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Altitudinal effects on reproductive success of three sympatric species of drosophila in Chamundi Hills

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Abstract
The amount of sperm and secretions from the male accessory glands that are transported to the paired females during mating determines the reproductive success of animals. Three sympatric species of *Drosophila*, including *D. malerkotliana, D. bipectinata,* and *D. nasuta,* were collected from Chamundi Hill for this study on the effects of altitude on accessory gland features and sperms transmitted by male to female during copulation and its consequences on fitness. Unmated males of all three sympatric species of *Drosophila* in low altitude were found to have much more larger major cells in their accessory glands, as well as a larger accessory gland and more accessory gland protein. They had longer copulations yet took less time to mate. Furthermore, compared to flies acquired from either moderate or high elevations, these ones were better able to transport more sperm and accessory gland proteins to mated females, as well as generate more eggs and offspring. In addition, among the three sympatric species, *D. malertotliana* considerably outproduced *D. bipectinata* and *D. nasuta* in terms of the amount of accessory gland proteins and sperm transported to mated females. Three species' subsequent reproductive success was in the following order: *D. malertotliana*>*D. bipectinata*>*D. nasuta*. Therefore, these investigations imply that in all three sympatric species of Drosophila, low altitude flies had higher reproductive success than those at intermediate and high elevations. Compared to *D.bipectinata* and *D.nasuta, D.malerkotliana* showed much better reproductive success

Keywords: fecundity, fertility, altitude, copulation duration, accessory gland secretory protein

Introduction

In nature, factors including temperature, humidity, rainfall, photoperiod, population density, age distribution, intra- and interspecific competition, as well as the accessibility and availability of food in a particular location, all affect how successfully a species reproduces (Dobzansky, 1956) [14]. Therefore, the existence or absence of a species in an ecological niche is determined by the ecological and biological variety of an ecosystem (Brncic *et al.*, 1985) [11]. In addition to topography and biotic parameters, altitudinal transect studies allow us to understand how species distribution, cohabitation, biotic and abiotic factors, which vary with elevation and seasons, relate to one another (Hegde *et.al,* 2010) [20]. As a result, the environment at various altitudes may play a significant role on population change and reproductive success of a particular species. Due to their remarkable sensitivity to minor environmental changes and the ability to investigate their evolutionary process, *Drosophila* flies are ideal for the research of population fluctuations and reproductive success (Bizzo and Sene, 1982) [9].

It is well recognised that temperature and precipitation variations frequently have an impact on vital *Drosophila* species traits such viability, fertility, reproduction, development time, and other elements that affect the rate of population expansion and survival (Bizzo and Sene, 1982) [9]. The endurance of the flies is also influenced by temperature. Temperature, precipitation, and light levels all have an impact on the availability of resources, particularly in connection to the flowering and fruiting seasons of varied vegetation, which provide the majority of the locations for oviposition and feeding for the community (Brncic *et al.,* 1985) [11]. Aside from these biotic parameters, survival and reproductive success in natural populations of *Drosophila* are also influenced by intra- and interspecific connections, population density, population age distribution, and competition between *Drosophilis* and their hosts and predators (Bizzo and Sene, 1982) [9].

In insects, the accessory glands, a secretory tissue of the male reproductive canal, contribute to reproductive capacity (Baldini *et al.* 2012; Findlay *et al.*2008; Gorshkov *et al.*2015; Mueller *et al.*2004; Sirot *et. al.*2008; South *et al.*2011) [6, 15, 18, 23, 30, 31]. The male accessory gland's development and secretory activity are tightly regulated by the insect endocrine system (Gillott, 1996; Gillott and Gaines, 1992) [16, 17] and play crucial roles in modifying the reproductive physiology and behaviour of the mated female. The accessory glands are derived from the genital disc during the late larval stage. The ingestion of sperm and seminal proteins is necessary for these physiological and behavioural modifications, which are collectively known as post-mating responses and include increased egg production, decreased willingness to remate, storage and utilisation of sperm, altered gene expression, and increased feeding [reviewed in (Avila *et al.* 2011; Sirot *et al.* 2009; Raviram and Wolfner 2007; Liu and Kubli 2003; Chapman *et al.* 2003) [4, 29, 27, 21, 12].

The bulk of persuasive investigations on the formation of accessory glands in male Drosophila showed that the genital imaginal disc's cells grow in various configurations (Nothiger *et al.*1997) [25]. Its fine structure is influenced by its developmental stage and the type of secretion it produces (Bairati and Aurelio, 1966) [5]. These glands are active in adults, and their secretion is important for a variety of reproductive systems (Wolfner, 1997) [34]. Between species (Chen 1984) [13], as well as between strains of the same species, accessory gland protein differs in structure and quantity (Raviram and Ramesh, 2002) [27]. Its structure and function are also known to be impacted by both inherent and external influences (Wolfner, 1997) [34]. The goal of the current study is to comprehend how elevation affects the reproductive success of three sympatric species of *Drosophila* in the Chamundi Hills, specifically *D. malerkotliana, D. bipectinata, and D. nasuta* (accessory gland characteristics and sperms to mated female).

Materials and Methods

In three different wild localities of Chamundi hill, Mysore, researchers looked at the altitudinal and seasonal variations in population density of three sympatric *Drosophila* species, *D. malerkotliana, D. bipectinata,* and *D. nasuta.* The locations of the chosen collection points were 25° 11' N latitude and 94° 55' E longitude. From January 2021 to December 2021, monthly fly collections were undertaken at elevations of 650 m, 800 m, and 950 m for this purpose. Methods such as bottle trapping and net sweeping were both employed.

Vegetation at 650 metres: Mango orchards, *Acacia catechu, Anacardium occidentale*, and other trees encircled the base of the hill. *Celastrus paniculata, Cipadessa baccifera, Clematis trifolia, Dalbergia paniculata, Dioscorea pentaphylla, Bombax ceiba, Breynearestusa, Cassia spectabilis, Ficus bengalensis, Ficus religiosa, species of Glyrecidia, Exercise sylvestres, Ichnocarpus frutescens, Lantana camera, Hibiscus malva Tectonagrandis, Sidaretusa, Phyllanthus species, Tamarindus indica, Thunbergia species, Pongamia glabra, and several shrubs, including cacti.*

The vegetation at 800 metres and 950 meters: There were a number of significant plants discovered in these areas, including *Albizzia amara, Andrographisser pellifolia, Argyria* species, *Bignonia species, Breynearestusa, Bridalia species, Cassia fistula, Cassineglauca, Eucalyptus grandis, Garcinia species, Lantana camera, Phyllanthus microphylla, Sidarhom bifolia, Terminal Acacia catechu, Anacardium occidentale, Autocarpus integrifolia,* and other plants can be found in the 950-meter-high vegetation at the summit of the hill. Species of jasmine, curcus, camera of lantana, *Murraya paniculata, Tamarindus indica, Zizipus jujuba, Leusaspera, Mallotus philippensis,* and *Tamarindus indica.*

Bottle trapping involved attaching 250 mL milk bottles to twigs beneath small plants that were three to five feet above the ground, along with ripe bananas that had been crushed and coated with yeast. Five traps were kept at each altitude. The bottles were retrieved from the bushes the following day after having their mouths sealed with cotton. The flies were moved to new bottles and fed with wheat cream agar medium, which is made of 100 grams of wheat flour, 120 grams of raw sugar, 10 grams of agar agar, and 7 millilitres of propionic acid that has been boiled in 1,000 millilitres of water and cooled.

The fruits used were *Musa acuminate* (Banana), *Citrus sinesis* (Orange*), Magnifera indica* (Mango), *Carica papaya* (Papaya), and *Artocarpus heterophyllus* (Jack fruit). All the fruits were crushed and the mixture was placed underneath the tree shades one day before collections, where the flies were attracted. Net sweeping was done on naturally rotting fruits available in the collection sites. For the bottle trapping method, 12 sweeps were conducted at each location. Milk bottles with a capacity of 250 ml were tied to small bushes at a height of 3 to 5 feet above the ground. The bottles' mouths were then covered with cotton after 24 hours, and the collected flies were transferred to new bottles with wheat cream agar medium (which contains 100g wheat powder, 120g raw sugar, and 10g ag). Flies were moved to the bottles holding fresh food after each sweep. In order to preserve consistency in collection in each locality, five sweeps were performed at each location. The lab was where the flies were transported, isolated, identified, and sexed. Using taxonomical characteristics such body pigmentation, sex combs, and genital plates, the collected males were identified. The collected females were put through isofemale lines because there are no such taxonomical markers in females of the *Drosophila* species. For species identification, male flies that were produced from the offspring of isofemale lines were used. The methodology and the quantity of baits utilised at the collecting sites were uniform, and this was maintained.

Individual females were put into a culture vial containing wheat cream agar media, and the progenies that were produced were used to identify the individual females. The collected *Drosophila* flies were categorised using a number of keys according to their taxonomic classifications (Sturtevant 1927; Thorckmorton 1962; Bock 1971) [32, 33, 10]. Premonsoon from February to May, monsoon from June to September, and postmonsoon from October to January were used as the divisions for the entire year in order to study seasonal variance.

In the current investigations, offspring from the isofemale lines of *D. malerkotliana, D.bipectinata,* and *D. nasuta* were used for the following experiments.

Altitude effects on accessory gland protein secretion, mating latency, copulation duration, fecundity, fertility

The amount of accessory gland protein, the length of the copulation, fecundity, and fertility are all impacted by altitude. For each of the three sympatric species under research, virgin females and mated/unmated males from low, middle, and high altitudes were utilised to examine how altitude affected the amount of accessory gland secretion. A virgin female aged 5 to 6 days and an unmated low, middle, or high altitude male were each aspirated into an Elens-Wattiaux mating chamber and studied for an hour in order to acquire mated males. Within an hour, unmated pairs were removed. If copulation takes place, the duration of each pair's copulation as well as the mating latency (the amount of time between the introduction of the male and female into the mating chamber and the start of copulation) were noted. Each mated female was aspirated into a fresh vial of wheat cream agar media once every 24 hours until she passed away shortly after copulation. It was recorded how many eggs were laid and how many offspring were born.

Males from various elevations, both mated and unmated, had their samples processed separately for the analysis of accessory gland protein. For each height, 10 pairs of accessory gland secretions from each route were dissolved in 25 l of sample buffers. The Bradford method was used to estimate the amount of accessory gland protein, with BSA serving as the reference. At 595 nm, the sample's OD was read against a blank. Each of the three groups of males those living at low, middle, and high altitudes had fifty replicates run individually. Separate experiments were run for each of the three sympatric species studied.

Using the SPSS 10.0 programme, a two-way ANOVA followed by a Tukey's test was performed on the data of mating latency, copulation duration, fecundity, fertility, quantitative accessory gland and sperm traits.

Effects of altitude on accessory gland size, the number of main cells (per lobe), and the size of the main cells in accessory gland

For each of the three sympatric *Drosophila*species following method was used to study the above traits. Males from low, intermediate, and high altitudes had their accessory glands individually removed using Medium A (Santhosh *et al.*, 2013) [28], and they were then preserved in 1N HCl for five minutes. Using a digital camera, accessory gland images were captured at X40. Using a tally counter and the method described by Santhosh *et al.*, 2013 [28], the size of the gland (measured in cm^2), the number of main cells in the auxiliary gland, and the size of the main cells were tallied in each gland at X40. Calculations of accessory gland size, cell number, and cell size were made for each altitude using a total of 50 repetitions. Separate experiments were run for each of the three sympatric species studied. Using the SPSS 10.0 programme, the method ANOVA was performed on mean cell number, cell size, and accessory gland size data.

Altitude effect on sperm counting

A virgin female (5–6 days old) and an unmated male (from low, middle, and high altitudes) were aspirated separately into an Elens–Wattiaux mating chamber and watched for an hour. Within 1 hour, unpaired pairs were removed. If copulation is successful, allow the couple to finish mating. For each height, a total of 50 replicates were performed. The reproductive organ of the mated female was removed shortly after mating and dissected out in the 20 l Beadle-Ephrusi Saline solution (128.3 mMNaCl, 4.7 mMKCl and 23mM CaCl2). Due to the possibility of sperm dissociation into the solution, 2% of lacto aceto-orcein was added to the slide without draining the saline, and the Olympus CX21 was used to count the quantity of sperm at a 40x magnification. For each of the three analysed sympatric Drosophila species, separate experiments were run.

Results and Discussion

The proteins from the male accessory glands and the sperms that are transported to the female during copulation can be used to explain the reproductive success of three sympatric *Drosophila* species from different altitudes of the Chamundi hills. The structure and function changes seen in the male accessory gland could be used to account for the accessory gland proteins. Previous research has demonstrated that the accessory gland's structure remained constant across all *Drosophila* species (Santhosh *et al.*, 2013) [28]. A single layer of the secretory epithelium rests on a thin layer of muscle cells in a typical *Drosophila* accessory gland. Binucleate hexagonal main cells, which make up 96 percent of the gland's secretory cells (100484 cells per lobe), are the most common form. The secondary cells, which are binuclear, spherical, and contain large vacuoles, make up the final 4% (439 cells per lobe). Different *Drosophila* species were shown to have comparable patterns of accessory gland secretion production and accumulation (Santhosh *et al.*, 2013) [28]. However, various species secrete varied amounts of accessory gland secretion (Anitha and Krishna, 2020) [3]. The secretion of accessory glands is affected by both biotic and abiotic stimuli (Raviram and Ramesh, 2002) [27]. Additionally, topography has an impact on the ability of several species that coexist in a certain environment to reproduce successfully (Guruprasad *et al.*, 2010) [19]. Unmated males from various elevations were used in the current investigation to examine changes in accessory gland anatomy and secretion associated with altitude.

Table 1a and b showed that three sympatric species of *Drosophila* in the Chamundi Hills, including *D. malerkotliana*, *D. bipectinata*, and *D. nasuta*, experienced altitude-related variations in the abundance of accessory gland proteins in unmated males. With increasing altitude, these species' accessory gland protein levels declined. Males living at low altitudes had the most accessory gland proteins, whereas males living at high altitudes had the least. This demonstrates that elevation is a significant characteristic that has been shown to affect the quantitative variation of accessory gland proteins. Prior research on altitude change has also highlighted elevation as a significant component that has been linked to biodiversity and population fluctuations (Alwyn *et al.* 2019) [2].

It is believed that differences in accessory gland size, accessory gland cell number and size, or accessory gland secretory activity with altitude could account for differences in the amount of accessory gland proteins in unmated males from different altitudes. This is due to the fact that as elevation changes, so do the biotic and abiotic elements that are prevalent at different elevations. Table 1a and b showed that accessory gland size was found to decrease with higher elevations in three sympatric species of *Drosophila*. At low altitudes, accessory gland size was determined to be the largest; when it ascended to high altitudes, it shrank in size. This shows that the size of the accessory gland, which determines how many accessory gland proteins are produced, is a crucial factor. The bigger the accessory gland, the more accessory gland proteins are produced (Santhosh *et al.,* 2013) [28]. Therefore, low altitude males in the current study in three sympatric species of *Drosophila* had larger accessory glands and produced more accessory gland proteins. In contrast, Ravi Ram and Ramesh (2002) [27] discovered no correlation between the size of the gland and the production of accessory gland proteins in *D. nasuta*. Table 1a and b also shows that main cell number and main cell size in the accessory glands of all three sympatric species of *Drosophila* at various altitudes in Chamundi Hill varied with altitude and decreased with increasing altitude, indicating that low altitude flies had significantly more main cells in their accessory gland. This shows that the shape of accessory glands is likewise influenced by altitude. Our findings support laboratory investigations of accessory glands in many *Drosophila* species (Abolhasan *et al.,* 2005, Anitha and Krishna, 2020) [1,3]. They discovered significant biotic and abiotic influences on the morphology of the accessory gland. In *D. melanogaster*, it has been found that the production of two particular accessory gland proteins, msp355a (Acp26Aa) and msp355b (Acp26Ab), is developmentally controlled, according to the research of Monsma (1990) [22]. While conducting research on *D. nasuta,* Ravi Ram and Ramesh (2002) [27] hypothesised that the amount of accessory gland protein synthesis may also be influenced by the secretory activity of accessory gland cells. Therefore, in the current study's three sympatric species of *Drosophila*, the effects of elevation on the structure and function of the accessory gland may be accounted for by differences in the quantity of accessory gland proteins in unmated males of different altitudes.

The form and amount of secretion in the accessory gland are influenced by both biotic and abiotic variables, according to earlier research on accessory glands in other species of *Drosophila* (Ravi Ram and Ramesh, 2002; Santhosh *et al.,* 2013) [27, 28]. The size of the flies, age, and temperature are other factors that are known to affect the number of main cells, main cell size, size of the accessory gland, and amount of secretion generated. The observed variation may be caused by natural variables present at various altitudes of the Chamundi Hills, including temperature, humidity, rain fall, photoperiod, intra- and interspecific relationships, like population density, population age distribution, and competition and relationships between *Drosophilids* and their hosts and predators. Additional altitudinal investigations have revealed that essential *Drosophila* species parameters including viability, fertility, reproduction, development time, and other elements that determine the rate of population expansion and survival are virtually always affected by temperature and rainfall variations (Alwyn *et al.,* 2019) [2]. The endurance of the flies is also influenced by temperature. The availability of resources is also influenced by temperature, precipitation, and light intensity, particularly in relation to the times when different vegetation are in bloom and bearing fruit, which provide the majority of the locations for oviposition and feeding for the community (Brncic *et al*., 1985) [11]. Therefore, elevation, a significant element that is known to impact the accessory gland's shape and function in species *of Drosophila* at various altitudes in the Chamundi Hills. The causes of the observed phenomenon can be attributed to changes that take place as one climbs an altitude transect, which may include changes in temperature, precipitation, partial pressure of atmospheric gases, atmospheric turbulence, wind speed, and radiation input, including short-wave ultra-violet radiation at various wavelengths (Barry 1992) [8].

Through the act of copulation, male *Drosophila* transmits accessory gland secretions to mated females. The quantity of accessory gland secretory proteins in mated males at various elevations was also examined in the current study. Table 1a and b demonstrates that the amount of accessory gland secretory proteins decreased with increasing altitude in all three sympatric species of *Drosophila*, even in mated males. This implies that there is a correlation between altitude and the amounts of accessory gland protein transmitted to the mated female. The amount of accessory gland protein transmitted was estimated in the current study by dividing the amount of accessory gland protein from unmated males by the amount from mated males. Table 1a and b shows that in all three sympatric *Drosophila* species under study, low altitude males transferred the greatest amount of accessory gland protein to the mated female, whereas high altitude males transferred the least amount of accessory gland protein. It is known that a variety of variables, including the length of copulation, the length of the mating process, the size of the male, and the status of the mating, affect the amount of accessory gland protein transmitted (Santhosh *et al*., 2013) [28].

In the current study, low-altitude males copulated longer and mated more frequently than high-altitude males (Figure 1 and Table 2 b). This implies that altitude-related modifications in Drosophila mating behaviours really occur. Additionally, this study raises the possibility that there is a connection between altitude, mating delay, duration of copulation, and the quantity of accessory gland proteins. One of the key elements of *Drosophila's* mating behaviour is mating delay, which also plays a crucial role in fitness (Hegde and Krishna, 1997) [20]. It is recognised as having an impact on the variations in qualities including fecundity, fertility, and lifespan (Hegde and Krishna, 1997) [20].

Males in *Drosophila* transport sperms to the mated female in addition to accessory gland secretion during mating. However, it was unknown whether or not the number of sperm and accessory gland proteins transported is affected by altitude. To better understand the relationship between altitude, the amount of accessory gland proteins, and the quantity of sperm transferred to the mated female, the quantity of sperm transferred by males at various altitudes in all three sympatric species of *Drosophila* was also studied in the current study. In all three of the sympatric species of *Drosophila* that were investigated, it was shown that low altitude males had much more sperm and accessory gland protein transmitted to the mated female than intermediate and high altitude males(Table 2a and b). This shows a relationship between sperm transported to mated females and the quantity of accessory gland protein. It is known that an accessory gland secretion delivered to the female during mating is linked to post-mating physiological changes in females, such as increased receptivity, fecundity, and fertility (Wolfner, 1997) [34].

In the current study, females mated with low altitude males received more accessory gland proteins and more sperm, which allowed them to produce greater numbers of eggs and reproduce more successfully and give birth to more offspring than females mated with high and moderate altitude males (Figure 2 and 3;and Table 2b).These *Drosophila* species studies thus imply that topography and season, in addition to physical and biotic factors, also influence species reproductive fitness at various altitudes. Across the current study, *D. malerkotliana* had the highest reproductive success among the three sympatric species of *Drosophila*, while *D. nasuta* had the lowest in all altitudes and study seasons in the Chamundi hills. This is because, in comparison to *D. bipectinata* and *D. nasuta* males, *D. malerkotliana* males significantly transported more sperm and accessory gland protein to the mated female in all three sympatric species of *Drosophila* under study (Table 2a and b). This shows that there is a positive correlation between sperm transport to mated females and the level of accessory gland protein. It is known that an accessory gland secretion delivered to the female during mating is linked to post-mating physiological changes in females, such as increased receptivity, fecundity, and fertility (Wolfner, 1997)[34]. In all Chamundi Hills heights, *D. malerkotliana* had noticeably greater reproductive success than the other two species. This indicates that *D.malerkotliana* was the dominating species among the three sympatric species, followed by *D.bipectinata,* which is followed by *D.nasuta.* The ability of the dominant species to take advantage of the circumstances present in certain habitats can be linked to the occurrence of one species dominating the others in any particular area. The results of our study are in agreement with those of Guruprasad *et al.* (2010) [19], who demonstrated that the quantity and density of *Drosophila* species decreased with increasing altitude at Chamundi Hill in Mysore, Karnataka. The present study corroborates the work of Muniyappa and Reddy (1981) [28]. In our study, the availability of lush vegetation, which supplied good supplies of food and a more hospitable climate at lower altitudes than at the higher altitudes, can be ascribed to the occurrence of more species at lower altitudes.

Therefore, these results imply that altitude had a sizable impact on reproductive success in three sympatric species in the Chamundihills. *D. malerkotliana*, one of three sympatric *Drosophila* species, surpasses *D. bipectinata* and *D. nasuta* in terms of reproductive success, becoming the dominating species across all heights.

Table 1a: Altitudinal variation on accessory gland traits in three sympatric Drosophila species

Species	Altitudes	Accessory gland traits					
		Accessory gland Main cell numbers	**Accessory gland size (c m ^{2})**	**Accessory Main cell size (mm)**	**Accessory gland protein in unmated males**	**Accessory gland protein in mated males**	**Accessory gland protein transferred to mated female**
D.malerkotliana	Low	1928.98±11.86^a	0.242±0.0082 ^{a}	0.018±0.0027 ^{a}	14.19±0.031 ^{a}	10.39±0.052 ^{a}	3.77±0.054 ^{a}
	Middle	1729.96±3.04^b	0.231±0.0068 ^{b}	0.013±0.0002 ^{b}	13.33±0.034 ^{b}	11.67±0.057 ^{b}	1.56±0.062 ^{b}
	High	1586.14±12.30^c	0.224±0.0053 ^{c}	0.079±0.0006 ^{c}	12.27±0.019 ^{c}	10.42±0.054 ^{c}	1.77±0.053 ^{c}
D.bipectinata	Low	1810.90±7.94 ^{a}	0.242±0.0011 ^{a}	0.054±0.003 ^{a}	11.24±0.0087 ^{a}	6.75±0.0038 ^{a}	4.48±0.012 ^{a}
	Middle	1505.98±8.72 ^{b}	0.265±0.0041 ^{b}	0.073±0.002 ^{b}	11.26±0.0083 ^{b}	7.07±0.0049 ^{b}	3.42±0.77 ^{b}
	High	1516.25±6.802 ^{c}	0.239±0.0047 ^{c}	0.070±0.0002 ^{c}	11.07±0.037 ^{c}	7.18±0.006 ^{c}	3.62±0.043 ^{c}
D.nasuta	Low	1538.08±4.24 ^{a}	0.271±0.0084 ^{a}	0.074±0.0042 ^{a}	13.74±0.050 ^{a}	10.64±0.068 ^{a}	3.13±0.023 ^{a}
	Middle	1475.28±6.37 ^{b}	0.286±0.0066 ^{b}	0.084±0.0003 ^{b}	11.96±0.031 ^{b}	9.89±0.054 ^{b}	2.11±0.031 ^{b}
	High	1296.32±11.94 ^{c}	0.288±0.0071 ^{c}	0.0093±0.0006 ^{c}	11.66±0.79 ^{c}	9.57±0.049 ^{c}	2.33±0.049 ^{c}

Different letters on bar graph indicates significance at 0.05 level by Tukey's post hoc test

Table 1b: Altitudinal variation in accessory gland traits in sympatric Drosophila species at Chamundi hills

Parameter	Source	Mean sum of square	Df	Sum of square	F-value
Accessory gland main cell number	Altitude	11286108.480	2	5643054.705	1902.097***
	Species	7872117.480	2	3936058.740	1326.723***
	Species*altitude	1641154.718	4	410288.679	138.296***
	Error	1308338.297	441	2966.754	
	Total	112716448.290	450		
Accessory gland main cell size	Altitude	1.003	2	6.372	0.863^{NS}
	Species	1.001	2	1.002	9.533*
	Species*altitude	0.002	4	1.004	3.408*
	Error	0.033	441	7.385	

	Total	0.0077	450		
Accessory gland size	Altitude	0.030	2	0.015	123.07**
	Species	1.218	2	1.109	811.08**
	Species*altitude	0.065	4	0.016	121.908***
	Error	0.006	441	1.342	
	Total	28.682	450		
Quantity of Accessory gland proteins(unmated)	Altitude	171.644	2	85.822	1549.139**
	Species	367.440	2	183.720	3316.247***
	Species*altitude	57.274	4	14.318	258.458***
	Error	24.431	441	0.055	
	Total	68288.391	450		
Quantity of Accessory gland proteins(mated)	Altitude	8.543	2	4.272	39.701**
	Species	1101.428	2	550.714	5119.569***
	Species*altitude	99.970	4	24.992	232.336
	Error	47.439	441	0.108	
	Total	40571.143	450		
Quantity of Accessory gland proteins transferred to mated female	Altitude	205.175	2	102.587	654.005**
	Species	139.217	2	69.608	443.762***
	Species*altitude	32.864	4	8.216	52.378***
	Error	69.175	441	0.157	
	Total	41581.234	450		

***significant at 0.0001 level;**significant at 0.001;*significant at 0.05 level; NS=non-significant

Table 2a: Altitudinal variation on sperm traits in three sympatric Drosophila species

Species	Altitudes	Sperms traits		
		Sperms in spermatheca	Sperms in seminal vesicle	Sperms in transferred
D. malerkotliana	Low	60.37±0.49[a]	178.14±0.80[a]	4625±7.14[a]
	Middle	58.69±0.54[b]	161.71±1.29[b]	4050±9.12[b]
	High	54.83±0.37[c]	147.97±0.611[c]	3825±4.15[c]
D. bipectinata	Low	26.19±0.015[a]	613±0.050[a]	4544±3.04[a]
	Middle	23.74±0.019[b]	145.93±0.08[b]	4239±0.72[b]
	High	22.78±0.011[c]	139.37±0.05[c]	4050±2.72[c]
D. nasuta	Low	16.22±0.01[a]	91.52±0.11[a]	2867±4.61[a]
	Middle	14.09±0.041[b]	82.72±0.44[b]	2361±5.22[b]
	High	12.09±0.14[c]	75.73±0.56[c]	1849±2.73[c]

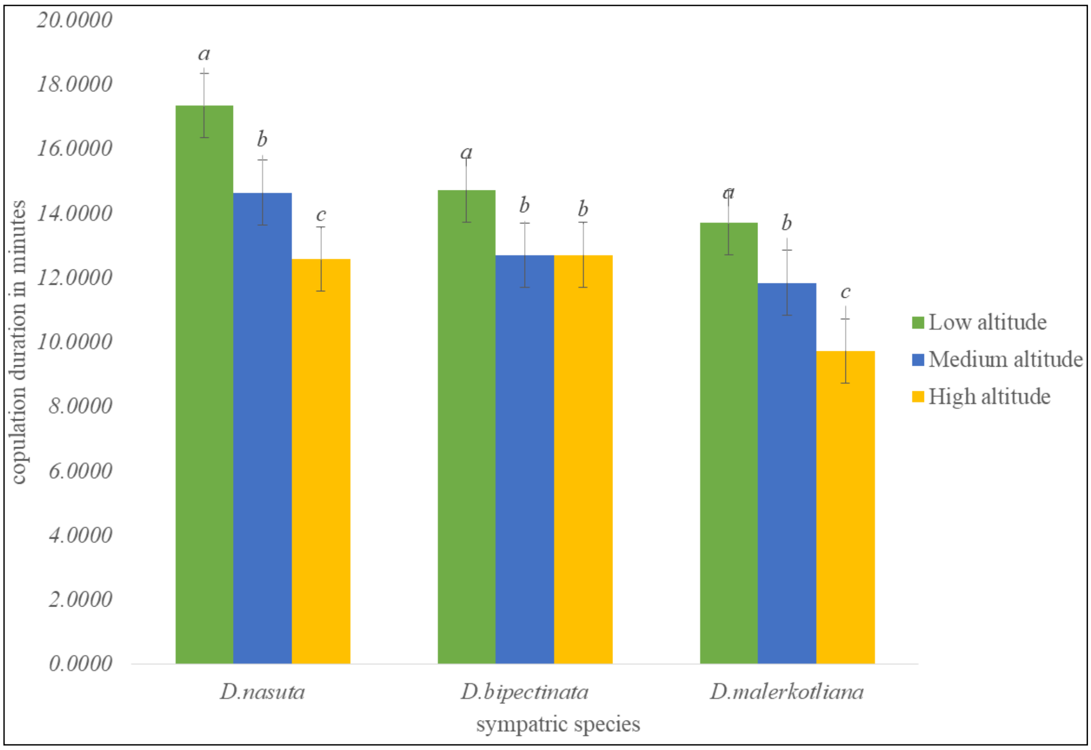
Different letters on bar graph indicates significance at 0.05 level by Tukey's post hoc test

Table 2b: Altitudinal variation in sperms and fitness traits in sympatric Drosophila species at Chamundi hills

Parameter	Source	Mean sum of square	Df	Sum of square	F-value
Sperms in	Altitude	2606.604	2	1303.302	350.508***
	Species	161686.156	2	80843.078	21741.802***
	Species*altitude	212.911	4	53.228	14.315***
	Error	1639.781	441	3.718	
	Total	616300.451	450		
Sperms in seminal recepticle	Altitude	85285.114	2	42642.557	2519.673***
	Species	498409.841	2	249204.920	14725.075***
	Species*altitude	22171.361	4	5542.840	327.517***
	Error	7463.416	441	16.924	
	Total	8036262.666	450		
Total sperms transferred to mated female	Altitude	61698461.362	2	30849230.681	7000.539***
	Species	371544611.111	2	185772305.555	42156.849***
	Species*altitude	3040594.770	4	760148.693	172.499***
	Error	1943351.772	441	4406.693	
	Total	6409582590.053	450		
Fecundity (in no.)	Altitude	24000.356	2	12000.178	277.726***
	Species	18082.689	2	9041.344	209.249***
	Species*altitude	1094.178	4	273.544	6.331*
	Error	3499.900	441	43.209	
	Total	794335.000	450		
Fertility (in no.)	Altitude	25894.867	2	12947.433	275.116***

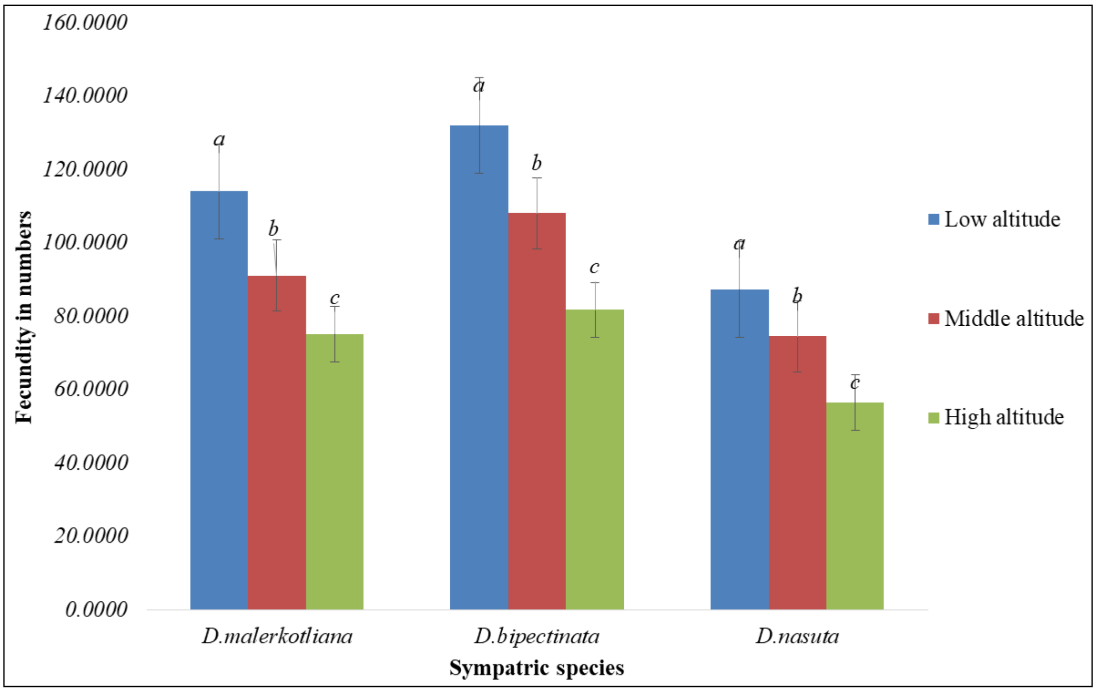
	Species	7908.867	2	3954.433	84.027***
	Species*altitude	1968.667	4	492.167	10.458***
	Error	3812.000	441	47.062	
	Total	430042.000	450		
Copulation duration (in min)	Altitude	1356.179	2	678.090	4467.206***
	Species	753.456	2	376.728	2481.856***
	Species*altitude	13.246	4	3.311	21.815***
	Error	66.941	441	.152	
	Total	79529.906	450		

***significant at 0.0001 level



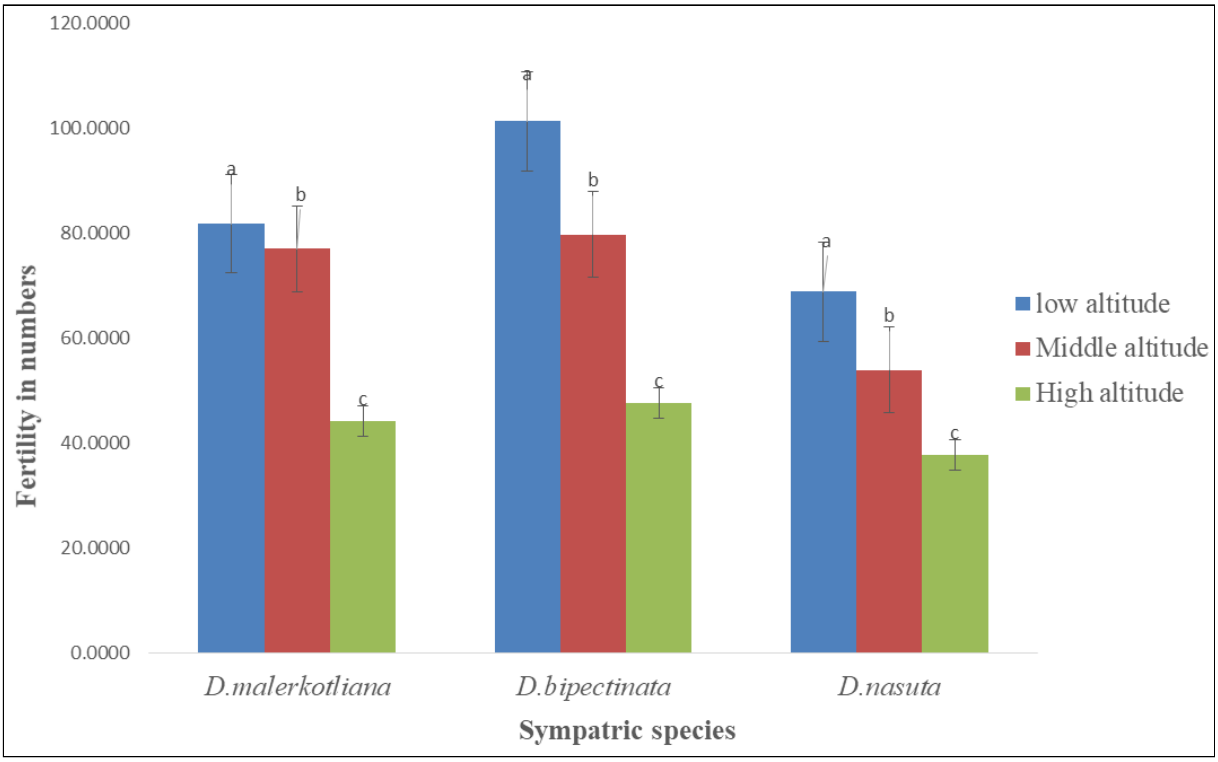
Different letters on bar graph indicates significance at 0.05 level by Tukey's post hoc test

Fig 1: Altitudinal variation on copulation duration in three sympatric Drosophila species



Different letter on the bar graph indicates significance at 0.05 level by Tukey's post hoc test

Fig 2: Altitudinal variation on fecundity in sympatric Drosophila species



Different letter on the bar graph indicates significance at 0.05 level by Tukey's post hoc test

Fig 3: Altitudinal variation on fertility in sympatric Drosophila species

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