REVIEW ARTICLE

Structural characterization of appendages of different butterfly species (Insecta: Lepidoptera) from Mohali, Chandigarh, India

Abstract

Insects form a very important and integral part of any ecosystem. Their contributions include agriculture, biodiversity, human health as well as conservation of natural resources. Butterflies belonging to this category falls under suborder of Lepidoptera called Rhopalocera. They have large and vividly coloured wings, which are used for both defence and communication.Butterflies play important role in the ecosystem. Lives of plants and butterflies are interlinked. These insects enhance the aesthetic value of environment by their wonderful wing colours. At rest, their wings are held together upright, which sets them off from all other insects. Butterflies are extremely important in pollination as most of them are important pollinating agent for many plant species, contributing to the enhancement of genetic diversity and stability within the ecosystem. However, their fascinating complex sensory systems go beyond their obvious beauty and importance in ecology.For instance, their legs are more than just organs of locomotion; they are actually very fine devices with sensilla. These sensilla function as receptors so that butterflies would be able to sense various aspects of their surroundings. This review article deals with different species of butterflies and the different sensory adaptations of these sensilla.By looking at these sensory structures, one can gather information about different behavioural patterns, ecological interactions and evolved tactics of an organism. Studying butterfly appendages provides critical insights into their morphological adaptations, ecological roles, behavioural strategies, offering a deeper understanding of their evolutionary success. It highlights the structural and functional adaptations of butterfly leg sensilla across species, providing valuable insights into behavioural patterns and ecological interactions, thereby advancing our understanding of insect- environment relationships.

Keywords: Ecosystem,Insects, Lepidoptera, Pollinating agents, Rhopalocera, Sensilla.

Introduction

Insects are very important due to their ecological role, effect on agriculture, diversity and social well- being. They are most prevalent fauna and found virtually everywhere. Number of insects is three times higher as compared to other animals. Some species are listed as pests and many species have not been exposed yet. Pests are plants or animals that harm people or causethem concern. It’s a useless insect or another animal that spells things like food, livestock, crops etc.(Khan;2021).Lepidoptera is the second largest insect order consisting of moths and butterflies. Itis among the most common insect orders and consists of most easily recognisable insects in the world today (Lin; 2020). Lepidopteran insects exhibit numerous differences in their basic body forms, which have evolved to improve their chances of survival. Almost every class of Lepidopteran insects has a few types of membrane- forming wings. Lepidopteran insects, which include moths, butterflies and nearly all other insects are holometabolous which means they undergo through entire mutation. While mating, adult insects deposit their eggs on plants which serves that serves as their larval host. The first stage of larvae isreferred as caterpillars. They have cylindrical shaped body with properly evolved mouth parts. As soon as they mature, the first stage of larva grows into a pupa. Following the pupa's mutation, an adult insect with sexual development appears.In addition,female insect in many classes can produce between two hundred and six hundred eggs, but in other classes, the variety can produce thirty thousand eggs in a single day. Butterflies are often polymorphic, and many species make use of camouflage, mimicry to evade their predators (McClure& Melanie; 2019).Butterflies are also very important from an ecological perspective as pollinators, as they assist in plant reproduction and thereby the diversity of life forms within the ecosystem. While drinking nectar, butterflies cluster on flowers and carry pollen from one flower to another supplying a abundance of plants with reproductive services, many of which yield edible goods for the organisms. Butterflies also plays an important role in food web as well, as they are food for birds, reptiles and other predators creating an equillibrium in nature. Their prevalence as well as their number clearly are the indicators of health of ecosystem especially when there are changes in environment such as pollution or land degradation. Therefore, butterflies are important for ecosystem stability and biome variety.

Modern technologies like high- resolution imaging and genetic sequencing, continues to uncover hidden facts of Lepidopterans such as their navigation mechansim during migration and their role in nocturnal ecosystems. These findings demonstrate how these delicate insects are actually essential component of biodiversity, unveiling the complex relationships that connect every aspect of the natural world.

Butterfies as contributors to Ecosystem

Pollination: "Pollination is the process in which pollens are transferred from male parts of flower to female parts of flower and reproduce sexually even over large areas. Nectar produced from flower contains nutritious vitamins, lipids, sugar, amino acids etc. which is important food source for pollinators" (Webb; 2008). "Baker demonstrates that nectar of many flowers, at which adult Lepidoptera feed, contains significant concentrations of a wide range of amino acids, which must contribute to its nutritional value" (Baker; 1973).

Genetic Variation in Plant Species:

"Kearney point out that butterflies collect nectar from plant species, which induce genetic variation in the plants. Some butterfly species migrate over long distance and share pollens across plants which are far away from one another. This helps plants to recover against disease and gives them a better chance at survival" (Kearney; 2015).

Reduce Pollution:

Some species of butterfly help to reduce air pollution. These species decrease carbon dioxide in air. The host plants of monarch butterflies and catterpillars absorb carbon dioxide and reduce amount of air pollution.

Provide Food for other Organisms:

Butterflies provide food for number of animals such as birds, reptiles, amphibians etc. and catterpillars provide an occassional meal for scorpions and ants. Eggs of some flies and wasps live as parasite inside catterpillar's body and feed on it. If populations of butterfly diminish, then population of birds, mice and other animals that rely on them as food source will also reduce. This loss will collapse the entire ecosystem. Stephen Dickie, explains: "Birds plan their whole breeding season around when caterpillars will be most abundant. If butterflies and caterpillars are depleted then there will be less food for developing chicks.

Predators:

Some butterfly larvae feed on harmful insects for example Hoverfly larvae are predators of aphids(Ehrlich;1984) so, catterpillars are also use as biological pest control.

Butterfly Body parts:

Body of butterflies is made up of three segments; head, thorax and abdomen.

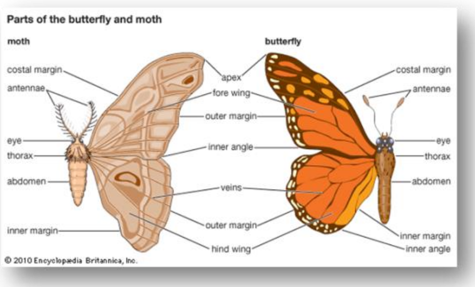

Fig1: Body parts of Moth & Butterfly.

Head: The head is globular in size and top of head is called as vertex; the anterior portion is called asfront. Large compound eyes are situated on each side of head between which antennae are present. Antennae help butterflies to find food or mate.The antennae of butterflies are always provided at the extremity with a club- shaped enlargement, and because of this clubbed form of the antennae the entire group are known as the Rhopalocera. The upper lip of butterfly is quite small and is called as labrum. Rudimentary mandibles are present on both sides of mouth.They have a long, straw- like structureknown as proboscis, which is coiled under their head. The proboscis is used for sipping nectar and water.The length of proboscis varies;at its base and on either side are place what are known as the maxillary palpi, which are very small.

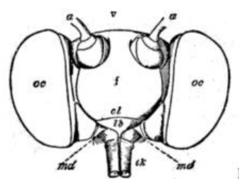

Fig2: Head of milkweed butterfly: (v) vertex; (f) front;(cl) Clypeus; (lb) labrum; (md) mandibles; (tk) proboscis.

Thorax: The central segment of butterfly's body is referred to as thorax.It is somewhat flattened upon its upper surface. Thorax is made ofthree fused segments, the prothorax, mesothorax, and metathorax, each with a pair of legs.(Carde; 2009).Legs are attached in pairs to these subdivisions of thorax. Therefore sometimes anterior pairs of legs is called as prothoeacic legs, the second pair as mesothoracic legs, and the latter pair as the metathoracic legs.

Anterior pair of wings are present on either side of mesothorax.

Abdomen: Normally it is made up of nine segments and in most butterflies it is shorter than hind wings. There are various appendages on the last segment which are mainly sexual in nature.

Butterfly Legs

Butterflies havesix legs arranged in three pairs. First part which is nearest to the body is called as coxa, with which a ring like piece trochanter is placed. Femur is attached with trochanter which is connected and forming an angle with tibia. To the tibia, tarsus is attached, the last segment of which bears the claws. These claws are very minute and blunt in butterflies and in moths they are strongly hooked. In some groups of butterflies anterior pair of legs is aborted or dwarfed, either in one or both sexes. It is useful in determining the location of species in their systematic order.

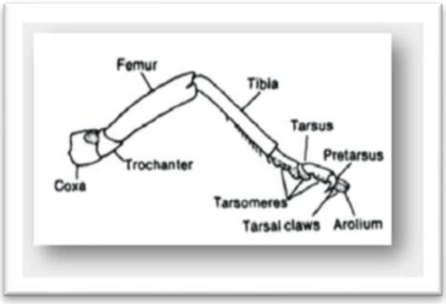

Fig3: Generalize structure of lepidoptera leg.

The morphology of the foreleg coxa, trochanter, and femur in butterflies is complex and three-dimensional, making it challenging to depict accurately on a two-dimensional printed page. Additionally, butterflies have hair-like structures on their legs called sensilla.Sensilla also plays important role in feeding. Members ofthe Diplura and Machilidaehave two pairs of abdominal processes in each segmentcalled styli (more laterally located) andeversible vesicles(more medially located) (Machida, 1981).

Lepidoptera also haveolfactory organs on their feet, which aid the butterfly in "tasting" or "smelling" out its food.(Heppner, 2008.). “In the larval form there are 3 pairs of true legs, with up to 11 pairs ofabdominal legs (usually eight) and hooklets, called apical crochets” (Cranston; 2004). “In some basal moths, these prolegs may be on every segment of the body, whileprolegs may be completely absent in other groups, which are more adapted to boring and livingin sand”.(Carde, 2009). “In addition to the structural changes, limbs have also been evolving in number. Most adult insects have three pairs of walking legs, juveniles display variations in both thoracic and abdominal appendages, only some of which are used for walking” (McGraw-Hill, 1935).

Nymphalidae

“In Nymphalidae forelegs in both sexes are vestigal and useless for walking. They have reduced forelegs which make them appear as if they have only have two pairs of legs. Therefore butterflies of this family are also called as “Four Footed Butterflies”. In many forms of these subfamilies, the forelegs are kept pressed against the underside of thorax and are often present in males” (Bingham, 1905). “Heliconianbutterflies (Nymphalidae) have reduced prothoracic legs as compared to mesothoracic and metathoracic legs. They apparently have no function in males, but are used by females to drum on the host plant prior to oviposition” (Renou1983;Thiele *et al.*, 2016).

Remaining two pairs of legs in Nymphalidae are extremely hairy and resemble brushes, so they are also named as "Feather legged butterflies".

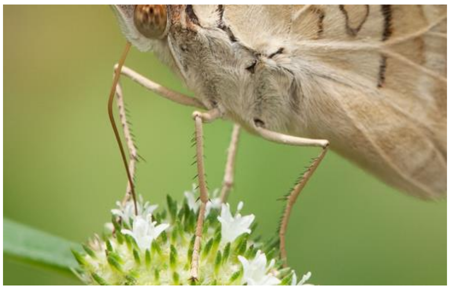

Fig4: Sharp spiny hairs on legs of Blue Pansy.

Fig 5: *Heliconius melpomene* rudimentary forelegs highlighted.

Several members of nymphalidae are considered model organisms in evolutionary biology(Joron, *et al*; 2006).

Papilionidae

All six les are present and well developed in all species of Papilionidae family. They have longest legs (measured from coxa to tarsus) as compared to the species in other families. They have more muscular looking femur as compredto species of other families.

Fig7: Long slender legs in Papilionidae species.

Tarsal segments are typically long. The body of a Papilionid butterfly is lifted high and some distance away from the food source when it puddles or uses its legs to grasp onto a flower while feeding. Majority of Papilionid species have tibia spurs and femur, tibia and tarsal segments may contain small spiny hairs which help in holding and gripping onto flowers as butterfly feeds. Butterfly's legs have noticable tarsal claws which help to hold on branches or leaves on which the butterfly is resting.

Fig6: Lime butterfly uses its sharp tarsal claws to hold something while resting.

Pieridae

Every member of Pieridae family has six completely grown and working legs.

Fig8: All six developed legs in Pieridae.

Legs of many species typically match the color of butterfly's abdomen or wings. There is usually a darker longitudinal stripe throughout the length of the leg.The legs of butterflies in Pieridae are shorter than those of Papilionidae. The femur is coated with soft, short hairs. They also have tarsal claws and fine hairs on the tarsal area of leg to help it hold on to its perch while it stops to rest or while feeding. They use all six legs to hold or balance on a branch.

Riodinidae

The Riodinidae is a large family of butterflies with a worldwide distribution. They are noted for extreme diversity in wing shape and pattern, which is perhaps the greatest among all families of the Papilionidae(true butterflies)(Harvey, Donald James; 1987). The Riodinids are unique because only female have six fully developed legs. In male specimens forelegs are diminished to hair like tufts similar tothose like in Nymphalidae and these legs are not functional. Females generally hold their forelegs tightly against the front of the thorax and it appears like they have only four legs to stand on.

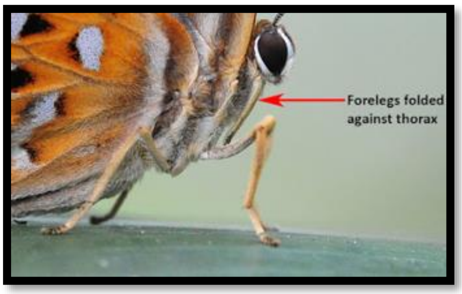

Fig9: Folded forelegs in females

Their legs are short and thick with tarsal segments, especially at the tips close together, resulting in a banned look. Legs of Riodinids also seems to lack noticable tibia spur that is found in other species of butterflies.

Lycaenidae

Lycaenidae is the second largest family of butterflies after Nymphalidae (brush-footed butterflies) with over 6000 species worldwide (Pierce& Naomi; 2002) Members of Lycaeidae ar also called Gossamer-winged butterflies. The diversity of leg structure among the numerous generaof Lycaenidae is truly remarkable. Lycaenid family also shows different leg pattern as compared to other families. Female Lycaenids havefunctional and completely segmented foretarsai, whilenearly all male species feature fused tarsomeres. Males also lack foretarsal claws. However, in both genders foretarsai are used for walking.As a rare exception (70 out of roughly 4500 described species ;Bridges; 1988), segmented foretarsai with claws occur in males of a few Lycaenidae genera. The legs of several species of the Miletinae subfamily (Lycaenidae) is quite amazing like in Miletus species.The legs taper towards the joints and seem flattened.

Fig10: Flat forelegs

The forelegs seem to be devoid of tarsal claws at least in one of the species, and the tarsal are wider towars extremeties. The legs of other species in the Theclinae subfamily are short, stout and ocassionally banned inblack and white while some other species have six legs that seems like that they are wearing white stockings.

Fig11: Six legged Theclinae with pure white legs

The tibia spur is also present in many species across the family. Tarsus is also covered with small spiny hairs which allow butterfly to hold on to its perch firmly.

Hesperiidae

Skippers are group of butterfles that are placed in family Hesperiidae within the order Lepidoptera. Previously they were assigned to distinct superfamily Hesperioidea. They are named so because of their quick, fluttering flight movements.All six legs of skippers are completely developed. In many species femur is covered with thick hairs which gives the illusion of being fairly heavy and muscular.

Fig12: Exceptionally hairy femur of some Hesperiidae species.

In some species the hairs extend to the next segment, tibia. The hairs on Tibia are thick and noticeable. In some species there is an additional pair of tibiaspurs on the hind legs. The hind legs of certain unique species, such as the White Banded Flat, have undergone further evolution to develop a noticeable hair taft that covers the tibia and extends beyond the leg.

Leg sensillain butterflies

A sensillum (plural- sensilla) is a sensory organ that emerges from cuticle of exoskeleton or sometimes rests inside or under it. Sensilla appear as small hairs over individuals's body. Inside each sensillum there are two or four sensory neurons. These neurons or receptors gather information about environment in which the arthropod resides (Alexander ;2007). Sensilla are specially shaped according to the type of information they are gathering. Certain similarities in the structure of sensilla in different arthropod groups can be the result of adaptaions to specific environmets. In holometabolous insects larval sensilla appear to be structurally quite advanced,and more complex than in adult.

Campaniform Sensilla

Campaniform sensilla are a class of mechanoreceptors found in insects, which respond to local stress and strain within the animal's cuticle. Campaniformsensilla function as proprioceptors that detect mechanical load as resistance to muscle contraction (Zill; 2012).Sensory feedback from campaniform sensilla is integrated in the control of posture and locomotion (Tuthill&Wilson;2016).Each campaniform sensillumconsists of flexible dome, which is embedded in a spongy socket within the cuticle and innervated by the dendrites of a single bipolar sensory. Campaniform sensilla are often oval- shaped with long axes. Campaniform sensilla are distributed across the body surface of many insects. Campaniform sensilla are located in regions where stress is likely to be high, including on the legs, antennae and wings (Dinges; 2020)

Campaniform sensilla on legs

On the legs, groups of campaniform sensilla are located close to the joints on all segments except for the coxa, with most sensilla located on the proximal trochanter (Harris; 2020). The number and location of sensilla on the legs varies little across individuals of the same species (Dinges; 2020) and homologous groups of sensilla can be found across species (Harris; 2020).

Function of leg Campaniform sensilla

Campaniform sensilla on legs are activated during standing and walking. Their sensory feedback is thought to reinforce muscle activity during the stance phase (Zill; 2015) and to contribute to inter-leg coordination, much like sensory feedback from mammalian Golgi tendon organs (Pearson; 2008). Feedback from leg campaniform sensilla is also important for the control of kicking and jumping.

Structure

A channel extending throughthe entire thickness of the cuticle, topped with a dome- shaped cap, and lined with a material exhibiting distinct staining reactions compared to the surrounding cuticle. Usually, the cap membrane arches over the surface of the cuticle, but sometimes it can be recessed such that its peak lines p with the surface. Sensilla are present in clusters in specific pattern.The single sensory cell is large and has a distal extension on the underside of the rounded cap’s centre by a highly refractive structure. In oval sensilla, this extension may also extend down the long axis. In some transmitted light configurations, this body makes the dome which seems to be a pore. Other modifies hypodermis calls are usually absent, however occasionally chitogenous cells appear.

Monarch Butterfly

There are six legs on the monarch butterfly. The two forelegs, situated just behind the head, are so tiny that they must be seen under a microscope or magnifying lens. Three pairs of tiny, copper- coloured needles are carried at the tips of each female foreleg.

Fig13: Male and female forelegs.

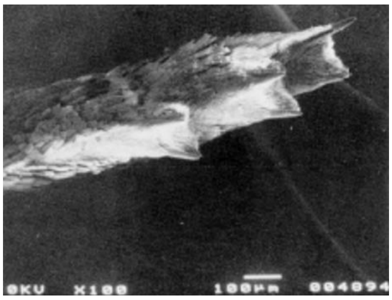

Fig14: Six needles of female foreleg magnified by SEM.

The female assesses if the milkweed is suitable for depositing her eggs by tasting and smelling the secretions. She uses the smell sensors on the tips of each antenna and the taste sensors on the bottom of the other four legs to ascertain this. Male forelegs are different in shape.

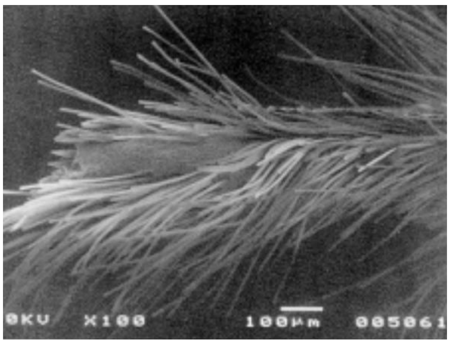

Fig15: Tip of male foreleg (x 100) by SEM.

While mating, the male monarch utilizes its forelegs to stay balanced while his other two pairs of legs grip the female. He uses his forelegs to grasp a flower or other item.

The taste sensors on the ends of the two large pairs of legs on both sexes are 2000 times more sensitive to tasting sugars than the human taste sensors. The red smelling sensors on each of the antenna tips are about 5000 times more sensitive to smell than the human nose sensor.

Scales cover the dorsal and lateral surfaces of the lycaenid male fore-tarsus, but not the distal ventral surface, where many trichoid sensilla occur. A- type and B- type trichoid sensilla on lycaenid male forelegs as well as distinctive setae that also may be trichoid sensilla.

1)A- type trichoid sensilla(spines): Spines are stout trichoid sensilla, sometimes called "bristles", that have fluted sides and occur primarily on the ventral surface of lycaenid fore-tarsus (Bates 1861). Similar spines occurredon ventral abdomen of some lycaenids and riodinids (Inoue and Kawazoe; 1966)

2)B- type trichoid sensilla: These sensillaarescattered over the dorsal, lateral and ventral sides of the lycaenid male fore-tarsus. At magnifications below about 1000 times, B- type trichoid sensilla on male lycaenid fore-tarsi appear to be smooth- walled (Robbins 1987a) but at magnifications above 8000 times, they have a variable fine structure.

Riodinidae

Unlike male lycaenid fore-tarsus, the male riodinid fore-tarsus is not used for walking (Bates; 1861), and is covered dorsally, laterally and ventrally with elongate scales.The male riodinid pretarsus is like that of lycaenids in that it lacks claws. The occurrence of spines (A- type trichoid sensilla) on male fore-tarsi differs between lycaenids and riodinids. Male riodinids lack forelegs spines (Bates; 1861). Usually one or two "microtrichia" is present at tarsal tip and sometimes a few B- type trichoid sensilla. Many setae on the tarsus resemble B- type trichoid sensilla at lower magnifications, but at higher magnifications appears to be scales with longitudinal ridges ad scutes.

Styx infernalis

The structure of the male *S. infernalis* fore-tarsus and pretarsus is similar to thoseof riodinids in some respects, and is unique in others. The *S. infernalis* male forelegs pretarsus lacks claws (Harvey; 1987). One foreleg had no spines (A- type trichoid sensilla) on the tarsus, whereas the other foreleg from same specimen had a spine on the second tarsomere. The fine structure of these trichoid sensilla is superficially more similar to that of 'macrotrichia' than to that of lycaenid and riodinid B- type trichoid sensilla.

Significance of different types of legs

Butterflies have evolved different types of legs that play crucial roles in their survival, reproduction and ecological interactions:

1) Forelegs for Grooming and Sensing:

In certain species, like Nymphalidae, the forelegs are reduced and used primarily for grooming and cleaning their eyes and antennae. These legs also have chemoreceptors that help butterflies detect the chemical composition of surfaces, such as host plants, for egg- laying (Dethier; 1956).

2)Middle and Hindlegs for locomotion:

The middle and hind legs are primarily used for walking and maintaining balance. In butterflies with strong hindlegs, such as Swallowtails (*Papilio spp*.), they may also assist in gripping surfaces and stabilizing during feeding or resting (Scoble; 1992).

3) Legs for Pheromone Dispersal:

In some butterflies, such as certain species in the Pieridae family, the males have specialized brushes on their legs that release pheromones during courtship. These pheromones play a vital role in attracting mates and ensuring successful reproduction (Lederhouse; 1995).

Evolutionary Adaptations

Butterflies are diverse and charismatic insect group that are thought to have evolved with plants and dispersed throughout the world in response to key geological events. Butterflies exhibit remarkable evolutionary adaptations that enhance their survival and ecological success.

1) Coloration and Camouflage:

Butterflies have evolved diverse colour patterns that serve several functions, such as camouflage, predator deterrence and mate attraction. Many species like Peppered Moth, exhibit cryptic coloration that helps them blend into their environment, making it difficult for predators to spot them. In contrast, others have evolved bright, to warn predators of their toxicity or unpalatability. The colourful wings of butterflies such as the Monarch (*Danaus plexippus*) are a prime example, signalling their toxicity and reducing predation(Vane-Wright *et al*., 1999). Some butterflies also engage in Batesian mimicry, where non- toxic species resemble toxic ones to avoid predation (Papke *et al*., 2016).

2) Metamorphosis and Specialization:

Complete metamorphosis of butterfly is a critical evolutionary adaptation, involving four distinct life stages: egg, larva, pupa and adult. This process allows specialization between the larval and adult stages. While caterpillars focus on feeding and growth,adults specialize in reproduction and dispersal, optimizing their survival strategy. This developmental strategy reduces competition between life stages, as caterpillars and adults occupy different ecological niches (Aardema; 2014).

3)Flight adaptations for Evasion and Dispersal:

The wings of butterflies are specially designed to help them fly easily, allowing them to move quickly and travel long distances. Their lightweight bodies, coupled with large, scale- covered wings, allow them to fly quickly and evade predators. Flight plays a vital role in dispersal, with migratory species like Monarch butterfly undertaking long-distance migrations to escape adverse environmental conditions. These flight adaptations enhance both survival and reproductive opportunities, enabling butterflies to colonize new areas and maintain genetic diversity (Zalucki; 2014).

4) Behavioural Adaptations (Migration and Seasonal activity):

Some butterfly species have developed migratory behaviour as a response to environmental pressures such as seasonal changes. The Monarch butterfly is the most famous example, migrating thousands of miles from North America to central Mexico to escape cold temperatures. These behavioural adaptations help butterflies thrive across a wide range of environments and maintain their population numbers (Reppert; 2010).

Conclusion

Insects are very important due to their ecological role, effect on agriculture; diversity and social well- being.Butterflies belonging to order lepidoptera play an important role in the ecosystem services. Butterflies are extremely important in pollination as most of them are important pollinating agent for many plant species, contributing to the enhancement of genetic diversity and stability within the ecosystem. Butterflies have six legs arranged in three pairs. Butterflies have evolved different types of legs that play crucial roles in their survival, reproduction and ecological interactions

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