

Intraspecific variations in wing venation pattern of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera, Nymphalidae) from Mohali, Punjab, India

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ABSTRACT: The wing venation of *Danaus chrysippus* (Linnaeus, 1758) was assessed and vein atrophy as well as the presence of spurs at the middle discocellular of both forewings and hindwings were observed. This is perhaps the first evidence of intraspecific variations in the wing venation of this species. The forewing spurs varied in number and positioning on the middle discocellular. In the hindwings, variation occurred with respect to the presence or absence of a spur, as well as a protuberance at the base of the third anal vein (3A). These findings enhance the understanding of morphological diversity within *D. chrysippus*, and also underscore the potential significance of intraspecific venational variation in refining the taxonomy of this butterfly. © 2026 Association for Advancement of Entomology

KEY WORDS: Wing veins, nymphalid butterfly, first evidence, vein atrophy, vein spurs, morpho-taxonomy

INTRODUCTION

Wing venation patterns are significant taxonomic characters that are useful for identifying and classifying insects, including butterflies (Comstock and Needham, 1898a, b; Nath and Devi, 2009; Khaing *et al.*, 2022). Their taxonomic utility was brought to light over a century ago by Comstock and Needham (1898a, b), who argued that all extant insect groups evolved distinct venation patterns that could be traced back to a common ancestor. They further reported on the disappearance of the main stem of the Median vein in numerous Lepidoptera families, leading to the formation of vein spurs that project into the discal cell. The typical venation

pattern of butterflies comprises eleven or twelve veins in the forewings and eight or nine veins in the hindwings (Bingham, 1905; Revathy and Mathew, 2013; Khaing *et al.*, 2022). In the forewings of *Danaus* butterflies, from the costal to the dorsal margin, these veins are the Subcosta (Sc), Radius (R_1 - R_5), Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A) veins, and in the hindwings, the Subcosta and the Radius veins coalesce, giving rise to the $Sc+R_1$ vein and an unbranched Radial sector (R_s); the other hindwing veins, in similar order to that of the forewings, are the Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A and 3A) veins (Bingham, 1905; Comstock and Needham, 1898a; Nath and Devi, 2009; Khaing *et al.*, 2022).

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Furthermore, the discal cell (DC) of both fore- and hindwings is closed by three cross veins: the upper discocellular (UDC), middle discocellular (MDC), and lower discocellular (LDC) (Bingham, 1905). Despite the significance of wing venation patterns in insect taxonomy, the factors that lead to their development are poorly understood (Bingham, 1905; Das Shimmi *et al.*, 2014; Banerjee *et al.*, 2020).

Danaus chrysippus (Linnaeus, 1758) (Fig. 1a, b), commonly known as the Plain Tiger, African Queen, or African Monarch, is an acrid and migratory butterfly that is prevalent throughout the continent of Africa, as well as some parts of Europe and Asia, including India (Tolman and Lewington, 1997; Perkovic, 2006; Liu *et al.*, 2022; Goswami *et al.*, 2024; Omanakuttan *et al.*, 2024). This butterfly is characterised by a tawny ground colouration bearing black and white markings (Lushai *et al.*, 2005; Smith *et al.*, 2005; Braby *et al.*, 2015; Vane-Wright, 2020). According to Vattakaven *et al.* (2016) the Plain Tiger prevails across India, from Punjab in the north to Kerala and Tamil Nadu in the south. It exhibits sexual dimorphism, with differences in the number of black spots at the centre of the hindwings. Females possess three spots, and males have four spots, with the additional fourth functioning as a specialised scent gland (Vattakaven *et al.*, 2016; Alam *et al.*, 2019).

The wing venation pattern of *D. chrysippus* has been demonstrated to conform to the general pattern observed in butterflies (Bingham, 1905; Mal *et al.*, 2015; Patil and Magdum, 2017). However, Bingham (1905) reported a forking of the 2A vein at its base occurring in all species of the genus *Danaus*; in addition, two vein spurs occur on the MDC of the forewings in *D. chrysippus*, and in the hindwings, the MDC lies perpendicular to the M_2 vein. Bingham (1905) used this perpendicular feature as one of the diagnostic characters to discriminate *D. chrysippus* from *D. genutia*, whose MDC curves towards the M_2 vein. Interestingly, in more recent studies, neither the forking of the 2A vein nor the occurrence of vein spurs has been reported (Mal *et al.*, 2015; Patil and Magdum, 2017). The inconsistencies between Bingham (1905) and more recent studies (Mal *et al.*, 2015; Patil and Magdum,

2017) suggest that either certain diagnostic features of venation may have been overlooked or that they have undergone alterations due to regional or phenotypic variability. The present study aimed to document and analyse the detailed wing venation pattern of *Danaus chrysippus* and assess its variability within populations. Particular attention was paid to the presence or absence of specific veins and vein spurs on the middle discocellular (MDC), which have been inconsistently reported in the literature.

MATERIALS AND METHODS

Specimens of *D. chrysippus* were collected from semi-urban landscape of Gharuan village, Mohali, Punjab, India (30.773°N; 76.556°E). Gharuan experiences a temperate climate, characterised by hot summers (mid-April to June), monsoon rains (July to September), and cool winters (December to February). The vegetation comprises mainly crops, trees, and shrubs, including *Lantana camara*, which serves as one of the nectar plants. The butterfly specimens were captured with insect aerial nets during the day, between 0900 and 1600 hours, from March to August 2023. After capturing, they were narcotised, stretched, pinned, dry preserved, labelled and stored using standard entomological procedure (Beige and Noetzel, 1978). Thirty-two permanent slides were prepared: 16 of the forewings and 16 of the hindwings. A standard protocol advocated by Lee and Brown (2015) was followed. The slides were examined under a compound light microscope (Labomed) at $\times 40$ magnification and were photographed with a digital camera (Huawei Y6S). For wing venation nomenclature, the system adopted by Comstock and Needham (1898a) was followed. Each wing specimen was examined for the number, branching pattern, and position of veins, crossveins, and discal cell configuration. Observations were compared with standard descriptions by Bingham (1905) and later studies (Mal *et al.*, 2015; Patil and Magdum, 2017) to assess intraspecific variation. All observations were repeated to confirm consistency in the configuration of veins and to minimise observational errors.

RESULTS AND DISCUSSION

Family: Nymphalidae; **Subfamily:** Danainae;
Genus: *Danaus* Kluk, 1780

Type species: *Danaus plexippus* (Linnaeus, 1758)

Species: *Danaus chrysippus* (Linnaeus, 1758)
Bold (Fig.1)

Material examined: India: Punjab, Mohali district, Gharuan; 4 ♀♀, 06.iv.2023; 2 ♀♀, 27.iv.2023; 2 ♀♀, 10.v.2023; 3 ♀♀, 04.viii.2023; 1 ♂, 06.iv.2023; 1 ♂, 27.iv.2023; 1 ♂, 10.v.2023; 2 ♂♂, 04.viii.2023; coll. Aaron Mboma.

Distribution: India: Punjab (Mohali district: Gharuan). This species is recorded from Gharuan for the first time.

Description: Detailed examination of the forewing and hindwing venation in sixteen specimens of *D. chrysippus* revealed a generally consistent pattern characteristic of the genus *Danaus*, although individual variations were also observed.

General venation pattern: The venation of *D. chrysippus* conforms to the typical venation pattern of butterflies, with twelve veins in the forewings, i.e., Subcosta (Sc), Radius (R_1 - R_5), Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A); and nine veins in the hindwings, i.e., Sc+ R_1 , Radial sector (Rs), M_1 - M_3 , Cu_1 and Cu_2 , 1A+2A, and 3A. The discal cell (DC) of each wing is closed by three discocellulars: upper discocellular (UDC), middle discocellular (MDC), and lower discocellular (LDC). A detailed pattern for the forewing and hindwing is provided below.



Fig.1a



Fig.1b

Fig. 1 *Danaus chrysippus* - **a** - Male with four black spots on each hindwing; **b** - Female with three black spots on each hindwing

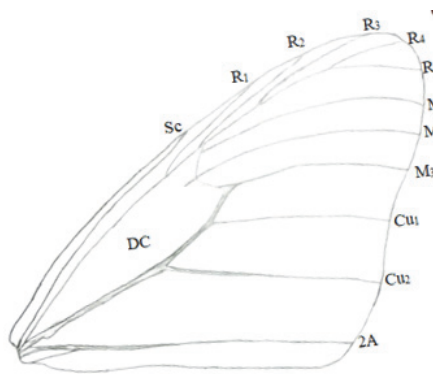


Fig.2a

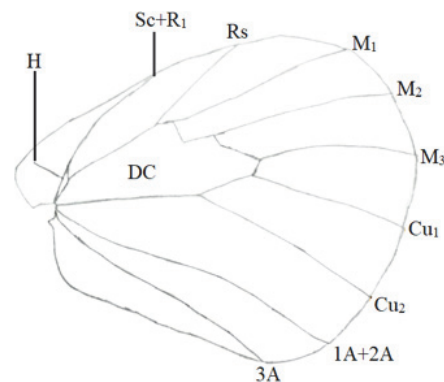


Fig.2b

Fig. 2 *Danaus chrysippus* - Generalised venation pattern - **a** - forewing; **b** - hindwing



Fig. 3 Forewing of *Danaus chrysippus* showing MDC and LDC (encircled in red to highlight variation)

Forewing venation (Fig. 2a): The forewing has four principal veins originating from the wing base: Subcosta, Radius, Cubitus, and Anal; discal cell closed by three cross veins: UDC, MDC, and LDC; UDC very short, MDC slanted inwards, upper half of LDC faded; Radius branches into five veins, R_1 to R_5 ; R_1 - R_3 branch to the costa, R_4 runs to the apex, and R_5 runs to the termen; the three median veins arise from the DC; M_1 and M_2 emanate from the MDC, M_3 emanates from the base of the LDC; Cu vein branches into Cu_1 and Cu_2 veins; a single anal veins (2A) is forked at the wing base; vein atrophies occur at the MDC and project as spurs into the DC.

Hindwing venation (Fig. 2b): In the hindwing, the four principal veins originating from the base of the wing are a coalescence of the Sc and R ($Sc+R$),

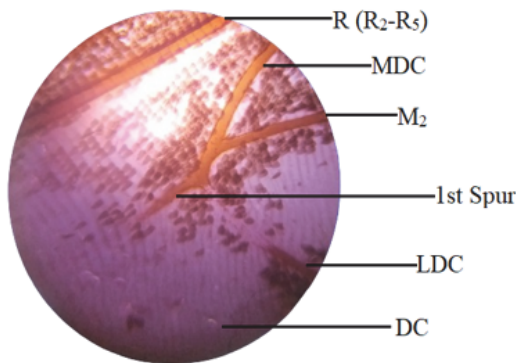


Fig.4a

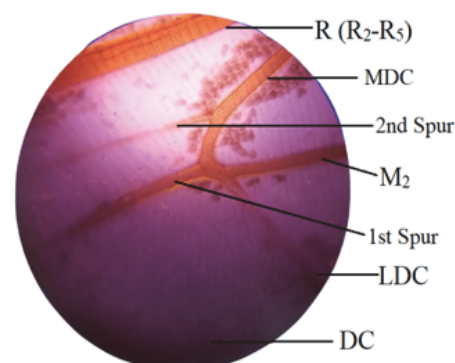


Fig.4b

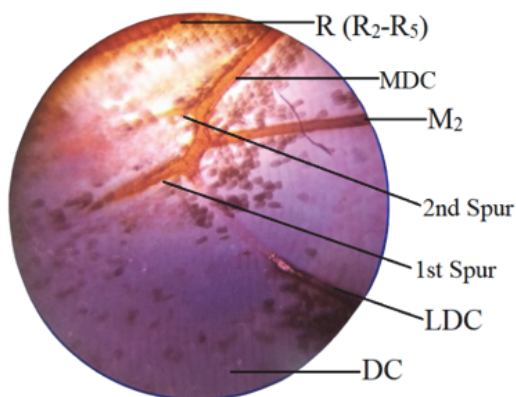


Fig.4c

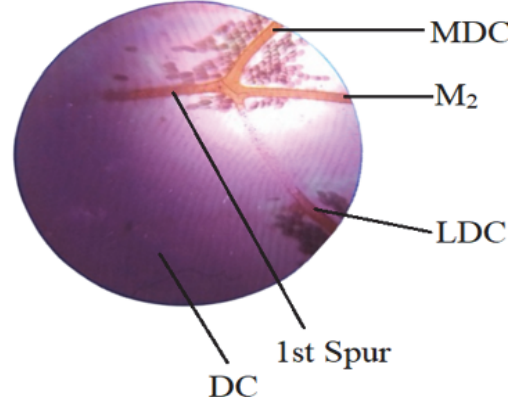


Fig.4d

Fig. 4 *Danaus chrysippus*: **a** - Forewing showing a single spur below the vein M_2 ; **b** - Forewing showing two spurs, with the first slightly above the vein M_2 ; **c** - Forewing showing two spurs, with the first spur below the vein M_2 ; **d** - Forewing showing a single spur occurring on the vein M_2

Cu, a coalescence of 1A+2A, and an independent 3A vein; DC closed by three cross veins: UDC, MDC, and LDC; a short humeral (H) vein branches out from the Sc+R, terminating before the costal margin; Radial sector (Rs) branches out of the Sc+R and runs to the costa; M_1 - M_3 veins arise from the DC; Cu branches into Cu_1 and Cu_2 ; a single vein atrophy occurs at the junction of the MDC and M_2 vein (MDC/ M_2 junction) and projects into the DC.

Intraspecific variations (Figs. 3, 4a, b, c, 5, 6a, b, 7a, b): The forewings exhibited variation in the number and position of vein atrophy at the MDC as spur(s). Of the examined specimens, 62.5 per cent (%) displayed two atrophied spurs on the MDC, one above the other. The lower spur occurred at or slightly above the M_2 vein. In these specimens, the upper spur varied in prominence, from conspicuous to significantly reduced to a notch; the remaining 37.5 per cent displayed only a single spur. Positionally, 12.5 per cent of the specimens displayed a spur below the M_2 vein instead of at or slightly above this vein.

The hindwings likewise exhibited variation in the form of vein atrophy at the MDC/ M_2 junction. About 56.25 per cent of the specimens displayed a very short spur, which was absent in the rest. The MDC of specimens with this spur lay almost perpendicular to the M_2 vein, while the MDC of specimens lacking this spur curved towards the M_2 vein. Additionally, 37.5 per cent of the specimens exhibited a protuberance at the base of the 3A vein, giving this vein a hooked appearance. However, this protuberance was absent in 43.75 per cent of the specimens, and inconclusive in the remainder due to damage to the area during preparation.

Perusal of the literature reveals that such intraspecific variations in the wing venation patterns have been reported across several insect taxa, including Lepidoptera (Albrecht and Kaila, 1997; Nath and Devi, 2009; Solonkin *et al.*, 2021, 2024). And in view of the diagnostic role of wing venation patterns, recording of such intraspecific variation is of considerable significance to insect systematics. Presently, the authors revisited the venation pattern of *D. chrysippus* to record intraspecific variations.

The ensuing account describes intraspecific variations in the examined specimens: vein atrophy in the forewing, altered angularity of discocellulars in the hindwing, and additional protuberance at the base of the 3A vein in the hindwing.

The presence of two vein spurs in the forewings of 62.5 per cent of the specimens corroborates earlier observations made by Bingham (1905). However, 37.5 per cent of the specimens exhibited only a single spur, which was not reported before. Furthermore, 12.5 per cent of the specimens exhibited displacement of the first spur, indicating developmental instability during wing morphogenesis; this feature has not been previously documented.

Bingham (1905) distinguished *D. chrysippus* from *D. genutia* (Cramer, 1779) primarily based on the angularity of the middle discocellular in the hindwing, arguing that it was perpendicular to the M_2 vein in *D. chrysippus* and curved in *D. genutia*. However, in this study, 43.75 per cent of *D. chrysippus* specimens displayed the MDC of the hindwing curving towards the M_2 , indicating that this feature is variable and not taxonomically stable.

Furthermore, in the hindwing, the presence of a protuberance at the base of the 3A vein in 37.5 per

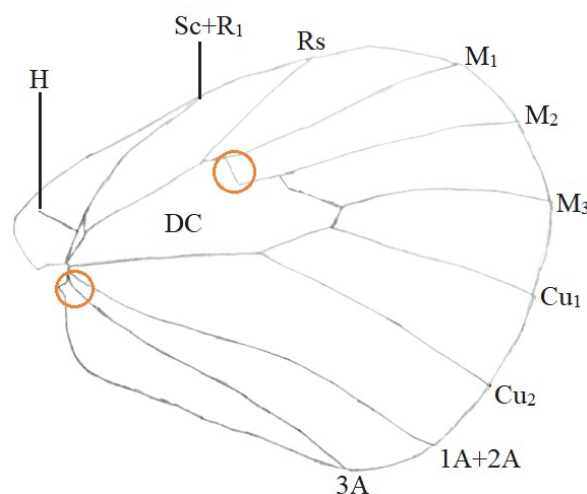


Fig. 5 *Danaus chrysippus* - Hindwing showing MDC and the base of the Anal veins (encircled in red to highlight the variation)

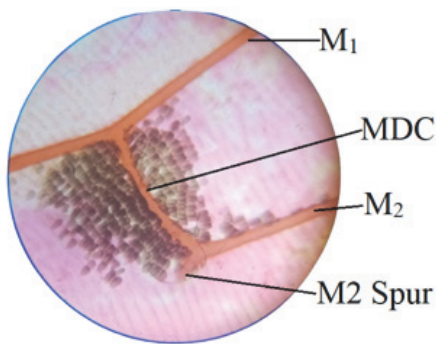


Fig. 6a

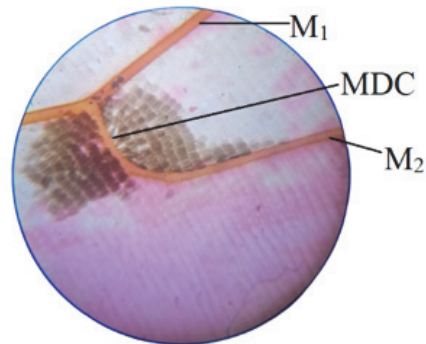


Fig. 6b

Fig. 6 *Danaus chrysippus*: **a** - Hindwing showing a short spur with the MDC perpendicular to the vein M_2 ; **b** - Hindwing in which the short spur is lacking and the MDC curves towards the M_2 vein

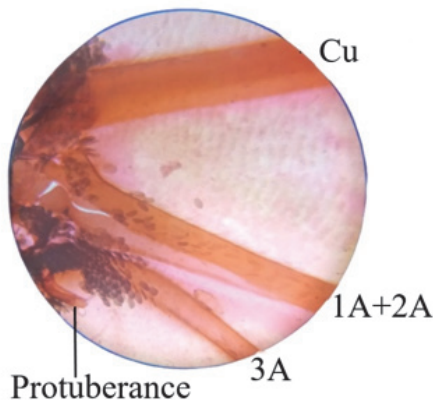


Fig. 7a

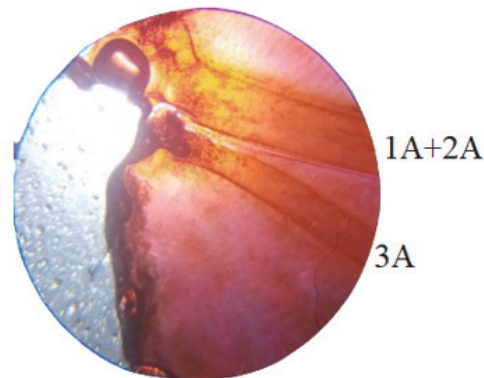


Fig. 7b

Fig. 7 *Danaus chrysippus*: **a** - Hindwing showing a hook-shaped 3A vein; **b** - Hindwing in which the protuberance in 3A vein is lacking

cent of the specimens represents a previously unrecorded structural deviation. This protuberance does not correspond to an atrophied vein; its formation may reflect localised sclerotisation or vein thickening during wing expansion, rather than a modification of the venation pattern. There is a further need for detailed morphometric and microstructural analysis to conclude its origin and whether it is aberrant or taxonomically relevant.

The absence of the presently reported variation in recent studies (Mal *et al.*, 2015; Patil and Magdum, 2017) could result from differences in the landscapes where specimens were collected, slide preparation methods, low magnification power of the

microscope, or simply a missed observation. Alternatively, vein spurs may have undergone significant reduction or have been completely lost in certain populations. This argument advocates for broader geographic sampling and the use of standardised modern imaging techniques. Although this study did not investigate the underlying causes of variability in venation patterns, which was beyond the scope of the present study, the observed vein atrophy is indicative of developmental instability. Solonkin *et al.* (2024) reported the influence of environmental factors, such as temperature and precipitation, on the development of wing veins in *Aporia crataegi* (Pieridae). This observation suggests that similar ecological stresses

could affect *D. chrysippus*. Thus, future studies must integrate morphometric, environmental, and genetic data from multiple populations of the species to understand the developmental mechanisms and adaptive significance of venation variability in *Danaus*. This study highlights that even morphologically 'stable' venational traits in *D. chrysippus*, or other species, may exhibit subtle yet significant variations, reinforcing the need for re-assessment of traditional diagnostic characters vis-à-vis taxonomy in Lepidoptera.

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