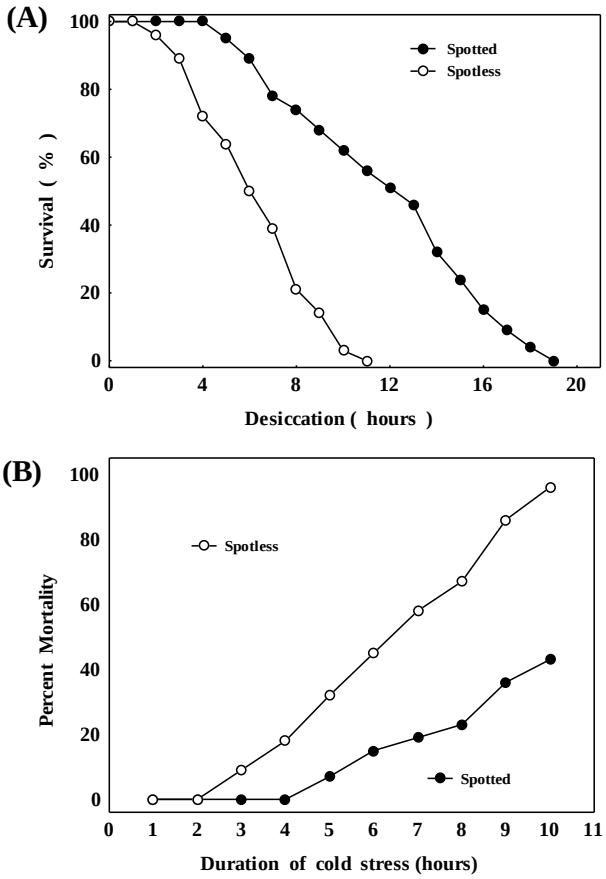

Figure 2. Survival (%) of spotted and spotless males with varying stress durations under desiccating conditions. (A) and during cold stress, (B) in males of *D. biarmipes*.

Discussion

A large number of studies have shown the role of abdominal melanisation in ecology of diverse insect taxa (Wittkopp *et al*., 2003). However, very few studies have directly tested the role of wing pigmentation. Interactions between an organism's morphology and its behavior are crucial to its fitness. Majerus and co-workers have done extensive studies on mating preference of color morphs of two-spot ladybirds (Majerus, 1994). Selection experiments for increased or decreased level of mating preference showed that this trait is genetically

controlled in two-spot ladybirds (Majerus, 1986). However, it has been shown that the maintenance of melanic polymorphism in two-spot ladybird is unrelated to any environmental factor (O'Donald and Muggleton, 1979). By contrast, in *Harmonia axyridis,* mating preference varied with season, *i.e*., in spring, both melanic and non -melanic preferred to mate with non-melanic males, while in the summer, melanics were over-represented in matings (Osawa and Nishida, 1992). The present study shows the impact of male wing spot dimorphism on mating success in warm adapted (*D. biarmipes*) species.

Fitness, a measure of relative performance of different genotypes at a locus, is generally assessed indirectly in terms of life history traits (Roff and Mousseau, 1987). Fitness consequences as survival under semi-field conditions for dark and light morphs of hoverflies (due to phenotypic plasticity) have shown clear adaptive differences, *i.e*., higher survival of lighter flies under summer conditions (Ottenheim *et al*., 1999). Further, two body color phenotypes (due to genetic polymorphism) of *Harmonia axyridis* differ significantly in development time and consumption rate (Soares *et al*., 2001). Thus, morphs resulting due to plastic or genetic effects show fitness consequences. In the present study, we have shown adaptive differences between spotted and spotless wing morphs of *D. biarmipes* for various ecophysiological traits. In the laboratory, male-choice mating experiments on spotted and spotless wing morphs showed the occurrence of assortative matings among spotted wing morphs.

Choosy females can increase the genetic quality of their offspring by mating only with males that will contribute good genes or compatible genes to their offspring. Good genes can be conveyed by condition-dependent traits (Rowe and Houle, 1996). For example, we have discussed that female guppies prefer to mate with longer males and peahens prefer to mate with males with larger eye-spots, because these males pass on to the offspring good genes that increase their fitness (Reynolds and Gross, 1992; Petrie, 1994). Mate choice also can be used to select males with compatible genes. Higher fitness of spotted wing males over spotless males is evident from measures of cold mortality as a function of duration of cold stress. Spotted and spotless males differ significantly in mortality due to cold stress. Finally, for desiccation resistance, there was lack of changes in the amount of cuticular lipids in spotted and spotless males. Thus, cuticular lipids cannot account for desiccation resistance in *D. biarmipes*. However, we observed significantly lower rates of water loss in spotted males and its impact on desiccation resistance. A major conclusion is that a spotted male is positively correlated with resistance to cold and desiccation stress. Thus, there are significant differences for various ecophysiological traits in spotted and spotless males of *D. biarmipes*.

Male vigor is measured by the number of females inseminated by a male in a given unit of time and·number of progeny produced from their insemined females. In this experiment, the males with patch inseminated more females and produced more progeny than males without patch, indicating that males with patch have higher vigor than males without patch. Thus, these studies have suggested that, in *D. biarmipes*, males with patch, fast mating abilities, and greater courtship activity convinced the females faster during both competitive and non-competitive situations. With these qualities, they are also able to mate quickly and produce more offspring. We have found significant effects on fecundity and egg-to-adult viability as well as moderate to high coefficients of genetic variation for these traits in *D. biarmipes*. These results indicate that there is genotypic variation among males that translates into variation in offspring viability. This variation provides the raw material for the accruement of indirect benefits resulting from female choice.

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In vivo and *in vitro* genotoxicity analysis of silver nitrate.

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Abstract

Silver nitrate is an inorganic compound which is toxic and corrosive. This heavy metal, when overdosed, leads to skin disease, blindness, and organ damage. The present study investigated the genotoxicity of silver nitrate in *Drosophila melanogaster* and human peripheral blood lymphocytes. Canton flies were exposed to 0.1M, 0.01M, and 0.001M of silver nitrate. Phenotypic analysis revealed discoloration of head and thorax in the treated flies and their progeny. The DNA from both parent and F1 was subject to Fragmentation assay to study the damages induced by the heavy metal, and the analysis showed significant shearing with fragmentation in the parent DNA. However, the F1 DNA depicted only shearing. To understand the type of mutation induced, Wing Somatic Mutation and Recombination test (SMART) was performed using trans-heterozygous larvae of *mwh/flr3* cross-over, exposed to the different concentrations of silver nitrate. Analysis of wings obtained from the emerging flies revealed spot formation characteristic of both the recessive markers. *In vitro* analysis