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Bruchid fitness on multigenerational rearing *vis-a-vis* their environment

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Abstract
Multigenerational rearing study is a useful approach in entomology, where insect biological responses are discrete and developmental rates vary from one generation to another depending on the prevailing environmental conditions and rearing host. Fitness components of a South Indian bruchid (*Callosobruchus maculatus* Fabricius, Thanjavur population) were studied for continuous twenty-five generations on susceptible *Vigna radiata*. Inherent reproductive potential of the female bruchids varied from 47.67 to 69.67 eggs during 2nd and 20th generations when the average max. temperatures recorded 28.59ºC and 37.33ºC respectively. Adult females exhibited shorter lifespan (11 and 12 days) compared to males (12 and 16 days) as observed from adult mortality (released for oviposition) and offspring longevity (after emergence) studies. Sex ratio was female biased (average 22.59 ♀s over 21.21 ♂s) in all the subsequent generations. Adult emergence pattern from infested seeds showed peak emergence (8.60 adults) on second day of its initiation per generation studied and declined consecutively (7.76, 6.19, 5.19 adults and so on). Bruchid fitness was strongly influenced by prevailing max. and min. temperatures ($r=0.515^{**}$, $r=0.434^{*}$ for fecundity and $r=-0.613^{**}$, $r=0.745^{**}$ for development) and relative humidity ($r=-0419^{*}$ and $r=0.423^{*}$). This study will assist in population ecology study and enable construction of several life tables to prepare a predictive model, which can be tested against natural population fluctuations under storage conditions in order to check infestation from reaching total seed damage.

Keywords: Fitness, generations, biological response, bruchid, environment

Introduction

Stored insect pests are considered one of the most important biotic constraints causing huge losses in grains (30-40%) especially during storage conditions[1]. Postharvest grain losses caused by storage insects are estimated from up to 9% in developed countries to 20% or more in developing countries [2] and have probably been associated with human stores of grain legumes for several thousand years [3]. In addition to damage inflicted (quantitative), these pests also cause qualitative losses such as contamination of food products through the presence of live insects, their products such as chemical excretions or silk, exuviea and body fragments. Among these storage pests, bruchids (*Callosobruchus maculatus* Fabricius) are the most destructive ones mainly in the tropics and sub-tropics due to the prevailing environmental conditions. It infests seeds of wild and cultivated legumes, especially *Vigna* spp [4]. At the time of harvest, bruchids are carried to storage warehouses or god owns, where they multiply and develop inside the seeds at a faster rate (within a month) and cause total damage of the seed lot at optimum environmental conditions [3].

During the past decades, agricultural uses for insects and their rearing have expanded dramatically in the development and support of new pest management technologies[5]. However, development of an effective pest management module is based on a thorough understanding of the biology and ecology of the pest. It also requires such population and fitness studies like developmental rates, fecundity, per cent survivability and survivorship along with their temperature and humidity regime during storage period [6]. Egg-laying behaviour and development of bruchids are influenced by environmental factors, chemical and physical characteristics of host species [7]. Every major company or laboratory that produces chemical insecticides and universities or institutes offering entomological studies maintains a multispecies, multigenerational insect rearing facility for bioassay and other experiments. Multigenerational insect rearing plays a critical role in entomology, wherein its scientific importance can be enhanced and emphasized through studies on insect ecology, population dynamics, behaviour, systematic and biology, which forms the basis for any host plant resistance work [8].

Bruchids have been mass-cultured over many generations for different purposes *viz*. laboratory experiments for host plant resistance, and other advanced studies on genetics and their evolutionary traits [9]. Changes in insect life history traits due to mass culturing have been observed, which includes increased fecundity, early reproductive maturity, reduced longevity, increased mortality and reduced resistance to environmental stresses [10]. Traits that are closely related to insect fitness such as life-history, biology and survival are affected by environmental variations associated with their underlying metrical development [5]. Like other insects, bruchids tend to spend a fixed percentage of their total biology in each developmental stage; however temperature and humidity have been reported to alter their developmental time during larval or pre-adult stages [11]. With this background, the present study was undertaken with an objective to evaluate and compare any changes in *C. maculatus* fitness traits/parameters during mass-rearing on the same susceptible host for multiple generations and study their responses with respect to rearing environmental conditions.

Materials and Methods

Insect source

Bruchid species (*Callosobruchus maculatus*, South Indian Thanjavur populations confirmed morphologically[12]) employed in this experiment were initially obtained from an infested mung bean culture collected from Indian Institute of Crop Processing Technology (IICPT), Thanjavur, Tamil Nadu. Laboratory populations were established and maintained on susceptible mung bean seeds (*var*. Co8) inside an incubator (30 ºC and 70% RH with light dark cycle of L:D 13:11 hrs) during the entire study period following standard method [13].

Experimental set-up

Fitness of an organism is its ability to survive and reproduce in a particular environment [3]. Fitness parameters *viz*. fecundity, survival expressed through insect emergence, development expressed as mean developmental period, etc. of *C. maculatus* were studied on a highly susceptible mungbean variety, VRM (Gg) 1 [14]. The experiment was conducted for a period of two years (from October, 2014 to September, 2016) under laboratory conditions at an average temperature of 32 ± 5˚C and 75 - 95% RH (Table.7). One pair of newly emerged bruchids and the subsequent emerging progenies *viz*. F_2, F_3, F_4, F_5 till F_{25}, from each subsequent generation were released per fifty seeds, laid out in CRD with three replications.

Observations

Observations on the number of eggs laid (oviposition/fecundity), eggs hatched (successful larval entry indicated by the presence of white frass and a small dot when eggs are scrapped off), number of adults emerged or hollowed seeds (one adult per seed for South Indian strain) were made in order to calculate various life parameters like hatching per cent, seed damage per cent, mean developmental period (MDP in days), per cent survivality and index of suitability (Howe's growth index) [12, 14-16]. Number of adults emerged were recorded daily to determine the duration of development from egg to adult. Newly emerged adults were separated by gender [17] and sex ratio was determined by the formula [11] Sex ratio = number of females / (number of males + females). For every bruchid generation, adult mortality and offspring longevity tests were performed consecutively and separate data for both sexes were recorded daily. Emergence pattern of bruchid adults was recorded and worked out for all the generations studied.

Correlation study between C. *maculatus* fitness and weather parameters

Possible influence of weather parameters *viz*. maximum temperature, minimum temperature and relative humidity on some important fitness/biological parameters of Thanjavur *C. maculatus* was studied. Meteorological data on prevailing room temperature (^{0}C) and relative humidity (%) from two years (October, 2014 to September, 2016) were obtained from Department of Agro-Climatic Research Centre, TNAU, Coimbatore (Table. 7).

Statistical analysis

Observed and recorded data on different components were compiled and statistically analyzed independently using AGRES package. General analysis of variance (ANOVA) using completely randomized design model was performed for all measured and derived quantitative data and means were separated using least significant difference (LSD) and standard error (SE) at 5% level (p=0.05). Correlation (Pearson's Correlation) analysis between important bruchid biological responses and weather parameters were determined with SPSS software for Windows®.

Results

Fitness parameters of *C. maculatus* on VRM (Gg) 1 on multi-generational rearing

Rearing of bruchid beetles was continued till the completion of twenty-five generations conducted for fitness trait study during a period of two years. Seeds were examined after 24 hrs of oviposition and seeds with single egg alone were separated out and regularly monitored for their development. Eggs, which initially appeared translucent, turned white due to frass produced by first instar larva filling the egg shell. This white coloured egg was also an indication for the successful entry of bruchid larva inside seed cotyledon by penetration into seed coat [17]. Period up to this is considered as the egg (incubation) period. Results on different fitness parameters are as follows:

C. maculatus fecundity (oviposition) and hatching per cent

From multigenerational rearing (Table.1), total number of eggs laid (fecundity) per 50 seeds by a single *C. maculatus* female recorded 48.00 eggs on mungbean variety, VRM (Gg) 1 in first (F_1) generation. Among all the generations studied, significantly highest fecundity was recorded in F_{20} generation (67.67 eggs per 50 seeds) followed by F_{21} (68.0 eggs per 50 seeds), which was on par with F_{22} (66.67 eggs per 50 seeds); whereas lowest fecundity was observed in F_2 generation with 47.67 eggs per 50 seeds of VRM (Gg) 1. Analysis of the mean for all subsequent generations in this continuous experiment showed that fecundity of South Indian *C. maculatus* strain varied significantly during its generational development on VRM (Gg) 1 seeds with an average 56.27 eggs per 50 seeds and ranged from 47.67 to 69.67 eggs (Table.1). Fluctuations in egg hatching per cent was observed showing highest percentage during first two generations, F_1 (95.85%) and F_2 (97.16%), which was at par (Table.1). A decreasing trend was recorded from F_3 (85.60%) till F_7 (86.06%), which again increased from F_8 (97.29%) up to F_{13} (95.18%), which showed significant differences. From F_{14} generation onwards, average hatching per cent in the range 80.01% to 88.77% was noticed. However, lowest hatching per cent was recorded in last generation, F_{25} (75.33%), which differed significantly from other generations.

C. maculatus adult emergence and sex ratio

Daily observation of *C. maculatus* adult emergence including number of male and female bruchids was recorded separately till its ceasation. On an average, 40.33 to 47.33 adults emerged from 50 seeds evaluated irrespective of generations studied (Table. 2). Maximum and minimum adult emergence were noticed during twentieth (F_{20}) and third (F_3) generation, respectively. Bruchid developmental generations from sixteenth to twenty-first registered higher adult emergence (46.00, 44.33, 44.33, 45.67, 47.33, 45.00 adults) when compared to generations at the beginning from F_3, F_4, F_5, F_6 (40.33, 42.00, 40.67, 43.67 adults) and F_{12} to F_{15} (43.00, 42.67, 43.33, 43.67 adults, respectively). Overall mean number of female adults (22.59) slightly dominated over males (21.21) but the sex ratio remained almost as 1:1 but was more in favour of female than male.

Seed damage by *C. maculatus* and its adult survival

Seed damage registered significant differences between some developmental generations (Table.1). Highest damage was noticed in F_{20} generation (94.67%), which was at par with F_8 (93.33%) followed by F_7 (92.00%) and F_{16} (92.00%), respectively. On the other hand, lowest damage by *C. maculatus* was recorded in earlier generation, third (F_3) (80.67%) and last twenty-fifth (F_{25}) generation (80.67%). Comparatively lower seed damage was noticed during the middle of all generations studied, i.e. at eleventh to fifteenth generation (88.00%, 86.00%, 85.33%, 86.67% and 87.33%, respectively). This trend increased to 91.33%, 94.67%, and 90.00% at higher generations (F_{19}, F_{20}, and F_{21}). Data on *C. maculatus* survival (Table.1) revealed maximum adult survival at second generation (95.71%), which also recorded the highest egg hatching (97.16%) although oviposition (47.67 eggs per 50 seeds) was the least. This was followed by 94.60% survival at eighth generation, which was significantly at par with the highest value. Adult emergence (survival) was relatively higher during the first few generations (F_1=92.37%, F_2=95.71%, F_3=82.87%, F_4=80.28%) and near the middle from F_9 to F_{15} (81.44%, 93.29%, 83.05%, 84.37 5, 88.28%, 83.34% and 81.00%, respectively). However, a gradual decrease in adult survival rate was recorded from sixteenth (F_{16}) generation onwards and minimum of 65.01% was observed in F_{22} generation.

C. maculatus development (mean developmental period) and growth index (index of suitability)

Mean developmental period (MDP) of *C. maculatus* on VRM (Gg) 1 studied continuously for twenty-five generations revealed an average developmental period of 28.59 days and ranged from 23.87 days (min.) to 34.13 days (max.) recorded during F_{21} and F_2 generations, respectively (Table.1). MDP of 33.97 days and 33.61 days were noted in F_3 and F_{14} which were at par with the highest value. Significantly lower MDPs were recorded during 7th to 10th generations (26.16, 25.09, 24.84 and 26.87 days, respectively). Whereas higher developmental periods were observed in first few generations (F_1, F_2, F_3, F_4) and again during 12th to 17th generations (31.09, 31.63, 33.61, 32.64, 32.06 and 29.55 days, respectively), which differed significantly. The index of suitability or growth index for Thanjavur *C. maculatus* population ranged from 0.057 (F_3 gen) to 0.079 (F_8) (Table.1). Significantly higher growth indices were noticed from seventh up to tenth generation (0.071, 0.079, 0.077 and 0.073). Similarly, bruchid generations from F_{20} to F_{24} recorded significantly higher growth indices or index of suitability (0.073, 0.076, 0.076, 0.074 and 0.073, respectively). In contrast, minimum value was observed during second (0.057) and fourteenth (0.057) generations, which were at par. Further, relatively lower values of 0.058 and 0.059 index of suitability were evidenced during F_2, F_{15} and F_4, F_{16} generations, respectively.

C. maculatus adult mortality after release for oviposition

Mortality of *C. maculatus* adults recorded after release revealed differences between males and females at different days (Table.3). Mean male mortality started on 4th day (2 Males) of its release increasing up to a mean of 13 males on 5th day and reached a maximum mean mortality on 7th day with 23 males. However, mean mortality of females recorded earlier on 3rd day (6 females), which increased on 4th day with a mean of 10 females and highest female mean mortality was observed on day 5 (20 females) after its release followed by 19 female adults on day 6. Mean adult mortality was noticed till 11th day for females whereas the last mean mortality for males was recorded on day 12 (Fig.1).

C. maculatus offspring longevity (or mortality) after emergence

Longevity of freshly emerged *C. maculatus* adults was recorded with respect to each developmental generation. Longevity irrespective of male and female was highest (16 days) during first generation (November, 2014), while it was lowest (2 days) during fifth generation (March, 2015). Offspring longevity (mortality) for both males (Table.4) and females (Table.5) differed significantly in the successive bruchid generations. Highest male longevity was recorded during F_1 generation with mortality starting from 12th day and longevity extended up to 16 days after bruchid release, followed by longevity at second and third generations (up to 14th and 15th day, respectively) (Table.4). Male longevity was found to be minimum during F_{19} generation extending up to 9 days and mortality starting earlier from 4th day, which was also noticed in F_5 generation but longevity extended up to 11 days. Maximum mortality was observed on 7th day with a total of 42 male adults during the entire period of twenty-five generations studied followed by 41 males on day 6 after emergence (Fig.2). In contrast to male longevity, female longevity was comparatively lesser with maximum longevity extended up to 12 days, recorded during F_{15} generation, whereas shortest longevity of only 2 days was recorded in fifth (F_5) generation. In addition, different pattern was noticed in case of female mortality where maximum and minimum values were recorded on 5th day (43 females) and 2nd day (1 female), respectively during the study of subsequent generations (Fig.2). This was followed by mean mortality on 7th, 6th and 4th day, respectively with 36, 34 and 32 total number of females (Table.5).

C. maculatus adult emergence pattern

Studies on the developmental and emergence pattern of *C. maculatus* adults for twenty-five multi-generations revealed that maximum adults (8.60) emerged on second day (day 2) once the emergence started irrespective of its date of emergence (Fig.3). This was followed by emergence on the third (7.76 adults) and fourth (6.19 adults) day, respectively. Adult emergence on the first day (day 1) was moderate (5.35 adults). Gradual decrease in bruchid emergence was observed from day 5 (5.19 adults) onwards and extending up to day 17 (1.00 adult) in few generations (F_{14}, F_{15}, F_{16}) when the temperature regime was low compared to other generations (Table.7). Bruchid adult emergence was recorded on different days during the successive generations.

Correlation between weather and fitness parameters of *C. maculatus*

Weather parameters *viz*. max. Temperature, min. temperature and relative humidity (average data, Table. 7) were correlated in order to find out their significant influence (if any) on important bruchid fitness/biological parameters. From Table.6, it was evident that *C. maculatus* oviposition had significant and positive correlations with max. Temperature (r=0.515**) and min. temperature (r=0.434*) and negative correlation with relative humidity (r=-0.419*). Similarly, there was a significant and positive correlation between mean developmental period and relative humidity (r=0.423*) and a high but negative correlation with max. Temperature (r=-0.613**) and min. temperature (r=-0.745**). In terms of adult emergence, number of males emerged observed a positive and significant correlation with only one weather parameter, min. temperature (r=0.408*). No correlation was recorded among other bruchid fitness parameters assessed such as total number of adults emerged including number of females and seed damage per cent (Table.6).

Discussion

Fitness parameters of *C. maculatus* on VRM (Gg) 1 on multigenerational rearing

Studies on fecundity, survival and development (fitness) of Thanjavur *Callosobruchus maculatus* population through twenty-five generations on susceptible VRM (Gg) 1 seeds indicated that bruchids completed their immature and pre-adult stages in 23.87 days (21st generation) during June, 2016 within the thermal ranges of 24.29 ^{0} C (mean minimum) to 33.58 ^{0} C (mean maximum) and 84.48% RH. The shortest developmental time (23.87, 24.03 and 24.67 days) was noticed in 21st, 22nd and 23rd generations during the months of June-July and the longest developmental periods (34.13 and 33.97 days) were recorded in 2nd and 3rd generations during Dec.-Jan. (Table.1, Table. 7). Low temperature in December and January has been reported to reduce the development of immature stages of bruchid in mungbean seeds, while the most suitable months for the development of pre-adult stages is suggested to be between July to October [18], which supported the present findings. Larval competition at initial stages also tends to increase the developmental period [19]. The first reasonably comprehensive account of the biology and pest status of *C. maculatus* was reported and summarized information is available to this date [20]. Since then, various reports were available on how environmental factors affected development, reproduction and ultimately the growth of this insect populations [21]. Fecundity of this Thanjavur bruchid female was highest (69.67 and 68.00 eggs per 50 seeds) in 20th and 21st generations when temperature regime recorded around 33 ºC and 24 ºC. Earlier, it was reported that *C. maculatus* females produced an average of 25-61 eggs throughout their lifespan, and the highest number of eggs/day/female was achieved at 30 ºC [22]. However, *C. maculatus* can lay up to 115 eggs/female in its life span compared to 65 eggs by *C. chinensis* (L.) female [23, 24]. This difference in the number of eggs laid may be attributed to the variations in bruchid strain and host seeds.

An interesting example of environmental effects on fecundity was provided that showed significant variation in female egg production with that of mating behaviour among *C. maculatus* males [25]. Similarly, several environmental variables may affect the phenotypic value for a behavioural trait such as the pattern of egg-laying among seeds. Population count (adult emergence) and percentage seed infestation was directly proportional to the level of *C. maculatus* infestation in the present study (Table.1). Oviposition potential of *C. maculatus* females reached a peak within 2 days after its commencement and then declined with time. The short period for egg laying potential in this bruchid species may be an inherent survival mechanism for perpetuation of generations given the correspondingly short period of adult longevity [21, 26, 27]. This could also ensure rapid multiple re-infestations of pulse seeds in storage with consequent rapid population buildup since they oviposit continuously without feeding after their emergence from the infested seeds, which was noticed during this investigation for twenty-five generations and during sub-culturing process. Moreover, this behaviour is particularly important because adult bruchids do not feed and have to depend on the energy and other essential nutrients reserve within their body while developing inside the host seed, which may also explain the decline in oviposition with time in the present study.

With regard to longevity of adult females, low mortality rate was noticed from the first to the third day of commencement of oviposition (Fig.2). This was followed by drastic increase in their mortality rates particularly between the third and fourth day of oviposition. It was also observed that all the female bruchids died before reaching 11th day after the commencement of oviposition. This clearly indicated the shorter lifespan of *C. maculatus* females than males [27]. On the contrary, adult longevity of female was more than male and the sex ratio was more in favour of male for *C. chinensis* biology study for eight generations [4]. This may be due to difference in bruchid species used for the study (*C. maculatus* in the present case). Bruchid adults were segregated into males and females after their emergence to work out the sex ratio by keeping the constant value of male as one. Data on number of emerged males and females and sex ratio of *C. maculatus* from susceptible mungbean variety [VRM (Gg) 1] showed that sex ratio was in favour of females in all the subsequent generations studied except few developmental generations, *viz*. F_7, F_8, F_{11}, F_{20} and F_{22} (Table.2), where it was equivalent to that of males. Similar trend was reported that stated significantly female biased sex ratio[28]. The genetics of sex determination is unknown and have not been studied in bruchids. However, this biased emergence trend may be explained with the assumption that female sex determination might have taken place during embryonic development since females play a more important role (egg laying/offspring production) for their continuous perpetuation in host seeds [15]. Correlation studies between bruchid fitness and weather parameters showed that some of the fitness traits were strongly influenced by prevailing max. and min. temperatures and relative humidity (Table. 6). *C. maculatus* can be easily raised in laboratories and has been used as a model organism in a number of ecological studies. Nevertheless, its development is strongly influenced by temperature and humidity[29], which was evident from the results of correlation studies. In this study, max. Temperatures above 30 ^{0} C (during 7^{th}, 8^{th}, 19^{th}, 20^{th}, 21^{st} generations, Table. 1, Table. 7) recorded to be highly suitable for bruchid development considering their reproductive potential (fecundity), capacity to damage seeds (damage percent) and mean developmental periods. However, it has been reported that extremely high temperature of about 35 ^{0} C causes significant decrease in development rate of combined immature stages in bruchids[30], which slightly deviates from the present findings.

Table 1: Fitness traits of *Callosobruchus maculatus* (Thanjavur population) reared on susceptible *Vigna radiata* [*var*. VRM (Gg) 1] for 25 generations

Bruchid generations	No. of eggs laid	Hatched eggs	Hatching%	Exit holes*	% Seed damage	MDP (days)	% survival	log% survival	Index of Suitability
F1	48.00^{a}	46.00^{cde}	95.85^{ij}	44.33^{defg}	88.67^{defgh}	31.70^{hi}	92.37^{m}	1.97^{jk}	0.062^{e}
F2	47.67^{a}	46.33^{cdef}	97.16^{j}	45.67^{ghij}	91.33^{ghij}	34.13^{k}	95.71^{m}	1.98^{k}	0.058^{bc}
F3	48.67^{ab}	41.67^{a}	85.60^{e}	40.33^{a}	80.67^{a}	33.97^{k}	82.87^{ijk}	1.92^{gh}	0.057^{a}
F4	52.33^{de}	44.67^{bc}	85.36^{de}	42.00^{abc}	84.00^{abc}	31.98^{i}	80.28^{hi}	1.90^{fg}	0.059^{d}
F5	54.67^{ef}	45.67^{cd}	83.55^{de}	40.67^{ab}	81.33^{ab}	29.46^{g}	74.40^{fg}	1.87^{de}	0.064^{f}
F6	50.33^{abcd}	46.33^{cdef}	92.03^{g}	43.67^{cdef}	87.33^{cdefg}	28.09^{f}	86.76^{kl}	1.94^{i}	0.069^{hi}
F7	62.33^{ajk}	53.67^{gh}	86.07^{ef}	46.00^{ij}	92.00^{hij}	26.16^{d}	73.90^{efg}	1.87^{cde}	0.071^{jk}
F8	49.33^{abc}	48.00^{def}	97.29^{j}	46.67^{ij}	93.33^{ij}	25.09^{bc}	94.60^{m}	1.98^{k}	0.079^{o}
F9	52.00^{cde}	48.33^{ef}	93.02^{gi}	42.33^{abcd}	84.67^{abcd}	24.84^{bc}	81.44^{ij}	1.91^{g}	0.077^{n}
F10	48.67^{ab}	44.67^{bc}	91.77^{g}	45.33^{ghij}	90.67^{fghij}	26.87^{e}	93.29^{m}	1.97^{k}	0.073^{l}
F11	53.00^{de}	48.67^{f}	91.86^{g}	44.00^{cdefgh}	88.00^{cdefgh}	28.64^{f}	83.05^{ijk}	1.92^{gh}	0.067^{g}
F12	51.00^{bcd}	47.33^{def}	92.80^{gh}	43.00^{cde}	86.00^{cde}	31.09^{h}	84.37^{jk}	1.93^{ghi}	0.062^{e}
F13	48.33^{ab}	46.00^{cde}	95.18^{hij}	42.67^{bcd}	85.33^{bcd}	31.63^{hi}	88.28^{l}	1.95^{ij}	0.062^{e}
F14	52.00^{cde}	44.33^{bc}	85.27^{de}	43.33^{cdef}	86.67^{cdef}	33.61^{k}	83.34^{ijk}	1.92^{gh}	0.057^{ab}
F15	54.00^{ef}	45.67^{cd}	84.64^{de}	43.67^{cdefg}	87.33^{cdefg}	32.64^{j}	81.00^{hij}	1.91^{g}	0.058^{bcd}
F16	59.33^{gh}	52.67^{gh}	88.77^{f}	46.00^{hij}	92.00^{hij}	32.06^{ij}	77.53^{gh}	1.89^{ef}	0.059^{cd}
F17	61.33^{hij}	52.67^{gh}	85.89^{ef}	44.33^{defgh}	88.67^{defgh}	29.55^{g}	72.32^{def}	1.86^{cd}	0.063^{ef}
F18	63.00^{jk}	51.33^{g}	81.48^{bc}	44.33^{defgh}	88.67^{defgh}	26.94^{e}	70.37^{cde}	1.85^{bc}	0.069^{h}
F19	64.67^{kl}	54.00^{h}	83.52^{cde}	45.67^{ghij}	91.33^{ghij}	26.33^{de}	70.62^{cdef}	1.85^{bcd}	0.070^{i}
F20	**69.67^{n}**	61.67^{j}	88.51^{f}	47.33^{j}	94.67^{j}	25.13^{bc}	67.95^{abc}	1.83^{ab}	0.073^{l}
F21	68.00^{mn}	58.67^{i}	86.27^{ef}	45.00^{efghi}	90.00^{efghi}	23.87^{a}	66.17^{ab}	1.82^{a}	0.076^{mn}
F22	66.67^{lm}	55.00^{h}	82.51^{bcd}	43.33^{cdef}	86.67^{def}	24.03^{a}	65.01^{a}	1.81^{a}	0.076^{m}
F23	65.00^{kl}	52.67^{gh}	81.06^{bc}	43.00^{cde}	86.00^{cde}	24.67^{b}	66.23^{ab}	1.82^{a}	0.074^{l}
F24	60.00^{hi}	48.00^{def}	80.01^{b}	42.00^{abc}	84.00^{abc}	25.44^{c}	70.00^{bcd}	1.85^{bc}	0.073^{kl}
F25	56.67^{fg}	42.67^{ab}	75.33^{a}	40.33^{a}	80.67^{a}	26.89^{e}	71.23^{cdef}	1.85^{cd}	0.069^{hi}
Mean	56.27	49.07	87.63	43.80	87.60	28.59	78.92	1.89	0.0670
Max.	69.67	61.67	97.29	47.33	94.67	34.13	95.71	1.98	0.079
Min.	47.67	41.67	75.33	40.33	80.67	23.87	65.01	1.81	0.056
S.Ed.	1.37	1.28	1.45	1.07	2.13	0.32	1.94	0.01	0.0007
CD (0.05)	2.74	2.57	2.91	2.14	4.29	0.64	3.89	0.02	0.0015
CD(0.01)	3.66	3.43	3.88	2.86	5.72	0.85	5.19	0.03	0.002
CV%	2.97	3.19	2.02	2.98	2.98	1.36	3.01	0.67	1.36

All values are mean of three replications; ***Exit holes** = Number of adults emerged; one adult emergence per seed for South Indian bruchid (*C. maculatus*); MDP – Mean developmental period (in days); Index of suitability = Growth Index; % survival = % adult emergence
Different letters within a column indicate significant differences at 5 per cent level by LSD.

Table 2: Number of newly emerged males and females and their sex ratio

Bruchid Generations	Males (♂)	Females (♀)	Sex ratio (SR)	Adult* Emergence
F1	22.00^{efgh}	22.33^{abcd}	0.496^{ef}	44.33^{defg}
F2	22.00^{efgh}	23.67^{de}	0.482^{bcdef}	45.67^{ghij}
F3	18.67^{ab}	21.67^{ab}	0.463^{abc}	40.33^{a}
F4	19.33^{bc}	22.67^{abcd}	0.460^{abc}	42.00^{abc}
F5	19.00^{abc}	21.67^{ab}	0.467^{abcd}	40.67^{ab}
F6	21.33^{def}	22.33^{abcd}	0.489^{def}	43.67^{cdef}
F7	23.00^{ghi}	23.00^{bcde}	0.500^{f}	46.00^{ij}
F8	23.33^{hi}	23.33^{cde}	0.500^{f}	46.67^{ij}
F9	21.00^{de}	21.33^{a}	0.496^{ef}	42.33^{abcd}
F10	22.33^{efghi}	23.00^{bcde}	0.493^{ef}	45.33^{ghij}
F11	22.00^{efgh}	22.00^{abc}	0.500^{f}	44.00^{cdefgh}
F12	21.33^{def}	21.67^{ab}	0.496^{ef}	43.00^{cde}
F13	20.33^{cd}	22.33^{abcd}	0.477^{bcdef}	42.67^{bcd}
F14	21.00^{de}	22.33^{abcd}	0.484^{cdef}	43.33^{cdef}
F15	20.33^{cd}	23.33^{cde}	0.466^{bcd}	43.67^{cdefg}
F16	21.67^{defg}	24.33^{e}	0.471^{bcde}	46.00^{hij}
F17	21.67^{defg}	22.67^{abcd}	0.489^{def}	44.33^{defgh}
F18	21.67^{defg}	22.67^{abcd}	0.489^{def}	44.33^{defgh}
F19	22.67^{fghi}	23.00^{bcde}	0.496^{ef}	45.67^{ghij}
F20	23.67^{i}	23.67^{de}	0.500^{f}	47.33^{j}

F21	22.00^{efgh}	23.00^{bcde}	0.489^{def}	45.00^{efghi}
F22	21.67^{defg}	21.67^{ab}	0.500^{f}	43.33^{cdef}
F23	21.33^{def}	21.67^{ab}	0.496^{ef}	43.00^{cde}
F24	19.33^{bc}	22.67^{abcd}	0.460^{b}	42.00^{abc}
F25	17.67^{a}	22.67^{abcd}	0.438^{a}	40.33^{a}
Mean	21.21	22.59	0.484	43.80
Max.	23.67	24.33	0.500	47.33
Min.	17.67	21.33	0.438	40.33
S.Ed.	0.76	0.76	0.013	1.07
CD (0.05)	1.53	1.54	0.025	2.14
CD (0.01)	2.04	2.04	0.034	2.86
CV%	4.39	4.12	3.18	2.98

*****Number of adults emerged = exit holes (one adult emergence per seed for South Indian *C. maculatus*) (Mitchell, 1991)
Sex ratio (SR) = [No. of males / (No. of males + No of females)] (Soares *et al*., 2014)
Different letters within a column indicate significant differences at 5% level by LSD

Table 3: Mortality of *Callosobruchus maculatus* adults (one pair) after release for oviposition on different dates for subsequent 25 generations

Days*	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Total#
Males	0	0	0	2	13	8	23	14	7	2	2	4	75
Females	0	0	6	10	20	19	13	3	2	1	1	0	75
													150

Values include replicated values
*Days after *C. maculatus* release
#Total number of adults (males + females) used in the whole experiment; 3 pairs for 3 replications per generation

Table 4: Longevity of freshly emerged male adults (offspring) released on different dates for subsequent 25 generations

Generations	Released dates	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	Total adults
F1	13/11/2014												6	3			1	10
F2	20/12/2014												6	3	1			10
F3	21/01/2015											1	5	2	1	1		10
F4	21/02/2015							4		3	3							10
F5	22/03/2015				1		1	1		1	5	1						10
F6	19/04/2015					1		1	3		3	2						10
F7	15/05/2015							5		3	2							10
F8	09/06/2015					2	1	1	3	2	1							10
F9	04/07/2015					1	2	4	2	1								10
F10	31/07/2015					0	2	1	3	2	2							10
F11	29/08/2015						1		4	3	2							10
F12	29/09/2015							2	3	3	1	1						10
F13	29/10/2015								1	1	2	3	2	1				10
F14	30/11/2015									1	2	3	2	1	1			10
F15	31/12/2015								1	2	2	1	2	2				10
F16	26/01/2016								1	3	3	2	1					10
F17	26/02/2016							3	4	1	1	1						10
F18	22/03/2016						3	5	1	1								10
F19	16/04/2016				1	2		3	3	1								10
F20	11/05/2016					1	4	2	3									10
F21	05/06/2016					2	2	1	3	2								10
F22	30/06/2016					1	4	2	2		1							10
F23	24/07/2016					2	3	4	1									10
F24	19/08/2016						1	2	3	2	1	1						10
F25	16/09/2016							0	1	2	4	2	1					10
Total adults		0	0	0	2	12	24	41	42	34	35	18	25	12	3	1	1	250

Table 5: Longevity of freshly emerged female adults (offspring) released on different dates for subsequent 25 generations

Generations	Released dates	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	Total adults
F1	13/11/2014							1	2	4	2	1						10
F2	20/12/2014									1	4	5						10
F3	21/01/2015								4	4	2							10
F4	21/02/2015				2			3	1	4								10
F5	22/03/2015		1		1	6		1			1							10
F6	19/04/2015				5	4			1									10
F7	15/05/2015				5		4	1										10
F8	09/06/2015				3	6	1											10
F9	04/07/2015					6	4											10
F10	31/07/2015					2	6	2										10

F11	29/08/2015				1	2	4	3										10
F12	29/09/2015					1	2	4	3									10
F13	29/10/2015						1		2	3	4							10
F14	30/11/2015							1	2	2	3	2						10
F15	31/12/2015							2		2	2	2	2					10
F16	26/01/2016						1	2	2	1	2	2						10
F17	26/02/2016					2	1	2	2	1	2							10
F18	22/03/2016			1	1	2	2	2	1	1								10
F19	16/04/2016			2	3	2	1	1	1									10
F20	11/05/2016			1	1	1	2	1	3	1								10
F21	05/06/2016			1	2	5	1	1										10
F22	30/06/2016				5	1	2	2										10
F23	24/07/2016				3	1	1	2	2	1								10
F24	19/08/2016					2	1	3	2	1		1						10
F25	16/09/2016							2	3	3	1	1						10
Total adults		0	1	5	32	43	34	36	31	29	23	14	2	0	0	0	0	250

Table 6: Correlation between fitness traits of South Indian *Callosobruchus maculatus* (Thanjavur populations) and meteorological data (weather parameters) between October, 2014 to September, 2016

Variables	Max. temp.	Min. temp.	RH (%)	Ovip.	Adults emerged	Male	Female	Damage %	MDP (days)
Max. temp.	1.000	0.721**	-0.537*	0.515**	0.309	0.360	0.075	0.309	-0.613**
Min. temp.		1.000	-0.250	0.434*	0.626	0.408*	-0.142	0.262	-0.745**
RH (%)			1.000	-0.419*	0.206	0.135	0.253	0.206	0.423*
Ovip.				1.000	0.282	0.277	0.173	0.283	-0.632**
Adults					1.000	0.932**	0.696**	1.000**	-0.219
Male						1.000	0.387	0.932**	-0.329
Female							1.000	0.696**	0.092
Dam%								1.000	-0.220
MDP									1.000

**Correlation is significant at 0.01 level of significance
*Correlation is significant at 0.05 level of significant
Values without any asterisk are non-significant
Max. temp. – maximum temperature, min. tempt. – minimum temperature, RH – relative humidity;
Ovip. – number of eggs laid / 50 seeds; damage% – seed damage; MDP – mean developmental period (in days)
Significant correlations are typed in bold font

Table 7: Meteorological data (average) from October, 2014 to September, 2016 used in this study

Bruchid Generations	Duration	Max. Temp. (^{0} C)	Min. Temp. (^{0} C)	RH (%)
F1	17-10-2014 to 13-11-2014	29.30	21.27	95.48
F2	16-11-2014 to 15-12-2014	28.59	20.49	89.60
F3	19-12-2014 to 19-01-2015	29.26	19.71	86.97
F4	20-01-2015 to 19-02-2015	30.77	19.83	75.19
F5	21-02-2015 to 20-03-2015	33.55	21.94	79.96
F6	23-03-2015 to 18-04-2015	35.10	24.29	81.33
F7	20-04-2015 to 14-05-2015	33.38	23.60	88.24
F8	16-05-2015 to 08-06-2015	32.80	23.44	89.08
F9	10-06-2015 to 02-07-2015	31.90	23.67	81.09
F10	05-07-2015 to 29-07-2015	31.97	22.92	84.92
F11	01-08-2015 to 26-08-2015	32.19	23.37	86.50
F12	29-08-2015 to 25-09-2015	32.98	23.83	82.11
F13	28-09-2015 to 25-10-2015	31.73	23.12	87.25
F14	27-10-2015 to 26-11-2015	28.97	22.39	92.58
F15	28-11-2015 to 27-12-2015	28.95	21.61	90.43
F16	30-12-2015 to 26-01-2016	29.94	19.36	87.25
F17	28-01-2016 to 23-02-2016	33.03	20.89	80.70
F18	25-02-2016 to 20-03-2016	34.97	23.69	83.16
F19	22-03-2016 to 15-04-2016	36.79	24.97	80.20
F20	17-04-2016 to 10-05-2016	37.33	25.46	80.88
F21	13-05-2016 to 04-06-2016	33.58	24.29	84.43
F22	06-06-2016 to 28-06-2016	31.87	22.96	80.74
F23	30-06-2016 to 23-07-2016	29.98	23.88	81.42
F24	25-07-2016 to 18-08-2016	32.18	23.80	81.00
F25	21-08-2016 to 15-09-2016	32.18	22.70	86.92
Average	-	32.13	22.70	84.70
Max.	-	37.33	25.46	95.48
Min.	-	28.59	19.36	75.19

Weather data Source: Department of Agro-Climatic Research Centre, TNAU, Coimbatore

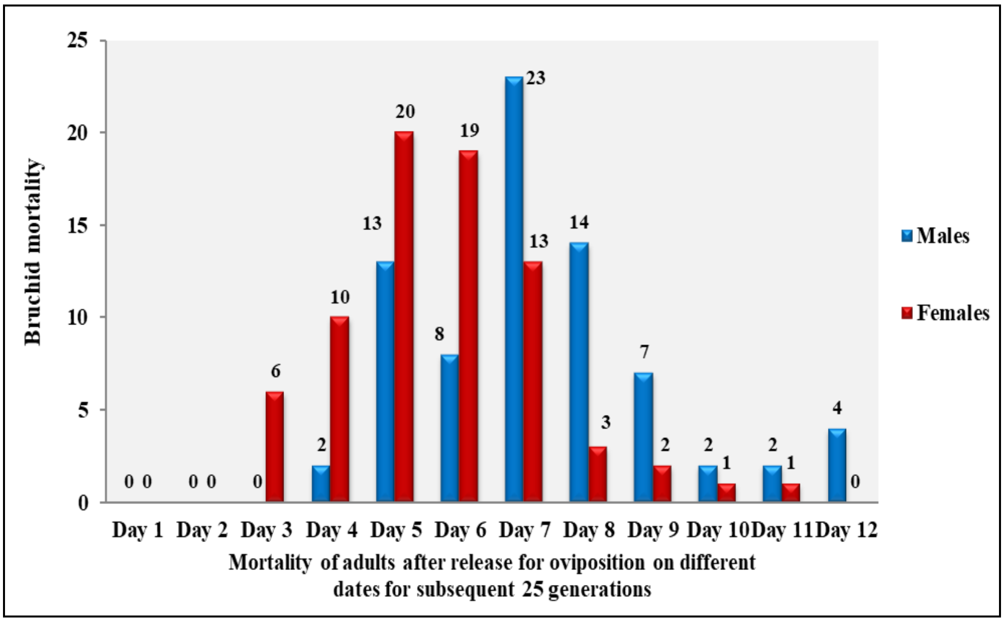

Fig 1: *Callosobruchus maculatus* Adults mortality

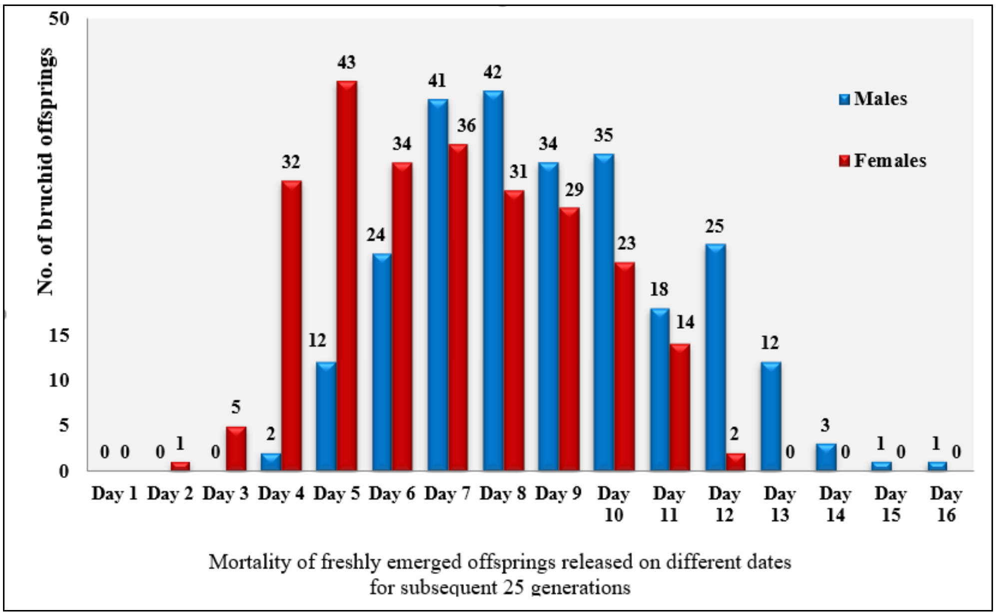

Fig 2: *Callosobruchus maculatus* offspring longevity

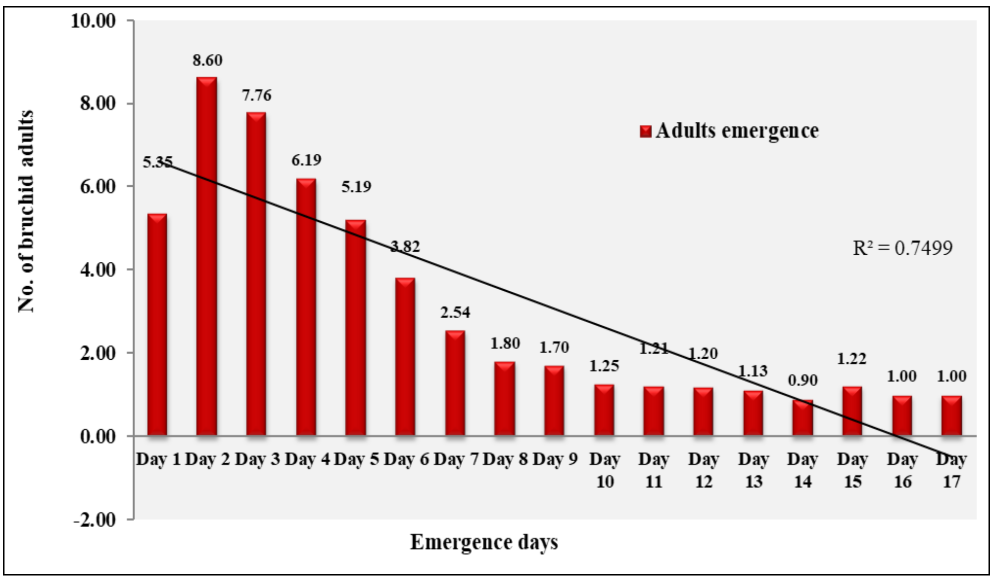

Fig 3: Emergence pattern of *C. maculatus* adults studied for subsequent 25 generations

Conclusion

Multigenerational rearing of *C. maculatus* for twenty-five generations showed that there were no major wide differences or changes on their fitness traits (parameters) and were at par throughout the subsequent generations except fecundity. The underlying rationale for this study was to ensure constant bruchid developmental traits over different time periods for its application in repeated resistance bioassays of host plant resistance studies during different seasons. In addition, such functional relationships between traits may be more accurately assessed in larger populations of *C. maculatus* that have been maintained in laboratory for many generations in varying locations and may provide insights in bruchid biotype development. This study will enable construction of life tables to prepare a predictive model, which can be tested against natural population fluctuations under storage conditions, in order to check insect infestation from reaching total seed damage. It also serves as an advantage in key aspects of bruchid biology to eliminate or manage their populations through manipulation of their physical and biological environments.

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