New host races of *Aphis gossypii* (Insecta: Hemiptera: Aphididae) from northeast India

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Insect herbivores show dynamic interaction with their food plants in space and time. Intra-species variations in response to new environments, both biotic and abiotic, though well-known in allopatric and sympatric populations, were very little documented on closely related hosts. Two new host races of *Aphis gossypii*, a polyphagous aphid species of agriculture and horticulture importance, are reported from Tripura, northeast India. Natural populations of this aphid from two congeneric hosts, wild taro (*Colocasia esculenta esculenta*) and cultivated taro (*Colocasia esculenta antiquorum*), showed significant differences in life history traits. Aphids from wild taro clones were bigger in size, showed higher mean relative growth rate and intrinsic rate of increase, and produced more offspring per generation than those from cultivated taro clones which were smaller in size and took longer development and generation time. Reciprocal transfer of aphids between the two host plant subspecies significantly dwindled their colonization success rate in successive three generations on the transferred hosts. Results suggest that asexual lineages of aphids show adaptation to human-imposed selection pressure in the host environment.

Keywords: Aphid, *Aphis gossypii*, host plant interaction, host races.

As obligatory parasites of plants, the survival and reproductive fitness of aphids are dependent on their host plants[1,2]. Several studies have recorded host races based on differences in biological and ecological performances of aphids[3–6], but very little is known of such differences in asexual populations of polyphagous aphids from the tropics[7–9]. Growth rate and intrinsic rate of increase, among other attributes, are considered to be important biological traits of adaptation in an aphid's life history strategy on a host plant[10,11].

Aphis gossypii Glover is a well-known pest and vector of viral diseases in agriculture and horticulture[12,13]. This aphid is one of the major pests of taro[14,15] and is a vector of the taro virus, dasheen mosaic virus[16]. Cultivated taro, *Colocasia esculenta* (L.) Schott *antiquorum* (Schott) Hubbard and Rehder, is widely cultivated in swamps or flooded lands; whereas wild taro, *Colocasia esculenta* (L.) *esculenta* Schott, grows in dry or semi-dry conditions along roadsides, near ditches, drains or water-logged areas in large parts of northeast India. Thus, the two taro subspecies occur in different niches without geographical isolation.

It was predicted that the sympatric populations of *A. gossypii* living in two different host environments of wild and cultivated taros might show differences in their ecological and biological fitness. It was tested by examining the population growth, intrinsic rate of increase, and developmental and reproductive attributes of *A. gossypii* collected from wild taro and cultivated taro plants. Aphids from the two hosts were also subjected to reciprocal host transfers to record the effect of a new host environment on the performance of aphids.

Apterous adult viviparous female of aphids is responsible for colonization of a host, whereas alate adults act as dispersal agent. Apterous adults were collected randomly from wild and cultivated taro plants in a rural location, about 10 km south of Agartala, northeast India (23.50°N, 91.25°E). Those aphids represented different asexual lineages and were used to raise stock cultures on their respective hosts in a greenhouse at a temperature of 24 ± 1°C and photoperiod of 16 : 8 L : D to correspond with the average climate of the study area.

Ten host plants of each subspecies of taro in early vegetative stage were maintained individually in 20 cm diameter plastic pots and these were held in water trays on benches illuminated with photosynthetically active radiation lamps[2]. Each plant was infected at random with a single fourth instar apterous aphid collected from wild or cultivated taro in the field. These were allowed to grow to adult stage, reproduce and increase in number. Aphid cultures on individual potted plants were confined in nylon net cages and separated from each other to prevent contamination. All the aphids produced from a single parthenogenetic mother on each of the plants by this practice consisted of the same genotype and, thus, constituted a clone. As a result, several independent clones of *A. gossypii* on two subspecies of taro were produced in the laboratory. In order to achieve maximum randomization effect on aphid–host relationship, fourth instar aphids from the stock culture were chosen randomly and were placed individually on the apical part of another set of 16–20-day-old pot-grown saplings at the early vegetative stage in a rearing cabinet (Sheldon, USA) (temperature: 24 ± 1°C; RH: 65%; photoperiod: 16 : 8 L : D). Thus, several sister clones of mixed origins of *A. gossypii* were raised in the laboratory on the two hosts. Observations were made at frequent intervals until each clone attained maximum increase in population and then started to decline. Sister clones were monitored individually several times a day. Aphids from these clones were used to record the following ecological and biological parameters.

Maximum population size (N_t), population growth rate and the time (T) taken to reach the N_t were determined for

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aphids on the two host plant subspecies[17]. Twenty replicates were used, ten on each plant subspecies. Population growth rate, denoting increase in the number of aphids of a clone per day per plant in the rising phase of population increase, was calculated by the formula

$$\mathrm{GR} = \frac{N_t - N_0}{\Delta t},$$

where N_t is the number of aphids recorded at the maximum count of the population on a plant, N_0 the number of aphids initially released on a potted plant, and ΔT is the difference in time between N_0 and N_t (ref. 18). Time taken to reach the maximum population size (T) was calculated by the equation $T = \sum$ No. of days to N_t/n, where n is the number of observations. Mean relative growth rate (MRGR) was determined by the following method[19]

$$\mathrm{MRGR} = \frac{\log_{10} \mathrm{AW} - \log_{10} \mathrm{BW}}{\mathrm{DT}} d^{-1},$$

where AW = weight of final molt (mg), BW = birth weight of aphid (mg) and DT = development time from the time of birth to the final molt. The intrinsic rate of increase (R_{max}), a measure of rate of increase of a population under controlled conditions, was calculated using the formula[20]

$$R_{max} = 0.738(\log_e \mathrm{MGT})/\mathrm{GT} \text{ (per day)},$$

where 0.738 is a constant, GT = generation time, MGT = number of nymphs laid during the time equal to GT.

DT, GT, reproductive duration (RD), and total fecundity (F) were determined for individual aphids of *A. gossypii* of the two host subspecies. This was done by allowing individual fourth instar nymphs to settle and feed on tender or young leaf of a potted plant in a temperature controlled cabinet at 24 ± 1°C (n = 10 for each host species). Nymphs were allowed to become apterous adults, to reproduce in the first 24 h and then the adults were removed. Only one new born aphid of an adult was retained and the rest removed. Its weight was recorded and allowed to develop to the final molt when it was weighed again and observed for the durations of pre-reproduction, reproduction and post-reproduction. The number of nymphs born to individual aphids was counted and all but one was removed. The remaining aphid was allowed to develop in experimental culture. As a result of this procedure, BW of nymphs within 12 h of laying by a mother aphid, AW at the final molt, DT from birth of a nymph to its final molt, GT from the birth of a nymph to the onset of reproduction by this nymph, RD from the birth of the first nymph to the last nymph laid by an apterous female and the lifetime F, representing the total number of offspring produced in life, were recorded.

Aphids of clones from the wild and cultivated taro hosts were subjected to reciprocal host transfer to record the colonization success, which is the ability of individual aphid to survive and reproduce in a new food environment. Two experiments, I and II, were set up using parental clones of *A. gossypii* from the two host plant subspecies. In the first experiment, aphids were transferred individually from the wild taro field host to the laboratory host, cultivated taro; and in the second experiment, aphids were transferred from the cultivated taro field host to wild taro as the laboratory host. Individual nymphs, 0–12 h old, obtained from the laboratory clones were released at the apical most part of potted plants of 12–16 days-old of field (control) and laboratory hosts (treatments). Aphids were allowed to settle and produce nymphs for the first generation. If successful, second and third generations were produced. Ten replicates of control and another ten replicates of treatment were used in each experiment to record the success rate of survival and reproduction by apterous viviparous aphids of a host plant leading to the establishment of colony. Aphids that either failed to develop to the adult stage in the first generation or failed to produce second or third generations were considered to be unsuccessful.

Data on the third generation aphids, wherever available, were used to compare results of ecological and biological parameters tested in this study. This was done to allow the field-collected aphids' sufficient time for acclimatization to the laboratory rearing conditions of greenhouse and rearing cabinet. All weights in this study were taken in a Mettler microbalance sensitive to 2 µg. All the parameters that were measured from the wingless aphids of *A. gossypii* clones met the criteria of normality and equal variance and these were compared using the two-tailed Student's t-test. Data of colonization success was tested by chi-square test. Origin 7 (www.originlab.com) was used for the analysis of data.

Population GR of *A. gossypii* clones from the two taro hosts did not show difference (mean ± SE: wild taro = 11.79 ± 1.04 aphids/day/plant, cultivated taro = 10.72 ± 0.71 aphids/day/plant; t value = 1.599, df = 18, $P > 0.05$ (NS)), but the maximum population size (N_t) attained by the clones on respective host plants showed significant difference (mean ± SE: wild taro = 287.12 ± 13.02 aphids/plant, cultivated taro = 202.84 ± 11.48 aphids/plant; t value = 2.99, df = 18, $P < 0.01$). The time taken by the aphids of the two clones to achieve the maximum population size (T) on their respective host plants also showed significant difference (mean ± SE: wild taro = 23.80 ± 1.37 days, cultivated taro = 20.20 ± 0.92 days; t value = 2.960, df = 18, $P < 0.01$). Mean values of MRGR and R_{max} from 10 replicates of the *A. gossypii* clones from wild taro were significantly higher than the mean values of clones that were reared on cultivated taro (Figure 1 *a* and *b*).

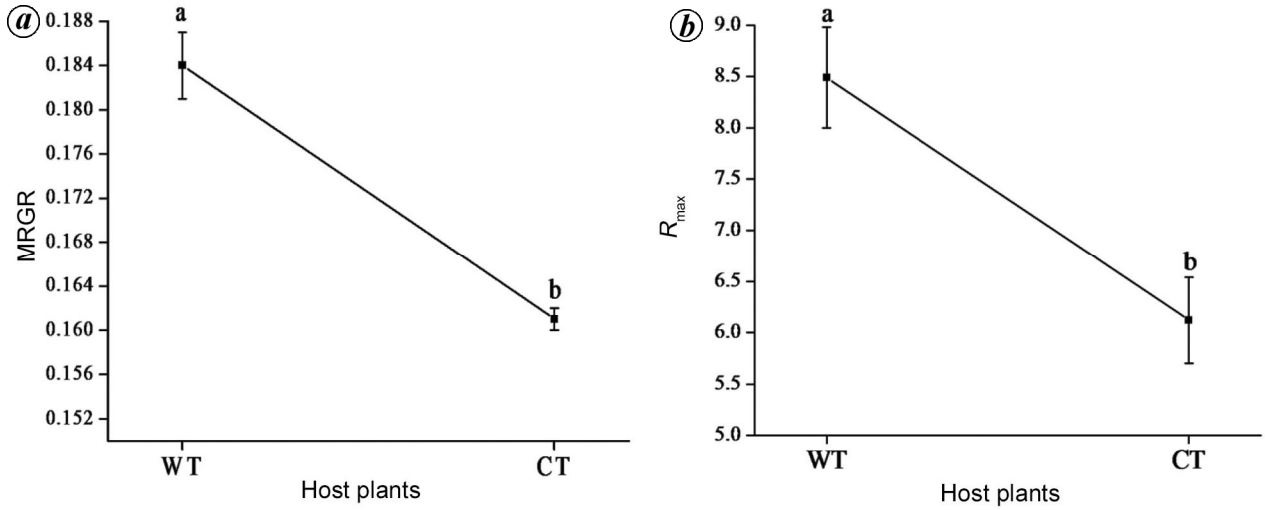

Figure 1. Mean values of (***a***) mean relative growth rate (MRGR) and (***b***) intrinsic rate of increase (R_{max}) of *Aphis gossypii* clones on wild taro (WT) and cultivated taro (CT) host plants respectively. Bars with mean values are standard error of means. Dissimilar alphabets appearing with bars denote significant differences between mean values by two-tailed student *t*-test at $P < 0.05$.

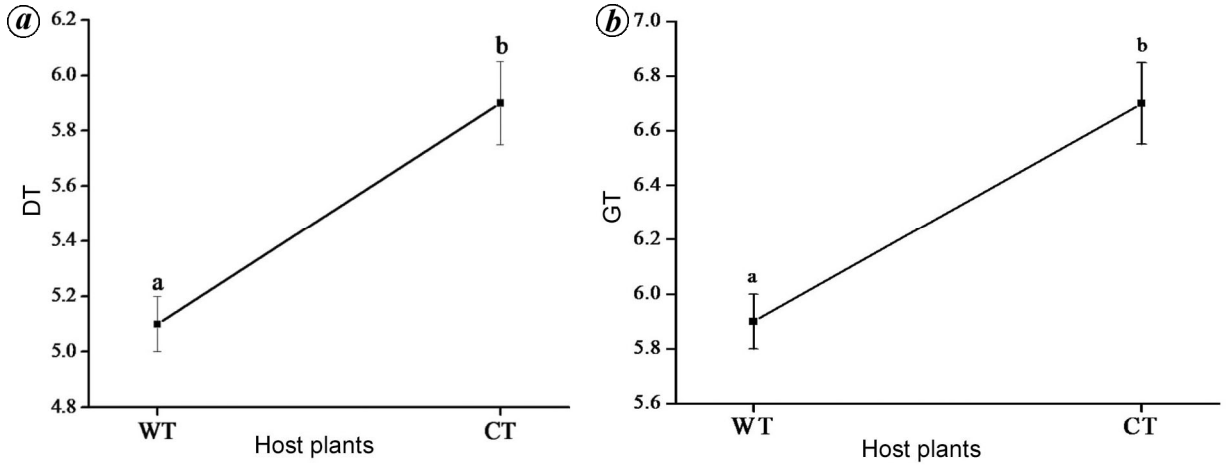

Figure 2. Mean values of (***a***) development time (DT) and (***b***) generation time (GT) in days of *Aphis gossypii* clones on wild taro (WT) and cultivated taro (CT) host plants respectively. Bars with mean values are standard error of means. Dissimilar alphabets appearing with bars denote significant differences between mean values by two-tailed student *t*-test at $P < 0.05$.

Significant differences in DT, GT, lifetime F and weight of aphids at birth and at the day of first reproduction in the aphids from the two taro hosts were also recorded. Aphids from wild taro were 1.3 times heavier at birth (t value = 4.70, df = 18, $P < 0.001$) and 1.25 times heavier on the day of first reproduction (t value = 7.31, df = 18, $P < 0.001$; Figure 2), and their DT and GT were significantly shorter (DT: t value = 4.58, df = 18, $P < 0.001$; GT: t value = 4.38, df = 18, $P < 0.001$) (Figure 3) in comparison to the aphids reared on cultivated taro. Individual aphids of wild taro clones, on average, produced about 32% more offspring in significantly shorter reproductive time than aphids of cultivated taro clones which took more time to produce less number of aphids (fecundity: t value = 3.58, df = 18, $P < 0.001$; Figure 4 *a*; reproductive time: t value = 3.58, df = 18, $P < 0.001$; Figure 4 *b*).

Figure 5 presents results of success rate of colonization by *A. gossypii* clones when transferred from their field hosts to laboratory plants in the greenhouse in the two experiments. Transfer of aphids from wild to cultivated species of taro recorded only 45% success in the third generation (third generation: $\chi^2 = 8.4$, $P < 0.01$), whereas the transfer of aphids from cultivated to wild species recorded 50% success rate (third generation: $\chi^2 = 7.3$, $P < 0.01$). Thus, the performance of the aphids in the two experiments was lowered when they were transferred to the other host.

This study has shown that *A. gossypii* on the two congeneric taro hosts exhibited differences in MRGR, intrinsic rate of increase, DT, GT, lifetime F, and sizes of aphids at birth and at the first maturity. When the aphids from the two hosts were subjected to reciprocal host

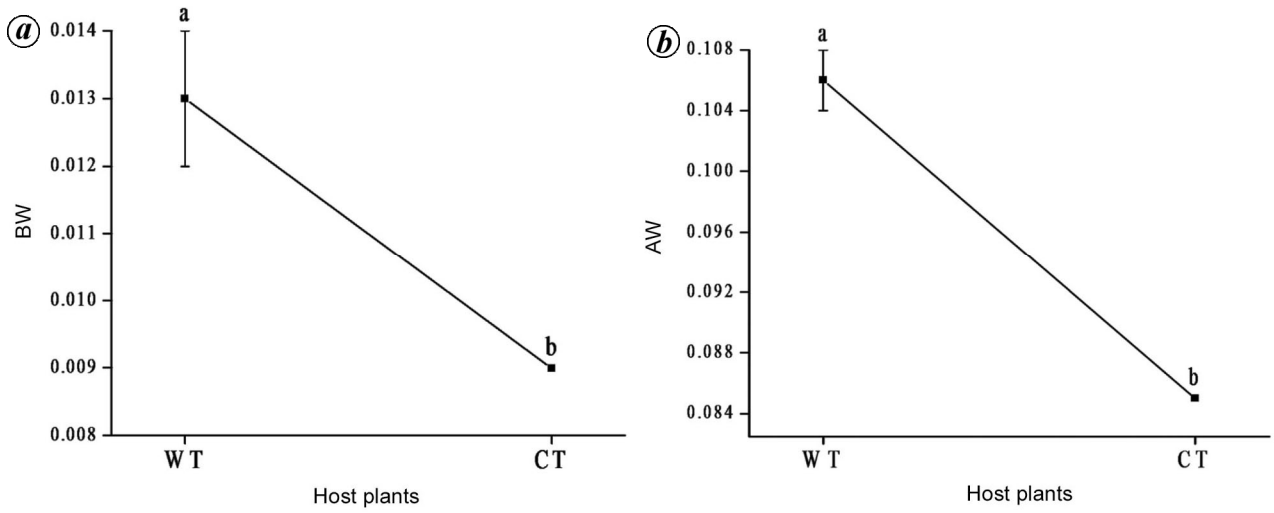

Figure 3. Mean values of (***a***) birth weight (BW) and (***b***) adult weight (AW) in mg at the first reproduction of *Aphis gossypii* clones on wild taro (WT) and cultivated taro (CT) host plants respectively. Bars with mean values are standard error of means. Dissimilar alphabets appearing with bars denote significant differences between mean values by two-tailed student *t*-test at $P < 0.05$.

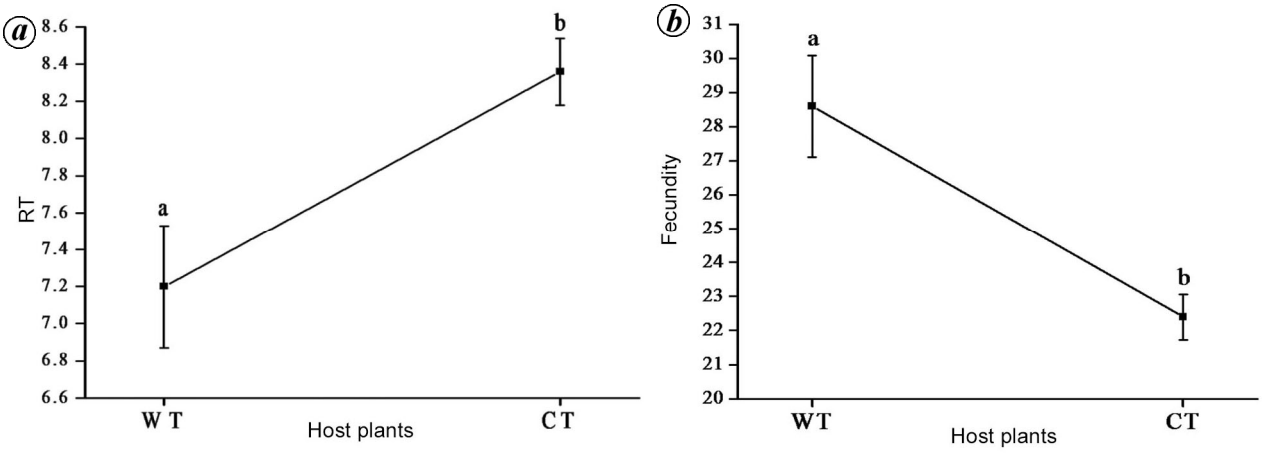

Figure 4. Mean values of (***a***) reproductive time (days) and (***b***) life-time fecundity of *Aphis gossypii* clones on wild taro (WT) and cultivated taro (CT) host plants respectively. Bars with mean values are standard error of means. Dissimilar alphabets appearing with bars denote significant differences between mean values by two-tailed student *t*-test at $P < 0.05$.

transfers, their survival declined significantly in the new host environment. Between the two taro hosts, wild species is primitive and less abundant, whereas cultivated species is domesticated and more recent, and more abundant. The two hosts also showed differences in morphology, nutritional quality of phloem sap and genetic structure[21]. *A. gossypii* on wild taro were more fecund and showed higher rates of relative growth and increase in comparison to less fecund aphids on cultivated taro of recent origin. Results showed intraspecific variation in host choice and suggested that aphids can select less rewarding food plant in the same geographical space. The selection pressure that might cause sympatric aphids to explore and adapt to novel host environment has been variously explained[7,8]. It has been demonstrated that genetic variation in populations of a species can cause individual insects to respond differently in terms of ecological and biological performances in a local environment; thus, the plasticity of a trait is adaptive across environments[22–24]. The degree of plasticity that can be expressed by insects depends on the capacity of the insects for making physiological, morphological and behavioural adjustments in response to the nutritional and chemical cues, and physical structure of the host plant and their relative abundance[25–27]. Between the two taro host subspecies, wild taro plants are perennial, relatively less abundant, and highly scattered in distribution in comparison to cultivated taro plants which are seasonal, more abundant, and grown intensively from India to Indonesia in the south of Asia and up to China in the north[21].

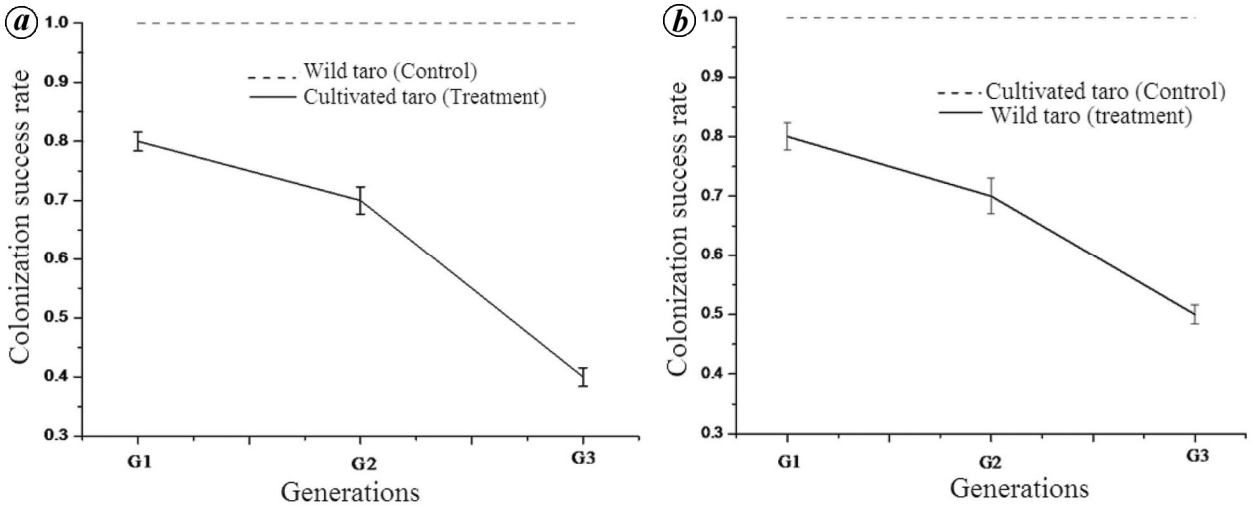

Figure 5. Mean proportion of success rate of colonization of *Aphis gossypii* clones through three generations on their field host (control) and across host plants. (***a***) Treatment I: *A. gossypii* of wild taro transferred to laboratory host cultivated taro; (***b***) Treatment II: *A. gossypii* of cultivated taro transferred to laboratory host wild taro. Bars with means denote standard error of means.

Several host-based races of cyclical populations of *A. gossypii* were identified in Japan, China and USA according to variations in their rates of increase on different host plant species[4,28,29]. Given that there has been no reported occurrence of sexual reproduction in *A. gossypii* in the tropics of Indian subcontinent, the chief factor that might contribute to the observed variability in acyclical populations of *A. gossypii* on the two taro subspecies could be the host plant specialization. In this scheme, asexual viviparous aphids undergo constant pressure of host selection in heterogeneous environment, and the choice of host selection is chiefly determined by the proximate causes of interaction between aphid phenotype and host environment[30]. Results highlight the importance of differences in host plants which can cause differentiation in life history traits of populations of an insect species without geographical isolation.

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Determination of the state of origin of the writer from the class characteristics in English handwriting

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In this research work, we discuss class characteristics in English handwriting of people from Tamil Nadu, Uttar Pradesh and West Bengal. The subjects had primarily studied in their local language, with English as their second language. The regional handwriting samples from 750 subjects were collected randomly in the age group between 18 and 60 years and their class characteristics such as writing movement, formation of letters, letter designs, pen-lifts, letter size, artistic ability, letter spacing and embellishments were examined. Several characteristic features peculiar to the individual linguistic groups were identified and the impact of the regional language script writing system was observed on the English handwriting of almost all the subjects.

Keywords: Class characteristics, forensic science, handwriting, linguistic groups.

THE multi-linguistic society in India provides ample opportunities for examining English handwriting of different linguistic group as the education system emphasizes on bilingualism. Thus, each child learns English and also his/her local language. The subjects selected for the study are from the states: Tamil Nadu (TN), West Bengal (WB) and Uttar Pradesh (UP)[1]. It is common that Tamilian children learn Tamil, children from UP learn Hindi and Bengali children learn Bengali in their schools and also at home. Exposure to a writing system[2] using characters different from the Latin script may give rise to the observed distinctive characteristics of different linguistic groups while writing English. In this communication, we study the class characteristics of the subject's English handwriting and explore possible influence of local language on English handwriting.

The Tamil writing system[3–5] is the most common Indian language used and learnt by the Tamilians in TN. It has 12 vowels, 1 rhythm and 18 consonants (Figure 1).

Figure 1. Tamil numerals, vowels and consonants[5].

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