Palaeodietary and Palaeoclimatic Reconstruction for Late Miocene Hipparionines from the Siwaliks of Pakistan

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A B S T R A C T

Dental materials of three hipparionine species from Dhok Pathan Formation (late Miocene) has been utilized in this study to reconstruct the palaeodiet and palaeoecology by using stable isotope analyses of carbon and oxygen of tooth enamel carbonate. A gradual increase in hypsodonty and complexity in plications indicate the drier environments with hard and tough food available during the late Miocene. The late Miocene hipparionines were expected to have inhabited a mosaic of woodland and grassland with predominant C_3 vegetation which latter shifted to C_3/C_4 grasses. For this purpose, fifteen dental samples (Five for each *Hipparion* species) from well dated late Miocene localities between 8.8-7.7 Ma are analyzed. All the $\delta^{13}C$ values (V-PDB) are in range of -11.27‰ to -3.36‰ which represent that these horses dominantly inhabited C_3 vegetation however, the C_4 grasses were being indicated as the diet of Hipparionines the latest Miocene. All these species are believed to inhabit more open environment with patchy grasslands. Non-significant differences were observed between the species which explains that the dietary niche of these species was more or less similar. However, the values for carbon isotopes are highly depleted for early hipparionine species (*Sivalhippus theobaldi*) and less depleted for succeeding species (*Sivalhippus perimensis* and *Cremohipparion antilopinum*) which represent dominancy of C_4 diet in more open habitat in later species. The values for $\delta^{18}O$ are also not significantly different between these species indicating that water intake behavior was also, more or less, same for these species. $\delta^{18}O$ (range between -11.4‰ to -3.8‰) variability may indicate a climatic shift in precipitation source or amount rainfall through time. We suspect that grasslands expanded at the expense of forests towards the latest Miocene while environment became drier and warmer. These analyses also reveal that hipparionines shifted from C_3 browsing to mixed C_3/C_4 and to C_4 diet at the end of Miocene.

INTRODUCTION

All three toed horses, present in Mio-Pliocene are categorized under a unanimous term: hipparionines. In this time span hipparionine horses were wide spread across the Old as well as New World (Colbert, 1935; Webb, 1969). Due to the ubiquitous nature of all these horses across the Old World, particularly, they are often termed as "Hipparion Fauna" and it is more related to Neogene time span. The description of Neogene horses is chiefly based on their dental character and also on the basis of post cranial features, but to a lesser extent (MacFadden, 1984).

Hipparionines are widely distributed in the Middle Siwaliks of Pakistan, most specifically during the late Miocene to Pliocene time span (MacFadden and Bakr, 1979;

Bernor and Hussain, 1985; Ghaffar, 2005; Wolf *et al*., 2013). The enamel pattern of these hipparionine species is relatively complicated as compared to living genera of horses. The complexity in dental structures of extinct hipparionine species indicates an addition of tougher food along the temporal boundaries of late Miocene. According to Wolf *et al*. (2013) different species of this group reported from the Siwaliks are *Sivalhippus nagriensis*, *Sivalhippus theobaldi*, *Sivalhippus perimensis*, *Sivalhippus anwari*, *Cremohipparion antilopinum* and *Cremohipparion* sp.

One of the most significant event of latest Miocene is the expansion of C_4 grasslands and the reasons behind this global phenomenon is the uplifting of Himalayan mountain belt, decrease in atmospheric CO_2 levels and warmer conditions. This decrease in annual rainfall patterns is interpreted from the $\delta^{18}O$ values of equid teeth (Nelson, 2005). According to literature review (i-e., Cerling *et al*., 1989; Barry *et al*., 2002; Nelson, 2005, 2007; Badgley *et al*., 2008; Damingo *et al*., 2009) late Miocene record at ca.

8.5 Ma represent a transition from wet monsoon forest to dry monsoon forest at ca. 7 Ma to savannah at ca. 6 Ma. Cerling *et al.* (1993) have investigated the paleosols and tooth enamel from various localities of Pakistan and North America and reported that C_4 expansion happened at ca.7 Ma - 5 Ma and according to authors, this expansion was the result of decrease in atmospheric concentration of CO_2 while Pagani *et al.* (1999) associated the C_4 expansion with increasing episodes of low latitude aridity and seasonal precipitation pattern on global scale.

The present study is an attempt to investigate the palaeoclimate and palaeodiet of three late Miocene hipparionine species by using the stable isotope analysis of carbon and oxygen. As it is known that hipparionine horses were widely distributed in Middle Siwaliks of Pakistan, which spans the late Miocene-Pliocene time period, so this study will help to understand the changes in environment and dietary patterns as well as to co-relate it with previous studies which were based on morphological and morphometric interpretations from the Siwaliks (Ghaffar, 2005; Ghaffar *et al.*, 2004; Wolf *et al.*, 2013) as well as stable isotope data (Cerling *et al.*, 1989; Nelson, 2005, 2007; Bibi, 2007).

Stable isotopic data for oxygen is associated to body water, which corresponds the water uptake behaviors of animals (Byrant and Froelich, 1995). The oxygen values explain weather the animal was an obligate drinker/ passive drinker or taken his water from open evaporating water holes/shaded ponds/riverine water (Kohn, 1996). While considering the evaporative enrichment factor, the leaf water values of plants are being recorded 10-30‰ more positive than meteoric water which allows us to test the hypothesis that whether hipparionines were dependent on their food or on water holes for their water intake (Yakir, 1997; Sponheimer and Lee-Thorp, 2001). On the other hand, carbon isotope values explore the dietary composition ingested by the animals (Quade *et al.*, 1992). Both these isotopic values together are quite helpful in understanding of dietary and climatic structure as well as water intake behavior.

Along the tropics and sub tropics (the Siwaliks system), C_4 pathway of photosynthesis is dominantly present in grasses which make such flora enable to thrive in heat stressed and seasonally arid conditions (Tipple and Pegani, 2007). The record of stable isotope studies indicate that C_4 vegetation did not expand in the Siwaliks promptly but gradually (Quade and Cerling, 1995). The emergence of C_4 vegetation in the Siwalik is estimated to be around 8.1 Ma. However, the first dominated habitats which were more exposed to faunal elements appeared at about 7.4 Ma (Quade *et al.*, 1989; Barry *et al.*, 2002). Now all these facts suggest that early hipparionine species must be feeding in

an open C_3 environment, C_3 grasslands more appropriately. Stable isotope analysis plays a vital role here as it can readily differentiate the C_3 vegetation from C_4 provided that C_3 flora represents $\delta^{13}C$ values in range of -35‰ to -22‰ while C_4 vegetation lies in the range of -19‰ to -9‰ as described by Bender (1971). But there are a number of factors which affect this ratio and alter it, these factors include alteration in partial pressure as well as $^{13}C/^{12}C$ ratios of atmospheric carbon dioxide (Passey *et al.*, 2002). It was estimated by Cerling and Harris (1999) that with respect to the plant source, the bioapetite of large animals (e.g. horses) shows an enrichment of about +14.1±0.5‰. After the estimation of all enrichment factors, Cerling and Harris (1999) reported that values range from -16‰ to -9‰ in the case of C_3 vegetation, while C_4 vegetation shows the $\delta^{13}C$ values between -2‰ to +3‰ for tooth enamel. The depleted values of $\delta^{13}C$ indicate a closed habitat (Forest land ecology) while enriched values indicate more open habitat (most likely woodlands and grasslands) (Nelson, 2005).

In our study, we used enamel rather than bone because enamel is resilient to diagenetic effects which can alter isotopic values in fossils (Lee Throp and Sponheimer, 2003). There are many reasons of using mammalian fossils over paleosols like straightforward identification and isotopic signals are enhanced in mammals due to selective feeding as reported by Cerling *et al.* (1997).

In this study, stable carbon and oxygen isotopic analyses have been done for fifteen samples of hipparions from Dhok Pathan Formation (Fig. 1) of the Siwaliks of Pakistan ranging from late Miocene to latest Miocene.
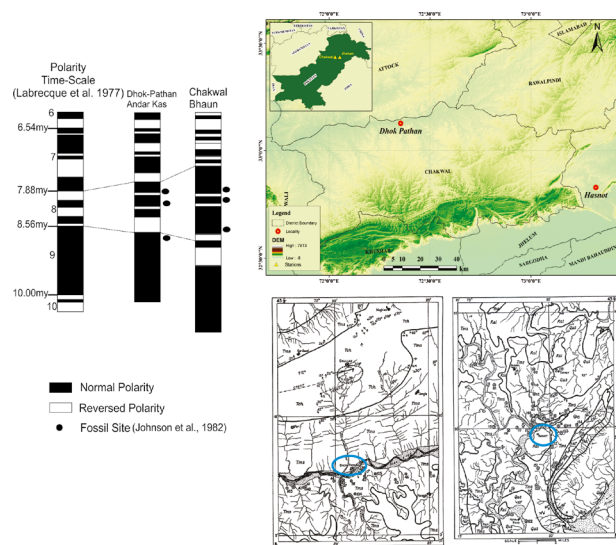

Fig. 1. The studied sites along with the localities.

MATERIALS AND METHODS

Sampling

Tooth enamel samples of three species of hiparionines, five samples from each species, *Sivalhippus* cf. *theobaldi, Sivalhippus* cf. *perimensis,* and *Cremohipparion* cf. *antilopinum* from the Siwalik of Pakistan were analyzed for the estimation of carbon and oxygen ratios in tooth enamel. Samples were collected from the Dhok Pathan Formation (late Miocene) of the Siwaliks. All the samples are preserved at Palaeontology and Environmental Biology Lab, Department of Zoology, University of the Punjab, Lahore, Pakistan. An excellent collection (~500 samples) of hipparionine horses having maxillae and mandibles in addition to upper and lower incisors, canines, premolars and molars has been housed at this university. Among this collection precisely identified specimens of *Sivalhippus theobaldi, Sivalhippus perimensis,* and *Cremohipparion antilopinum* were picked for isotopic analyses because in our collection very few specimens belong to *Sivalhippus nagriensis*. The fossil collection of specimens under study along with their respective ages has been given in Table I.

Enamel extraction

To obtain a drilled sample of tooth enamel, samples first washed, dried and scrubbed with wire brush to remove adhesive dust or contamination. The samples were then drilled by M-3 Champion drill machine to get the enamel extract in powder form. Drilling was done from root to crown direction from the lingual side of the tooth in a closed chamber (Fig. 1). The powder was collected and transferred to plastic vials by using a fine tipped brush. Each vial contains 15 mg of drilled enamel powder. For the analyses of enamel extract the samples were subjected to the Isotope Ratio Mass Spectrometer in PINSTECH (Pakistan Institute of Nuclear Science and Technology) Islamabad. Statistical analysis was done by using SPSS (Statistical Package for the Social Sciences) 16.0 version. Normality test was applied to check weather our data is normally distributed or not and then ANOVA (Analysis of Variance) test was applied with Duncan test for further results.

Pre-treatment and analysis of samples

The powdered sample of enamel was treated with H_2O_2 and CH_3COOH to counter the effects of inorganic and organic contaminants as proposed by Koch *et al.* (1997). Then enamel was further treated with 30% H_2O_2 for overnight, washed with de-ionized H_2O, reacted with 0.1 N CH_3COOH for 4 h and samples were washed. Ethanol was added and samples were left for drying out for 24 h. For CO_2 production, the enamel samples were dissolved in 95% H_3PO_4. The CO_2 was analyzed with an IRMS (Isotope Ratio Mass Spectrometry) at PINSTECH, Islamabad.

One solid carbonate ($CaCO_3$) and two gaseous (CO_2) standards were prepared and calibrated against V-PDB for internal use. The reproducibility of results and accuracy of mass spectrometric analysis was within the internationally accepted limits of 0.1 – 0.2‰.

Mass spectrometric analysis of CO_2 is performed through isotopic masses $^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O$, $^{12}C^{16}O^{16}O$, $^{12}C^{16}O^{18}O$, $^{13}C^{16}O^{17}O$, $^{12}C^{17}O^{17}O$, $^{12}C^{16}O^{17}O$. For carbon-13 analysis, we measured mass 45 versus combined mass 44 + mass 45 in CO_2. Similarly, for the analysis of oxygen, we measured mass 46 versus the combined mass 44 + mass 45 in CO_2.

Table I. The stable isotope values of carbon and oxygen along with the localities and age information.

Sample code.	PUPC No.	Local-ities	Ages (Ma) (John-son *et al.*, 1982)	δ ^{13}Cc ‰ (V-PDB)	δ ^{18}Oc ‰ (V-PDB)
EB-01	83/80	DP 3	7.7	-5.10	-7.12
EB-202	85/53	DP 3	7.7	-3.36	-7.14
EB-203	86/153	DP 2	7.7	-4.94	-3.81
EB-102	00/24	DP 4	8.0	-6.83	-8.40
EB-103	08/718	DP 4	8.0	-6.67	-7.71
EB-101	67/339	DP 4	8.3	-7.27	-8.98
EB-105	86/98	DP 4	8.3	-7.75	-9.21
EB-204	02/119	DP 4	8.3	-7.93	-9.57
EB-205	08/568	DP 4	8.3	-8.80	-8.18
EB-02	94/66	DP 4	8.3	-8.50	-8.01
EB-03	11/15	DP 4	8.3	-7.92	-11.03
EB-205	08/568	DP 4	8.3	-8.80	-8.18
EB-05	06/15	DP 5	8.4	-9.50	-11.48
EB-104	83/317	DP 6	8.4	-9.94	-9.90
EB-201	68/720	DP 5	8.4	-9.45	-6.89
EB-04	83/802	DP 5	8.8	-11.27	-10.38

For the ages of the specimens, the sections of Johnson *et al.* (1982) are correlated with geo-magnetic polarity time scale of LaBreque *et al.* (1977).

RESULTS

Carbon isotope ratios

Variations between all the hipparionine samples was found to be 8‰, ranging from -11.27‰ to -3.36. The mean values for *Sivalhippus* cf. *theobaldi, Sivalhippus* cf. *perimensis* and *Cremohipparion* cf. *antilopinum* is

-8.458‰, -7.692‰ and -6.896‰ respectively. Normality test indicates that all the values are normally distributed. The two-way ANOVA was performed along with Duncan test which indicated that the differences between these three species are not significant. Most of the values are carbon depleted while one value of *Cremohipparion* cf. *antilopinum* shows a value of -3.36‰.

Oxygen isotope ratio

The δ18O values for all the samples range between -11.48‰ to -3.8‰. Means for *S.* cf *theobaldi, S.* cf. *perimensis and Cremohipparion* cf. *antilopinum* are -9.604‰, -8.84‰ and -7.118‰, respectively. Shapiro Wilk test revealed that all the δ18O values are normally distributed (P> 0.05). ANOVA indicated that difference between these species is not significant (p = 0.106).

Table I shows the stable isotope values of carbon and oxygen along with the localities and age information. For the ages of the specimens, the sections of Johnson *et al.* (1982) are correlated with geo-magnetic polarity time scale of LaBreque *et al.* (1979).

Fig. 2. Extraction of enamel from the *Hipparion* molar along the vertical axis. Scale bar indicates 10mm.

DISCUSSION

Palaeodiets

Generally, carbon isotope values (Table I, Fig. 3) follow the palaeodietary investigations based on the dental morphology. Most of the values of δ13C were depleted while one sample of *Cremohipparion antilopinum* was relatively enriched in δ13C value (-3.76‰). Carbon isotope

values were however, not significantly different, but revealed fine variations between dietary behavior of these species. *Sivalhippus theobaldi* represent the most depleted values for carbon isotopes averaging -8.4‰ as compared to *Sivalhippus perimensis* which shows an average value of -7.6‰. *Cremohipparion antilopinum* is most enriched in carbon isotope values averaging -6.8‰ (Fig. 3). These minor variations in mean values of respective species indicate insignificant change in feeding niche which can be explained on the basis of niche breadth over time. The dietary behavior changed from exclusive feeding on C_3 grasses to a mixture of C_3/C_4 vegetation as it is indicated by dental morphological studies. More negative values for earlier species (*S. theobaldi*) indicate a less tougher food compared to succeeding species i.e. *S. perimensis* and *C. antilopinum*. Rather than niche shift, it was increase in niche breadth represented by these species. C_4 vegetation appeared in the Siwaliks around ~7.4 Ma as reported by Barry *et al.* (2002). Before this time period, there was dominant C_3 vegetation on which these species relied. The present study strongly supports the hypothesis that early hipparions inhabited C_3 vegetation while latter species experienced a mixture of C_3/C_4 vegetation with woodlands and patchy grasslands.
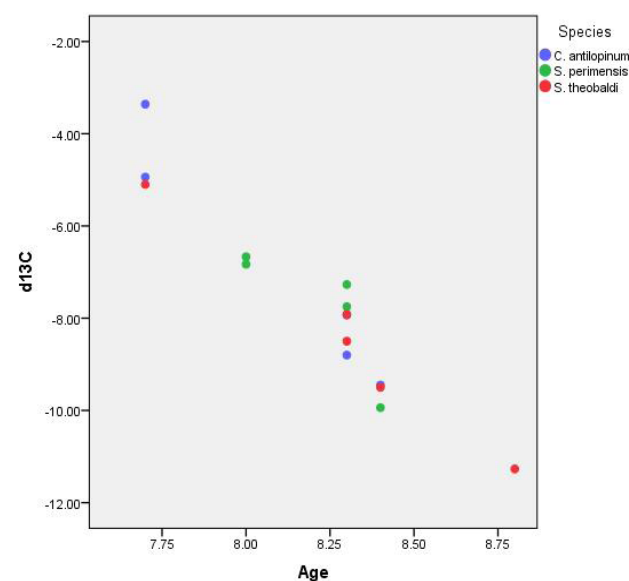

Fig. 3. The stable isotope of carbon along the age of the samples.

During late Miocene, a change in environment occurred globally, resulting into C_4 expansion. The C_4 expansion mainly depends upon temperature and partial pressure of atmospheric carbon dioxide as reported by Ehleringer and Monson (1993). Expansion of C_4 is

characterized by the higher temperatures, aridity and low pCO_2 as C_4 plants show the anatomical characters to reduce the loss of CO_2 from leaf. Emergence of C_4 ecosystem first appeared in low latitudes and was reported from paleosols studies of sediments from the Siwaliks of Pakistan by Morgan *et al.* (1994). Initial hypothesis for this change was made by Cerling *et al.* (1993) as he proposed this change was a consequence of global reduction in pCO_2. In our study which spans from 8.8 Ma to 7.7 Ma, a trend of positive isotope shift has been noted which, in turn, reveals the increase in C_4 consumption in late Miocene by these hipparion species.

Palaeoclimate

The creation of more open habitats and decrease in close canopy forests and woodlands which resulted due to environmental change, exerted a pressure on inhabiting fauna and trend shifted from browsing/grazing on C_3 vegetation to C_4 grasses (Cerling *et al.*, 1993; Nelson, 2005; Bibi, 2007). As reported by Barry *et al.* (2002), late Miocene was a period of great faunal and floral change, however, this study notices a fine niche expanded by the hipparion fauna due to this changing climate which represents that about 10.7 Ma, the degree of faunal change was not as rapid as afterwards. The depleted values of carbon (-11.27‰) at 8.7 Ma indicate forestland/woodland setting with humid conditions ($\delta^{18}O$ = -10.38‰). The environment became warmer and drier with the passage of time as at 8.3 Ma the forest lands were more constricted and open lands (woodlands/grasslands) expanded more ($\delta^{13}C$ = -8‰) while rainfall became more seasonal ($\delta^{18}O$= -9‰), may be due to uplift of Himalaya which happened in late Miocene (Quade *et al.*, 1989). At 7.7 Ma C_4 vegetation was a part of the ecosystem and the climatic conditions were warm and drier while monsoon shifted the most of the annual rainfall to the summer months April-July (Nelson, 2005). The comparative data of East Africa with the Siwaliks reports that the equids adapted to C_4 dominant environment more rapidly compared to the Siwaliks (Fig. 4). This hypothesis is in-line with the hypothesis developed by Cerling *et al.* (1993) that C_4 expanded earlier in low latitude regions.

These isotope shifts and C_4 expansion can be associated with other factors rather than decrease in pCO_2 as described by the following authors. These factors include regional dynamics like higher aridity and change in precipitation rates (Fox and Koch, 2004). This increase in seasonality and aridity may be attributed to uplift of Himalaya and consequent tectonic changes (Quade *et al.*, 1989; Wang *et al.*, 2006). Now all these changes triggered the weathering of silicate rocks which ultimately resulted in fall of atmospheric pCO_2 (Ehleringer and Monson,
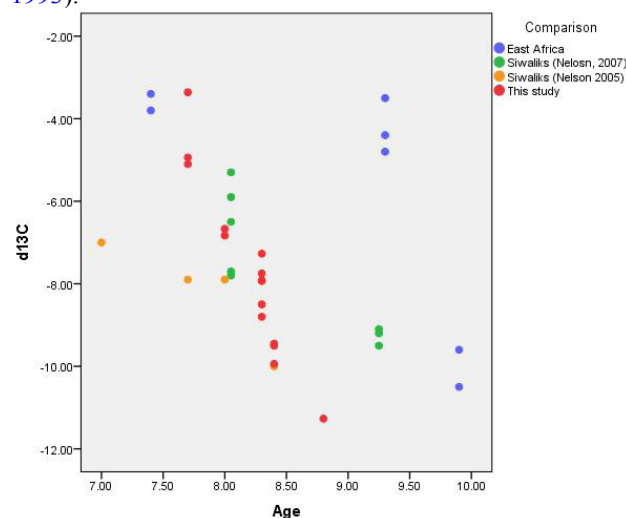
1993).

Fig. 4. A comparative picture of stable isotopes of carbon for the family Equidae across the late Miocene of the Siwaliks and East African habitats. Reference data taken from Nelson (2005, 2007) and Uno *et al.* (2011).

Cerling *et al.* (1993) reported the values of $\delta^{13}C$ for equids indicating that before 8 Ma the diet of these equids was dominantly C_3 but after 8Ma the trend started to shift towards the C_4 dietary niche. We attribute all above reported changes as the reason of faunal turnover and since animals were forced to expand their dietary niche they encountered a new habitat consisting of open grass and lands dominantly. The animals who survived this faunal turnover adapted to new habitats (Marino *et al.*, 1991) while others became extinct or migrated. Less depleted values of carbon isotopes along the temporal gradient in late Miocene (from 11.27‰ to 3.36‰) indicates a shift in vegetation pattern. Our study supports all these evidences and suggests that in late Miocene there was C_4 expansion between 8-5 Ma.

Oxygen isotope ratios

The means of oxygen isotope ratio (Fig. 5) for these three species are not significantly different. The highly depleted values are reported for grazers by Sponheimer and Leethorp (1999). Mainly oxygen values depend upon many factors like feeding from canopy tops (enriched) in comparison to feeding from understories (depleted), which can be seen in Bovids (Bibi, 2007), water intake from evaporating water holes (enriched) in comparison to water intake from rivers (depleted).

These horses can be attributed as obligate drinkers from standing water which indicates that *S. theobaldi* relied on the shaded ponds for their water intake (average

$\delta^{18}O$ = -9.6‰) while the later species were taking water from more open evaporative water holes as indicated by higher oxygen isotope values (average $\delta^{18}O$ = -7.9‰).
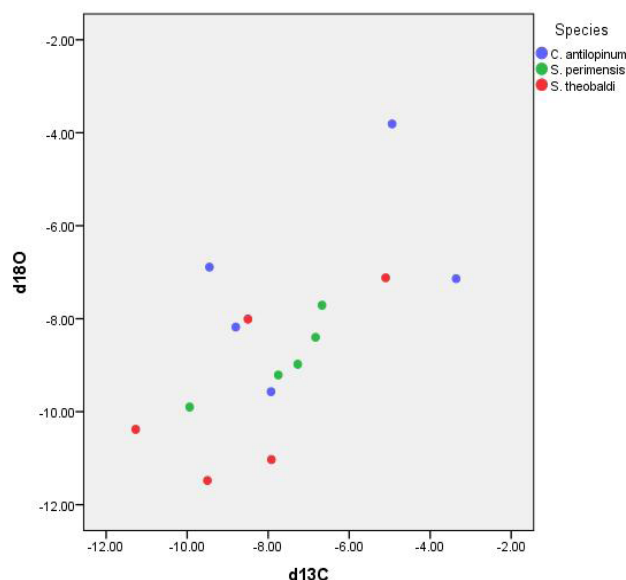

Fig. 5. Carbon and oxygen stable isotopic values for dental specimens of hiparionines.

On the basis of isotopic analysis of the fossils materials of hipparionine, we can argue;

(1) that the C_3 vegetation was dominantly present in the Siwalik continental deposits till 8.8 Ma Ma and then there was shift toward C_3/C_4 vegetation (8 Ma) or more dominantly C_4 vegetation started (7.7 Ma). So, we can conclude that till ~10–5 Ma both C_3/C_4 type of vegetation was present but for ~10–7 Ma, more C_3 and less C_4 type of vegetation was present and after that ~7–5Ma more C_4 and less C_3 type of vegetation was present. According to different authors (Cerling *et al.*, 1997; Quade *et al.*, 1994; 1995; Zachos *et al.*, 2001) the monsoon season was much likely similar in the Dhok Pathan Formation as of present days in which both types of vegetation are present in the Siwaliks.

(2) Till date we do not have any material of *Sivalhippus nagriensis* from Nagri Formation, but we collected very few specimens from Dhok Pathan Formation as compared to other three species mentioned in this study (*S.* cf *theobaldi, S.* cf. *perimensis* and *Cremohipparion* cf. *antilopinum*).

(3) Based on dental morphology and paleoecology, we agree that *S. theobaldi* has less derived characters as compared to *S. perimensis* and the oldest occurrence of this species confirms the fact while the younger stratigraphic range of *S. perimensis* suggests that this species might be derived from *S. nagriensis*.

(4) Higher values of $\delta^{13}C$ in one sample of *Cremohipparion* cf. *antilopinum* reveal that this species was present at the time of higher monsoon season while the hypsodonty index of *S. theobaldi* and *S. perimensis* suggest that these species relied more on C_3/C_4 and even more C_4 type vegetation as compared to C_3 vegetation.

(5) The gradual complexity in the teeth of hipparionines indicate that these species were trying to adapt with new vegetational type and were successful until C_4 became very dominant hence Hipparions replaced by Equids by the Pliocene time span.

CONCLUSION

Fossil Hipparionine samples are subjected to stable isotopic analysis belonging to the late Miocene of Pakistan. The carbon isotope values suggested that the early hipparions were dominantly relied upon C_3 vegetation while latter species consumed a mixture of C_3/C_4 grasslands for their diet. Our study follows the hypothesis developed on the basis of morphologic studies and also discriminates between open and closed environment in the late Miocene. However, this study also helps us to understand the palaeoclimatic conditions more deeply.

Values greater than -9‰ are reported from our study indicate the emergence of C_4 grasslands around 8Ma which may be attributed to low pCO$_2$ and tectonic uplift of Himalaya. It is evident that not only single factor was involved in the late Miocene faunal turn over but a sequence of factors which may be responsible for the uplift of Himalaya, resulting the low pCO$_2$ which in turn was responsible for the emergence of C_4 grasslands. Our study also reports that in late Miocene the environment was warm and arid with open habitats. The variations between the isotopic values of these hipparionine species support the niche breadthening over niche shift during late Miocene in the Siwaliks.

Statement of conflict of interest

The authors have declared no conflict of interest.

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