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Ecotype selection of *Acanthaspis pedestris* Stål (Heteroptera:Reduviidae) for mass multiplication by UPGMA cluster analysis

P.J. Edward George, Jeya A. Celin , A. Antonysamy* and Dunston P. Ambrose#
Eritrea Institute of Technology, Mai Nefhi, Eritrea, North East Africa.
[*]Principal, St. Xavier's College, Kathmandu, Nepal.
[#]Entomology Research Unit, St. Xavier's College, Palayankottai – 627 002, India.
Email: pjegeorge@yahoo.co.in

Abstract: *Acanthaspis pedestris* Stål is an important reduviid predator found in the scrub jungles and agroecosystems of India predating on a number of insect pests and it is used as a bio-control agent. But its use is limited due to its scarcity at times when pests are often most abundant. The only possible way to overcome this difficulty is to mass culture this predator in the laboratory and supply them to the farmers. The practice of mass culturing leads to inbreeding which renders the species ultimately less effective against the pests. In order to overcome this shortfall, the morphological and biological characteristics of *A. pedestris* collected from seven ecotypes were subjected to UPGMA cluster analysis and the distantly related ecotypes were identified. By this method, it is possible to select and use two distantly related ecotypes for mating which can restore the genetic variability. Thus, the suggested approach makes the biocontrol agent effective even under mass culturing.
Keywords: *A. pedestris*, ecotype selection, mass multiplication, UPGMA cluster analysis, pest management, biocontrol.

Introduction

Prolonged inbreeding of any species under definite condition renders the species ultimately less genetic vigour which sometimes referred as genetic erosion. To decrease the loss of genetic variability due to inbreeding in mass rearing programmes of reduviids, selective breeding of distantly related ecotypes is suggested. The morphological and biological parameters of the reduviid *Acanthaspis pedestris* Stål collected from seven isolated habitats were analyzed by Unweighted Pair Group Method cluster Analysis (Kaufman & Rousseeue, 1990) to identify the distantly related ecotypes. The selective breeding of such distantly related ecotypes thus identified is recommended to reduce the loss of genetic variability in mass rearing programmes.

Though, the role of predators in biological control was realized by the Indian entomologists more than 200 years ago, the impact of predaceous arthropods in agricultural crops is receiving more attention in recent years. The importance of the predators in the suppression of insect pests is coming into closer focus, based on modern ecological investigations and experiments (Whitcomb, 1987). Several authors reported the suitability of reduviid predators as biological control agents (Ables, 1978; Schaefer & Ahmed, 1987; Ambrose, 2002).

Acanthaspis pedestris Stal is a reduviid predator widely distributed in the tropical rainforests, scrub jungles and semiarid zones of peninsular India. It is reported as a predator on many insect pests. However, the use of this predator as a biological control agent has been limited due to its scarcity in periods when pests are often most abundant. The only possible way to overcome this difficulty is the mass culture of this predator in the laboratory. Mass culturing predatory Heteroptera for augmentative release was attempted for species such as *Podisus bioculatus* (F.), *P. maculiventris* (Say) (Hemiptera: Pentatomidae), *Pristhesancus plagipennis* Walker, *Rhynocoris kumarii* Ambrose and Livingstone (Hemiptera: Reduviidae) (De Clereq & Degheele, 1999; Hough-Goldstein & Whalen, 1993; Cloutier & Baudin, 1995; Grundy *et al*., 2000; Claver & Ambrose, 2001a,b). However, the mass culture of these predators poses numerous challenges such as the cost of maintaining large insect – prey cultures (Cohen, 1985; Grundy *et al*., 2000), the risk of prey shortages due to facility mismanagement (De Clereq & Degheele), cannibalism (Grundy *et al.,* 2000; Waage *et al*., 1985; George, 2000; George & Ambrose, 2000), reduction in fecundity, longevity and prey searching ability due to inbreeding (Ridgeway *et al*., 1970; Jones *et al.,* 1978) and diseases (Helms & Raun, 1971). A combination of these difficulties is responsible for the limited number of successful mass rearing programmes for natural enemies.

In mass rearing even though there are many challenges, the selective breeding resulted in the improvement of different

biological characteristics and the maintenance of genetic variability (AtmaRam, 1971; Mackauer, 1976). Hence, an attempt was made to minimize the loss of genetic variability due to inbreeding to a considerable level. Analysis of distribution of assassin bugs suggests that individuals of many species exhibit biological dissimilarity even though they are found in close habitats both geographically and ecologically. For instance, *A. pedestris* collected from seven different ecological and geographical habitats exhibited dissimilarity in size and biological parameters and yet members of one habitat could have successfully bred with members of another habitat (Ambrose, 1980; Ambrose & Livingstone, 1988). The seven morphological and twenty five biological characteristics documented for geographically isolated ecotypes of *A. pedestris* were analyzed by Unweighted Pair Group Method (UPGMA) cluster analysis. Such an analysis enables us to identify the distantly related ecotypes. The selective breeding between the distantly related ecotypes at specific intervals during mass rearing can incorporate diverse biological characteristics thereby it can overcome the shortcomings in inbreeding.

Materials and methods

The 7 ecotypes of *A. pedestris* and the morphological and biological parameters considered for such distinction are provided here (Table 1 & 2).

Table 1. The ecotypes of *A. pedestris*

S.No:	*Place*	*Ecology*
1	Azhagarmalai	tropical rainforest
2	Chandrapuram	semiarid zone
3	Malumichampatti	semiarid zone
4	Maruthamalai	scrub jungle
5	Mathukarai	scrub jungle
6	Mettupalayam	tropical rain forest
7	Sathiyar	semiarid zone

The values were then subjected to heirarchial clustering by Unweighted Pair Group Method (UPGMA) cluster analysis (Kaufman & Rousseeue, 1990). The Euclidean distances between each pair of insects was first calculated by the formula

$$d(i,j) = \sqrt{(xi_1 - xj_1)^2 + \text{----------} (xi_p - xj_p)^2}$$

Where xi_1 and xj_1 are the first characteristics of first and second locality and xi_p and xj_p are the last characteristics of the first and second locality (if i = 1, j = 2) where d (i,i) = 0. The Euclidean distances calculated between each pair of objects were represented as a dissimilarity matrix. The smallest value in this matrix was compared with the respected row and column and considered as the first cluster.

Table 2. The morphological and biological characteristics chosen to compare the ecotypes of *A. pedestris*

Parameters	*Characters*
Morphological	1. Head length
	2. Aantennal length
	3. Rostral length
	4. Prothoracic length
	5. Foretibial length
	6. Tibial pad length
	7. Abdomen length
Biological	1. Incubation period (days)
	2. Stadial period (days)
	3. Nnymphal mortality (%)
	4. Adult male longevity (days)
	5. Adult female longevity (days)
	6. Sex ratio
	7. Age at which first batch of eggs laid (days)
	8. Index of oviposition
	9. Number of eggs laid
	10. Number of nymphs hatched
	11. Hatching percentage
	12. Frequency of 0% hatching
	13. Frequency of 100% hatching
	14. Spermatophore capsule length (mm)
	15. Spermatophore capsule width (mm)
	16. Spermatophore width (mm)
	17. Egg length (mm)
	18. Egg width (mm)
	19. Opercular length (mm)
	20. Opercular width (mm)
	21. Weight of I instar nymph (mg)
	22. Weight of II instar nymph (mg)
	23. Weight of III instar nymph (mg)
	24. Weight of IV instar nymph (mg)
	25. Weight of V instar nymph (mg)

Subsequent clusters were then analyzed by UPGMA.

Results and discussion

The Azhagarmalai, a scrub jungle at low altitude transforming into a tropical rain

forest at higher altitude in Madurai district is a offshoot of Eastern Ghat range of South India. Except Azhagarmalai (Azhagar Hills) and Sathiar (both in Madurai district) all the other five ecosystems are very closer to the Western Ghat ranges of South India (Coimbatore district). The Chandrapuram is a dry semiarid zone southeast of Coimbatore bordering agroecosystems and far away from the tropical rainforests. The Malumichampatti, a semiarid zone of Coimbatore district also borders agroecosystems. The climatic conditions of Malumichampatti is almost similar to that of Chandrapuram. The Maruthamalai scrub jungle is one of the eastwardly directed offshoot of Western Ghats in Coimbatore district. The Mathukarai scrub jungle lies adjacent to the Walayar tropical rainforests of Kerala, south west of Coimbatore. The Mettupalayam scrub jungle in Coimbatore district is closer to the tropical rainforests of Western Ghats. The Sathiyar semiarid zone in Madurai district is closer to scrub jungles and agroecosystems and it lies adjacent to Eastern Ghat ranges.

Analysis of distribution of *A. pedestris* in these seven habitats clearly suggests that individuals of many ecotypes exhibit biological dissimilarity while inhabiting geographically closer and ecologically similar habitats. However, members of different ecotypes were found readily breeding when they were brought together. These convincing observations may have far reaching significance in the interpretation of species concept.

The life stages of *A. pedestris* collected from Maruthamalai scrub jungle was found very closely related to those collected from Mathukarai scrub jungle in having close agreement with most of the biological characters analysed (Fig.1). This affinity may be correlated to the existing similar ecological characteristics of these two scrub jungle ecosystems of Western Ghat range. Moreover, the prey faunal richness and its diversification are moderate in these two ecosystems. Similarly *A. pedestris* from Azhagarmalai tropical rainforests exhibited close affinity to *A. pedestris* of Mettupalayam. Though Azhagarmalai, a offshoot of Eastern Ghats, comprised of scrub jungles at low altitudes and tropical rainforests at higher altitude, the microhabitats of *A. pedestris* is predominantly scrub jungle. These microhabitats almost closely resemble those of Mettupalayam scrub jungle. Interestingly, the higher altitudes of Mettupalayam, ie., the Nilgiri Hills is a typical tropical rainforest and devoid of *A. pedestris*. Both these ecosystems harbour comparatively a scarce and less diversified prey fauna. Interestingly the three semiarid zone ecosystems viz., the Chandrapuram, Malumichampatti and Sathiyar exhibited affinity. All these three ecosystems are closer to human habitations and agroecosystems rather than to scrub jungles or tropical rainforests. The prey fauna and their diversity are comparatively richer due to the presence of host plants (agricultural crops) and all these three ecosystems exhibit similar ecological conditions including the red soil.

The observations suggest that the biological and ecological interactions operate in a special manner on this reduviid leading to ecotypical specialization without adversely affecting the genome that might cause reproductive isolation (Ambrose, 1980; Ambrose & Livingstone, 1987;1988). This type of ecotype specialization has been considered by Mayr (1963; 1969) as ecological races or habitat race, otherwise referred to as ecotype. According to Mayr there is no evidence to distinguish an ecological race because every race is simultaneously a geographical race and an ecological race. The studies on the ecotypic variations of *A. pedestris* of peninsular India unequivocally corroborate the view held by Mayr (1963).

The results observed for *A. pedestris* clearly indicate that in mass rearing programmes, one can reduce or prevent the loss of genetic variability due to inbreeding in one hand and increase genetic variability through out breeding on the other hand by incorporating distantly related ecotypes of a species.

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Fig. 1. Dendrogram showing the ecotypic diversity of *A. pedestris* from seven habitats of Tamil Nadu, India.