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Phenotypic plasticity in *Callosobruchus maculatus* (Fab.)- A critical review (Bruchidae: Coleoptera)

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Abstract
Callosbruchus maculatus (Fab.) is a cosmopolitan species of bruchids and is popularly called as cowpea weevil as it is a major pest of *Vigna ungiculata* (L.). However it attacks a number of edible legumes in addition to *V. ungiculata* in different regions. Moreover it also shows variable behavior during its life cycle, being a field type in some parts and storage type species in others. It also acts as an intermediate species spending some part of its life in the field and some part in the store.
C. maculatus has been reported to appear in two forms during the course of the year, a normal form and an active form or flight form which is more active and much less fecund than the normal form. The active form shows facultative reproductive diapause. The behavior of this form is quite different in different parts of the globe, with its Indian strain showing complete sterility. We have carried out detailed investigations on the Indian strain of *C. maculatus* during last many years. The present report includes a review of our own work as well as other relevant studies on this subject in order to project its phenotypic plasticity in the Indian strain as of exceptional type. Suggestions for its true status have also been recorded.

Keywords: Bruchidae, *Callosobruchus*, Store pest, phenotypic plasticity

1. Introduction
Phenotypic plasticity is all about phenotype. A phenotype can be produced by several genotypes due to relative dominance of different alleles of a given gene. Also, a single genotype can produce many phenotypes depending on various contingences encountered during development. A phenotype is thus an outcome of a complex series of developmental processes that are influenced by environmental factors as well as genes [1]. The process of appearance of different phenotypes during development caused by the influence of different environments and genes is called phenotypic plasticity. Plasticity can be passive when it is produced by direct influence of the environment on chemical, physiological and developmental processes, which is not anticipatory [2]. Such plasticity is also called ‘reaction norm’. The reaction norm, as pronounced by many workers, is produced when phenotypes change gradually with graded changes in an environmental variable. Nijhout [1] believes that it is caused by differential effect on the rate and timing of biochemical processes, by a change in the level of expression of certain genes or by the expression of new genes and repression of others. On the contrary, active plasticity is caused by some environmental cue that results in the modification of developmental pathways and regulatory genes and is anticipatory [2]. This type of plasticity is known as polyphenism. Polyphenic development can occur if the organism faces an environment that varies abruptly or discretely. The environmental variable has a direct effect on development via allelic sensitivity and by changing the pattern of hormone synthesis. Polyphenism is expressed in many ways, as for example, change in the proportion of different body parts, altered food preferences, change in reproductive characteristics and variation in the colour patterns of body. In all these processes including behavioral and physiological polyphenism, the environment faced by an individual during developmental stages produces the adult phenotype which is adaptable to the predicted environment.
The bruchids attack their legume hosts in the field as well in the stores. These are accordingly categorized as ‘field bruchids’ and ‘store bruchids’. Suzuki [3] also recognizes a third type the members of which can breed both in the field and in the store. This category is named by him as ‘intermediate type’ besides calling the field bruchids and store bruchids as field type and store type. The field bruchids generally are univoltine and usually host specific, showing a long period of reproductive diapause (imaginal diapause).

The storage type bruchids breed continuously in the abundance of food source, without entering diapause. The intermediate type bruchids enter reproductive diapause for a short period in the field and then breed in the stores without stopping reproduction. The phenotypically plastic species show facultative diapause under the changing environment of different seasons unlike the obligatory diapause depicted by field species.

Callosobruchus maculatus (Fab.) is a cosmopolitan species and is popularly called as cowpea weevil, being a primary pest of *Vigna unguiculata* (L.) (Rongi). It is however associated with a number of edible legumes in different parts of the globe. *C.maculatus* is reported to be both a field and storage form in many regions but the strain met with in India behaves as a storage form only. In our extensive surveys of different areas, it has never been intercepted from any of its seven hosts in the fields.

We review in this article our intensive investigations during the past many years on the phenotypic plasticity of an Indian strain of *Callosobruchus maculatus* (Fab.), a common pest of edible legumes, alongwith results of other relevant works. Our results differ from all such studies in different regions and are thus of great ecological significance.

2. Observations

C. maculatus is known to occur in two forms. It was Utida [4] who first recorded these forms and named them as 'flight form' and 'normal form' in a Mexican strain of this species. According to him, the flight form is much more active and leaves the store due to high density, for breeding in the field. The same flight form from Nigeria was called as active form [5, 6],the females of which lay low number of eggs or no eggs. The active form of *C. maculatus* has also been reported by other African workers namely, Taylor and Abaje [7], Taylor and Aludo[8] and, Quedraogo and Huignard [9]. Utida [10, 11] reported delayed development of ova in the females of the flight form which also lay a low number of eggs. Messina and Renwick [12] also found a majority of the females of this form not laying any eggs. On the contrary, the flight form in the Indian strain, called abnormal form by the authors [13], is totally sterile with the ovary showing stunted growth without the formation of any ova. The four pairs of male accessory glands in this strain also show extremely poor development [14]. Although showing enhanced flying activity, the members of this strain however never visit fields and remain restricted to the stores. Complete sterility in the Indian strain was also confirmed by George and Verma [15, 16]. The occurrence of sterility in Indian strain is so deep rooted that pheromone production and reception centers are completely disrupted so that the abnormal females no longer attract the abnormal and normal males. Likewise, the abnormal males are no longer attracted towards the normal females. There is a quantitative and qualitative change in the antennal sensilla of abnormal males that make them non attractive towards females [17]. Pajni and Gupta [18] conducted antennectomy studies on the males of *C.maculatus* and found that only the flagellar segments can perceive the female pheromones. They also observed that the pedicel of this species has only one type of trichoidea sensilla whereas all the flagellar segments carry two types of trichoidea sensilla and one type of brasciconia sensilla. They concluded that at least one type of trichoidea or brasciconia sensilla is involved in pheromone perception. Moreover, the number of sensilla present is also important in pheromone perception [18].

Biochemical studies performed on the Indian strain revealed that the sperm nucleus is similar in normal and abnormal males [19, 20] although their survival period is relatively less in abnormal form. The free amino acid pool also does not show any difference among the two forms [21]. Sidhu *et al* [22] found less amount of free fatty acids, total lipids and cholesterol in the sterile form.

The occurrence of sterility in the Indian strain is perfected by serious structural changes that take place in the male genital organ. Besides being of much increased length, the saccus region of the endophallus loses the paired lateral chitinised and dented plates which are present in the males of the normal form. It is rather surprising that the presence of paired chitinised plates in the saccus part of endophallus is a characteristic feature of genus *Callosobruchus*. All the species so far studied possess these paired chitinised plates [13, 23, 24] (Fig. 1-4).

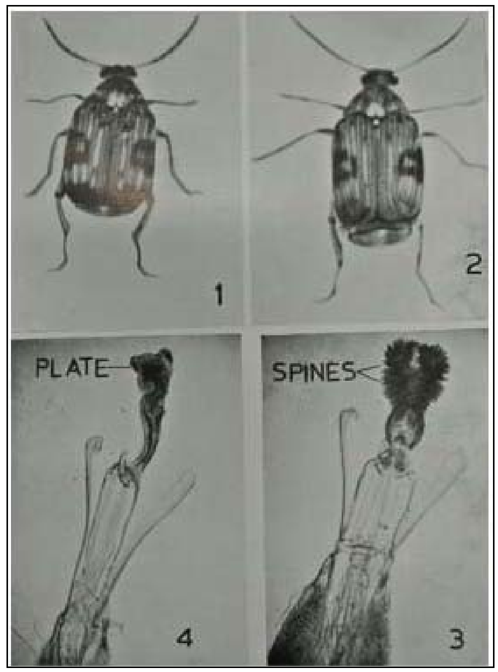

Fig 1-4: 1. Elytral colour pattern of abnormal made of *C. maculatus*. 2. Elytral colour pattern of normal made of *C. maculatus*. 3. Photograph of the everted genitalia normal made of *C. maculatus*. 4. Photograph of everted genitalia of abnormal made of *C. maculatus*.

This is a unique feature in the abnormal form of Indian strain which makes its status very exceptional in the phenotypic changes which occur in any species of bruchids. In addition to the changes in the pheromone apparatus and male genitalia, the Indian abnormal forms also depict increased melanization as also reported by the other workers [25, 4, 10, 11, 14]. Kapoor [26] and Kumar [27] also reported increase in the length of wings and male genital organ in the abnormal form.

The Indian abnormal form is produced by the rise in temperature and decline in relative humidity. The appearance of the abnormal form in the laboratory cultures as well as in the small stores and shops coincides with the sudden rise in temperature and fall in relative humidity in mid April. Starting with an initial strength of 5-7%, its population gradually rises to reach peak population of 80-90% in the second half of June when the day temperature fluctuates around 40ºC. With the onset of cool atmosphere due to monsoon during July, the population of abnormal form starts declining between July and September which completely disappears during October onwards. Our studies [13, 14, 23, 28] and Kaur [29] confirmed the starting and concluding date for the appearance of the abnormal form. We also performed some laboratory trials by changing temperature and relative humidity during developmental period. When seeds containing 9 day-old larvae (Second instar) were transferred from a culture maintained during January (temperature range 12 ºC-21 ºC) to four different temperatures of 20 ºC, 25 ºC, 30 ºC and 35 ºC for further development, 60% and 80% abnormal adults emerged from the jars maintained at 30 ºC and 35ºC respectively and no abnormal form emerged from the other two jars [23]. The second instar larvae from lab cultures during June and August when transferred to 20 ºC and 25 ºC for further development also did not produce any abnormal form, although original culture produced 50% and 20% abnormal adults respectively. Moreover, rearing *C.maculatus* under any constant condition of temperature (20 ºC, 25 ºC, 30 ºC and 35 ºC) and relative humidity (30% and 70%) does not induce the formation of abnormal forms as also observed by Sano [30], Utida [10] and Taylor [8] in the Mexican strain. The second stage larvae are also sensitive to a major change from high humidity (70%) to low humidity (30%) but only when bred at 35ºC. Accordingly, a threshold temperature of 35 ºC is essential to produce any results by the decrease of humidity. We also raised bruchid cultures under conditions that simulate the atmospheric conditions prevailing during May and June fixing temperature at 35 ºC during daytime and 20 ºC at night time with corresponding relative humidity of 30% and 70% respectively. The treatment results in the appearance of more than 50% abnormal population.

The workers outside India have held adult and larval density, rise in temperature and water content of the seeds [31, 10, 30, 8, 12], photoperiod [32, 33, 34] and parental condition [35] responsible for the production of the abnormal form. It was also reported that second instar larvae were sensitive to the high temperature for producing the active form adults. Zannou *et al* [36] simply referred to reproductive diapause in the life history of the flight morph females of *C. maculatus*. Suzuki [3] concluded his studies on *C. maculatus* by stating that the reproductive diapause is induced by the increase of temperature caused by larval density and the same was advantageous because such plasticity enabled weevils to lay more eggs during the harvest time and to enter reproductive diapause during the planting season in the field and, once in the storage, the constant resource availability leads to the evolution of non plastic adults with high fecundity and short life cycle.

The sterile female and male of Indian strain also appeared when reared in airtight cultures maintained for several generations. Sano [30] opined that carbon dioxide produced in the jars increases with the rise of temperature and that induces the active form production. According to Dawson [37] exposure to 100% carbon dioxide atmosphere for 8 minutes reduced the life time fecundity in *C. maculatus.*

3. Discussion

The exceptional phenotypic plasticity revealed by the Indian strain of *C.maculatus* is mainly due to the fact that this strain behaves as a storage type and breeds in the stored beans only. This active form shares some of the characteristics reported in the flight form of this species, as for example, the rise in temperature, change in relative humidity and exposure to CO_2 producing these forms in different strains of this species. Moreover, the sensitive stage during development (second stage larvae) is also reactive to the environmental cues that ultimately produce the abnormal/ flight form. However, similarity ends here because the Indian strain does not enter the long period of diapause and never visits the field for reproduction. The type of reduction in fecundity is also different in the two forms. As compared to less fecund flight form, the abnormal form of India is completely sterile. Although both the forms are active fliers, the sterile Indian strain expresses an unusual phenotype with structural changes in the male genitalia and other factors associated with the induction of sterility.

The altered phenotype in the abnormal form is totally irreversible and actually presents the end point because it does not survive further for any activity. As is well known moderate amount of adaptive plasticity is relevant for evolution in novel environments and high level of plasticity produces the likelihood of genetic change [38]. In fact degree of reversibility differs in different situations, whereas biochemical responses can be reversed in a short time but developmental plasticity tends to be irreversible. There is no fundamental difference between an irreversible phenotype and genetic polymorphism except for dependence of the former on environment for its appearance [2].

Indian strain of *C. maculatus* under discussion does not fit into any of three possible types of flightless forms of this species. It is neither a field type nor an intermediate type. It is a storage type strain showing a short reproductive diapause which is of exceptional type. The special phenotype is short lived, irreversible and involves total sterility and drastic changes in the male genital organ. It is though quite active like the flight forms of other authors, it never shifts to the field for further reproduction, being incapable of breeding.

Separately, in a bid to produce sterile males and females for use in Sterile Insect Release Method (SIRM) against bruchids, we exposed eggs, larvae, pupae and adults of different ages of *C. maculatus* at one day intervals to different doses of gamma radiation under a cobalt 60 source. It was found that pupal stage not only needs relatively lower doses but also produces 100% sterile adults not accompanied by mortality and mutations. Moreover, the sterile males and females are as good as normal individuals in different parameters of reproductive process. Exposure of fresh and one –day old pupae to 2000 rads, two-day old pupae to 2500 rads and three-day old pupae to 3500 rads produced 100% sterility in the males. Complete sterility in the females was caused by the exposure of two-day, three-day and four-day old pupae to 2000, 2500 and 3500-4500 rads. Both normal and sterile adults are equally competitive in different processes [39, 40].

The induction of sterility by these two factors is an equivalent phenomenon although the environmental cues and the reactive stage are different in the two cases. It is likely that the structural change in the temperature-dependent sterilization is in fact, also a mutation that results in producing a unique mutant, which can be established by detailed genetic experimentation. Kasiwagi and Utida [41] have reported a black coloured mutant in *C. chinensis* which is usually different from brown coloured adult and behaves as a single recessive chromosomal trait in the crossing trials. There is also a strong plea being made in favour of genetic structure acting at the base of polyphenism meaning that phenotypic changes are genetically based rather than the result of phenotypic plasticity [42]. Evans and Wheeler [43] also hold genetic architecture behind polyphenism involving interplay between genome, gene expression and phenotype. At least, both environmental and genetic cues can be involved in the evolution of phenotypic polyphenism as the phenotypic variants can be induced either by environmental or by allelic variation [44].

Reproductive diapause coupled with significant structural changes in *C. macualtus* is not at all adaptive to the environmental conditions faced by the altered phenotype. It can thus be concluded that phenotypic plasticity in the Indian strain of *C.maculatus* is only meant to decrease its population during the harsh environmental conditions of high temperature and low relative humidity. This is so because the alternative phenotype does not survive for long period or reverses back to continue further development like the flight form of this species in other regions. As mentioned above it is the sudden and discrete change in temperature and relative humidity which causes the noted plasticity in the present strain. Constant conditions of temperature and relative humidity, irrespective of low or high values of these factors, do not lead to the production of sterile phenotype. According to Nijhout and Wheeler [45] environments that change rapidly or that are coarse grained in some other way favour the evolution of discrete alternative phenotypes unlike the adaptive reaction norms produced by gradually changing environment.

It is interesting to note that two congeneric species of *C.maculatus* namely, *C.analis* Fab. And *C. chinensis* Linnaeus are also storage type species in India but neither of them shows a polyphenism behaviour similar to *C.maculatus*. On the other hand, both the species show seasonal changes in the coloration of pygidial setae. The three forms of *C.chinensis* have black, white or brown pygidium, all of which differ in fecundity during different seasons of the year. Likewise, *C.analis* appears in five forms again based on the colour of the setae on pygidium. These forms show very little difference in their fecundity. Obviously, the changes noticed in these two species during the course of the year only express reaction norm which does not result in polyphenism [23, 46].

Although Fujii [47, 48, 49] did not find any difference in the fecundity, fertility and longevity of four strains of *C.chinensis* procured from Japan and Iran. Applebaum *et al* [50] noted differences in the antennae and setal coverage on prothorax and pygidium in the Israeli and a Japanese strain of this species. As against all these observations on different strains of *C. chinensis,* Nakamura [51, 52] reported two forms in a Japanese strain, one of them more active and less fecund than the other. He also stated that larval density, temperature and parental status (age of parents) are the possible factors for the emergence of the active form. George and Verma [53] and Silim Nahdy *et al* [54] also noted the existence of two forms in this species. Whereas Tiwary and Verma [55] pointed out the role of juvenile hormone in melanin synthesis, Fairbairn [56] and, Dingle and Winchell [57] found the role of juvenile hormone titer in the relationship between the flight activity and reproductive diapause.

Another congeneric species among Indian *Callosobruchus, C. theobromae* (Linn.) attacking *Cjanus cajan* (Linn.) is the only field species which is monovoltine and shows prolonged over winter reproductive diapause. It surprisingly attacks the summer crop of the host sparing the other crop cultivated during winter months.

4. Conclusion

The noted novel phenotype in the Indian strain of *C. maculatus* is an example of extreme plasticity producing a form that is totally sterile, shows marked morphological alterations, is short – lived and irreversible. High levels of plasticity facilitate the induction of genetic change which tends to be irreversible. There is hardly any difference between an irreversible phenotype and genetic polymorphism although factors producing them are different, being environmental cue in the former and genetic change in the latter. There are reports suggesting genetic structure at the base of phenotypic changes rather than phenotypic plasticity [42, 43]. In fact phenotypic variants can be induced either by environmental or allelic variation [44]. The observed phenotypic change is therefore of no evolutionary significance. On the other hand, moderate amount of adaptive plasticity may result in evolutionary change through such processes as genetic canalization, genetic accommodation and genetic drift providing suitable ground for the operation of natural selection for ultimately generating a new species.

It is also likely that the unique phenotype under discussion is in fact a mutant which however cannot be established as it cannot be subjected to crossing trials due to its inability to reproduce. Mutants with twisted wings and damaged antennae and legs also appeared in our observations on irradiation of young developmental stages of *Callosobruchus maculatus* [40]. A black colored mutant which behaves as a single recessive trait in crossing trials has also been reported in *C. chinensis* [41].

Accordingly, the status of this disputed unusual phenotype remains unsolved and is open for further research.

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