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Analysis of Plant-Insect Pollination Network—A Case Study on the Exotic Plants as Nectar Resource of Butterflies across Darjeeling District of West Bengal, India †

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Abstract: In ecology bipartite network involving higher and lower trophic level components is a reflection of community interaction. The present investigation on butterflies and exotic nectar plant community across Darjeeling district of West Bengal, India is a significant event in generating awareness for protection of such plant communities. Analysis of such bipartite network characterizing butterfly-plant community interaction could help in elaborating different aspects of species assemblage. Different indices (based on unweighed links and weighed links) were used for exploration of such network. A total of 28 exotic plant species served as nectaring resource for 44 butterfly species. Some ecologically significant descriptors of this network includes network dimension (no. of species in higher trophic level: 44; no. of species in lower trophic level: 28), links per species (1.042), connectance (0.061) and network asymmetry (−0.222), generality (3.608), vulnerability (3.166), linkage density (3.387) and Shannon's evenness of network interaction (0.441). Thus the above predictions provide a probable clue to the involvement of exotic plant species to the maintenance of community structure. Significantly, exotic plants serve as key service providers to a community's pollinator assemblages, thereby attempting to fill up an otherwise "empty coevolutionary niche".

Keywords: bipartite network; butterflies; community; exotic plant; pollinator

1. Introduction

Feeding relationship between different individuals belonging to a particular species usually involves a complex network of food web series. Although food web are occasionally compartmentalised by habitats they, however retain connections to interact among each other [1,2]. Significantly, compartmentalisation within food web helps in creating mutually beneficial, heterogeneous, nestedness within such webs. Mutualistic network theory is centered on animal-mediated pollination with an integrative approach highlighting trophic level interaction and reproductive preferences [3,4]. Mutualistic networks are found to be compartmentalised or modular with a group of species, well connected amongst themselves but weakly connected with others within the same network [5]. Interesting observations have revealed the structure and co- evolutionary dynamics within ecological networks [6–11]. Interaction networks are considered to be a direct indicator of habitat conditions altering trophic interactions [12–14]. Among pollination network, specialised species associate with few species in contrast to generalist species interacting with many [15]. Significantly the occurrence of exotic plants may influence the availability and utilization of nectar and pollen resources of pollinators over the entire season which in turn directly influencing the network properties [16]. Thus such interactions network could be considered to be a suitable tool monitoring community-wide influences of exotic plant species on pollinators [17–20].

Therefore, the major objectives of the present study are to address the contribution of exotic nectar plant species of butterflies in determining the plant- pollinator network structure. In this regard, the present study will also investigate the different exotic nectaring plant species of adult butterfly community across the Darjeeling district of West Bengal, India. The construction and analysis of bipartite network characterising butterfly-nectaring plant resources and its relevant role in species assemblages was investigated. Several ecologically relevant descriptors of such network structure (links per species, connectance, network asymmetry, generality, vulnerability, linkage density and Shannon's evenness of network interaction) were also analysed.

2. Materials and Methods

Study Area: The entire study was conducted across the montane broad leaved temperate forests in Darjeeling district, West Bengal, India. Sampling was undertaken at forest patches during the study period (July 2020–June 2021)

2.1. Sampling Design

Sampling of butterfly visiting nectar plant species at different quadrates was undertaken weekly in a month with the help of two trained field assistants. Sampling procedure was repeated at an interval of seven days. Plants were identified from published literature [21–24] along with assistance from the plant taxonomist as and when required. Specific observations were made on each plant species visited by butterflies.

Observation on nectar plants were carried out twice a day (i.e., between 1000–1300 h in morning and 1400–1600 h in afternoon). The flower visitors were observed for 5–8 min per flowering stand from a distance of 1.5 m. Nectar probing by species was ascertained from the moment of inserting the proboscis in the corolla till the end of its withdrawal.

The butterflies were observed (using Bushnell binoculars) and photographed (using Nikon COOLPIX-P90) occasionally for the identification from published literature [24,25].

2.2. Data Analysis

A bipartite graph depicting association between lower trophic level species and higher trophic level species was produced. Such a mutualistic relationship was represented by connecting links between two trophic nodes. Several different indices based on weighed links and unweighed link were investigated.

(a) Indices based on weighted links (Quantitative webs):

(i) Generality: It represents the mean number of prey species per predator. In case of weighted links, H_j, the Shannon diversity of interactions for predator species j has been calculated as follows [26]:

$$H_j = -\Sigma[(a_{ij}/A_j) \times \ln(a_{ij}/A_j)]$$

(ii) Vulnerability: It represents the mean number of predator species per prey. In case of weighted links, H_i, the Shannon diversity of interactions for prey species i is calculated as follows [26]:

$$H_i = -\Sigma[(a_{ji}/A_i) \times \ln(a_{ji}/A_i)]$$

(iii) Weighted Linkage density: Since the generality and vulnerability are known. The weighted linkage density (L_q) is obtained as their arithmetic mean. In this case L_q is calculated as follows [27]:

$$L_q = 0.5(H_j + H_i)$$

(iv) Interaction Evenness: Shannon's evenness of network interactions has been calculated as follows [28]:

$$E_s = \frac{-\sum_i \sum_j p_{ij} \ln p_{ij}}{\ln L}$$

(b) Indices based on unweighted links (Quanlitative webs):

(i) Links per species: The mean number of links per species has been calculated as follows [28]:

$$L_x \text{ (mean)} = l/(I + J)$$

(ii) Connectance: It is represented by the realised proportion of possible links. Connectance (C) has been expressed as [29]:

$$C = L/(IJ)$$

(iii) Web asymmetry: It denotes a balance between the number of species in two trophic levels. Web asymmetry has been calculated as follows [30]:

$$W = (I - J)/(I + J)$$

3. Results

A total of 28 exotic plant species served as nectaring resource for 44 butterfly species across the entire study site. Butterfly species representing five subfamilies i.e., Nymphalidae: 50%, Lycaenidae:18.18%, Papilionidae: 15.91%, Pieridae:11.36%, Hesperiidae:4.54% were reported. Further investigations into this bipartite network revealed the existence of 75 connecting links between 28 lower trophic level species and 44 higher trophic level species (Figure 1).

Tirumala limniace, Argyreus hyperbius, Vanessa indica, V. cardui, Junonia orithiya, J. atlites, Hypolimnas misippus, displayed a maximum of three connecting links with their respective nectar plants. In contrast *Anemone japonica* was associated with a maximum of six butterfly species. Additionally *Argemone mexicana, Asclepias curassavica, Croton bonplandianus, Lepidium sativum, Ranunuculus repens, Tridax procumbens* and *Veronica persica* showed linkage with 4 butterfly species (Figure 1).

The mean number of links per species in the above bipartite mutualistic interaction network was 1.042. The network connectance of the above plant-pollinator network was found to be 0.061 (6.10%). Additionally the web asymmetry of studied network was −0.222 with negative values being an indicator of the assemblages of higher trophic level species. Values of indices based on weighed links (quantitative webs) were also obtained by analysing the above mutualistic plant-pollinator network (Table 1). Generality (Shannon diversity of interaction for predator/butterfly species) was 3.608. Additionally, vulnerability (Shannon's diversity of interaction for prey species/exotic nectar plant species) was 3.166. Therefore the weighted linkage density was deduced as 3.387. Finally Shannon's evenness of this network interaction was found to be 0.441 (Table 1).

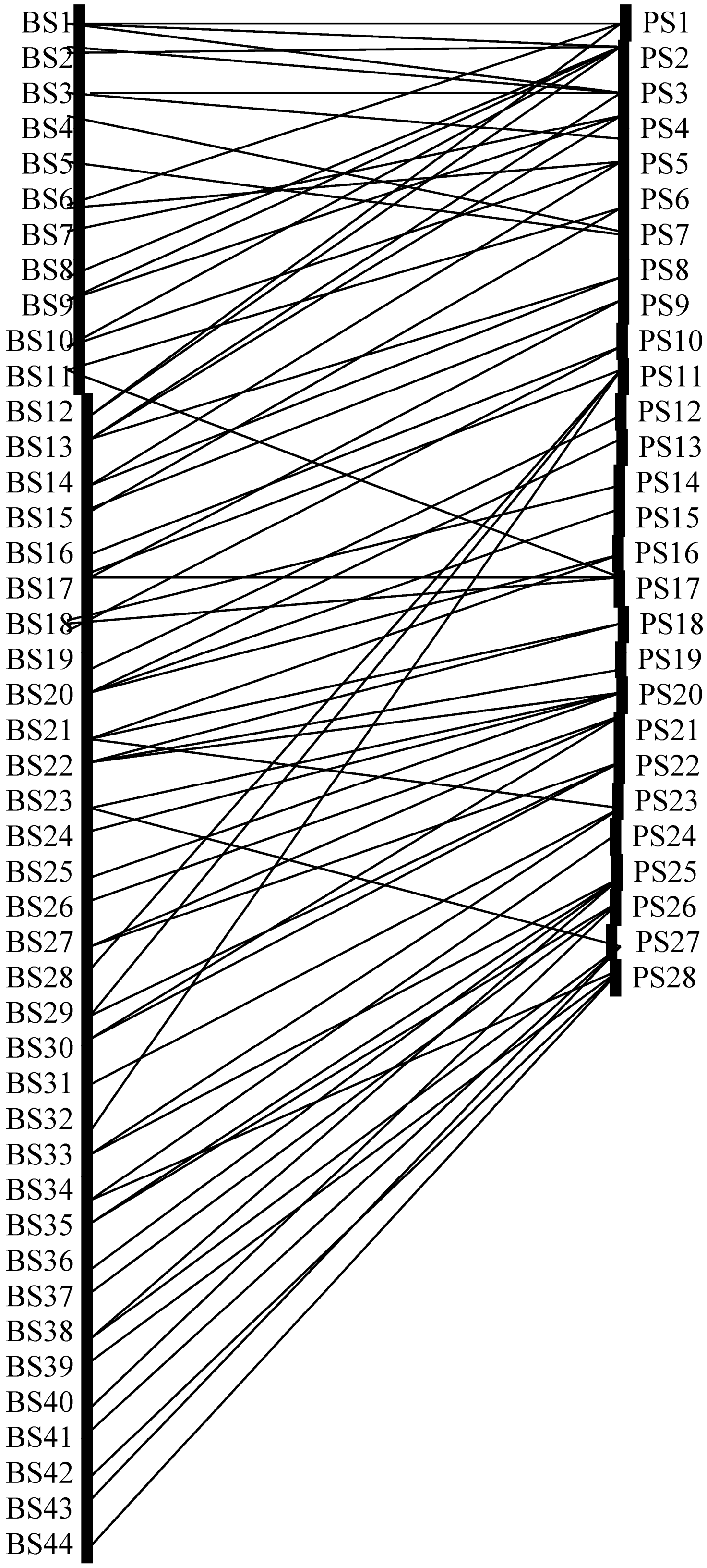

Figure 1. Bipartite graph representing two trophic levels (ie. butterfly species expressed as BS and nectaring plant species expressed as PS). where: BS1: *Tirumala limniace*, BS2: *T.septentrionis*, BS3: *Danaus genutia*, BS4: *D. melanippus*, BS5: *D. chrysippus*, BS6: *Parantica aglea*. BS7: *Euploea sylvestor*, BS8: *E. mulciber*, BS9: *E. core*, BS10: *Ypthima baldus*, BS11: *Y. sacra*, BS12: *Arceea violae*, BS13: *Argyreus hyperbius*, BS14: *Cethosia cyane* BS15:*Athyma perius*, BS16: *Ariadne merione*, BS17: *Vanessa indica*, BS18: *V. cardui*, BS19: *Aglaia cashmirensis*, BS20: *Junonia orithiya*, BS21*: J. atlites*, BS22*: Hypolimnas misippus*,

BS 23: *Deudorix epijarbas*, BS24*: Rapala manae*, BS25*: Spindasis lohita*, BS26: *Lycaena phlaeas*, BS27*: Heliophorus brahma*, BS28*: Prosatas nora*, BS29*: Zizeeria karsandra*, BS30: *Pseudozizeeria maha*, BS31*: Sarangosa dasahara*, BS32: *Pseudoborbo bevani*, BS33*: Eurema hecabe*, BS34*: Catopsilia pyranthe*, BS35*: Appias lyncida*, BS36*: Pieris brassicae*, BS37*: Delias eucharis*, BS38*: Graphium sarpedon*, BS39*: G. agamemnon*, BS40*: Papilio helenus*, BS41*: P. polytes*, BS42*: P. polymnestor*, BS43: *P. paris*, BS44*: Aristolochia hector*. PS1: *Ageratum haustomiianum*, PS2: *Anemone japonica*, PS3: *Argemone mexicana*, PS4: *Asclepias curassavica*, PS5: *Cassia siamea*, PS6: *Catharanthus roseus*, PS7: *Chenopodium ambrosoides*, PS8: *Cleome rutidosperma*, PS9: *Coronopus didymus*, PS10: *Crassocephalum crepidiodes*, PS11: *Croton bonplandianus*, PS12: *Drymaria villosa*, PS13: *Erigeron karwinskianus*, PS14: *Eupatorium adenophorum*, PS15: *E. ligustrinum*, PS16: *Galinsoga parviflora*, PS17: *G. quadrifolia*, PS18: *Ipomoea purpurea*, PS19*: Kerria japonica*, PS20*: Lepidium sativum*, PS21*: Linoria cymbalaria*, PS22: *Mikania micrantha*, PS23: *Passiflora foetida*, PS24: *Peperomia pellucida*, PS25*: Ranunuculus repens*, PS26*: Setaria geniculata*, PS27*: Tridax procumbens*, PS28: *Veronica persica*.

Table 1. Table indicating different values of network indices.

Network indices based on unweighted links (Qualitative webs)		
Sr. no.	Network indices	
1	Links per species	1.042
2	Connectance	0.061 (6.10%)
3	Web asymmetry	−0.222
Network indices based on weighted links (Quantitative webs)		
Sr. no.	Network indices	
1	Generality	3.608
2	Vulnerability	3.166
3	Weighted linkage density	3.387
4	Interaction evenness	0.441

4. Discussion

The construction of the above mutualistic bipartite interactive network helps to highlight the significance of nectaring exotic plant resources of butterflies in the Himalayan landscape of Darjeeling, West Bengal, India. Analysis of this bipartite network generated new network indices [26,31], which in turn explored different patterns among them [15,32–34]. This in turn also contributed to the ongoing evolutionary processes affecting different communities [35–37].

Dual trophic level species interaction in bipartite networks appears to be highly unstable both in temporal and spatial scale [38,39]. The present study emphasizes on an area where the utilization of exotic plants as nectaring resource could probably be driven by a need to explore such resource as the native plant species have been replaced by exotic invasive species. One possible reason behind the switching over of preference towards exotic plant resource could be the greater abundance of predator/butterfly species as displayed by higher values of "generality" as compared to "vulnerability". Such dynamism in switching over species preference could probably explain the asymmetrical food web (as denoted by negative values) due to greater number of higher trophic level members. Such an asymmetry in plant-pollinator network could probably be attributed to the presence of specialist species, a common phenomenon as observed by previous studies [3,34].

Several studies have hypothesized that the species abundance and morphological traits are the key factors of a mutualistic network [40,41]. It could also be assumed that utilization of exotic or introduced plants could lead to homogenization of butterfly fauna. Such exotic plants (ornamental or cultivated plants) have also facilitated the entry of butterflies into previously unexplored region [42]. Such supplementation of the flowering native plants by the exotic resources (pollen and nectar) could help enriching the plant-pollinator integration network further [43,44].

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