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Changes in host discrimination behaviour of *Callosobruchus maculatus*: shifting from habitual to ancestral hosts

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ABSTRACT

Background: *Callosobruchus maculatus* (the cowpea weevil) can cause considerable damage to important leguminous food crops. Its ancestral host is cowpea (*Vigna unguiculata*), but it can feed on other leguminous seeds as well. We reared a laboratory colony of *C. maculatus* on chickpea (*Cicer arietinum*) for 45 generations. The chickpea is termed the 'habitual' host of these weevils.

Questions: When given a choice, do *C. maculatus* from our laboratory colony prefer their ancestral host (cowpea), their habitual host (chickpea), or a third leguminous host (lentil, *Lens culinaris*) with which they are unfamiliar? If we observe a shift from chickpea back to cowpea, does it happen as soon as chickpea becomes available to the population, or does it occur gradually, generation after generation? Does it depend on the number of host choice tests weevils are confronted with? Is there a relationship between the adaptive evolution of female oviposition behaviour and larval feeding biology under free- and no-host-choice tests.

Methods: Experiment I: Allow weevils to choose freely between chickpea, cowpea, and lentils. Experiment II: Force females to use one and only one of the three weevil resources. Measure their reproductive success on each. Carry out each experiment for six generations.

Results: In the first generation, in both free- and no-host-choice tests, *C. maculatus* favoured the habitual host, chickpea. But, beginning with the third generation, weevils expanded what they used to include the ancestral host as well. Thus, *C. maculatus* can swiftly recognize and adapt to host opportunities, rapidly diversifying its host range and thereby increasing the damage it can inflict. Furthermore, results revealed that *C. maculatus* behaviours were greatly affected by the host-choice tests, by hosts and by generation number. Demographic traits

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adapted to the novel host evolved in concert with the elevation of population fitness on cowpea and lentil over the course of only three to four generations in both free- and no-host-choice tests.

Keywords: behaviour, *Callosobruchus maculatus*, host discrimination, host shift.

INTRODUCTION

The colonization of new habitats by organisms of short generation time offers one of the best contexts for studying natural selection, host discrimination behaviour and, potentially, adaptive evolution. In general, populations of herbivorous insects appear to maintain sufficient genetic variation to allow rapid adaptation to novel host plants (Carroll *et al.*, 1998). In addition, rapid local adaptation may in turn help to explain the high degree of ecological specialization in this group of organisms (Feder *et al.*, 1995; Messina and Karren, 2003). Furthermore, the incorporation of a new host in the diet is thought to necessitate changes in female oviposition behaviour as well as physiological changes in larval performance and the ability to use the novel food source (Jaenike, 1990). Fricke and Arnqvist (2007) believed that theory predicts females should preferentially oviposit on hosts that improve larval performance, and thus that the two traits should become genetically correlated.

Among herbivorous insects, the cowpea weevil *Callosobruchus maculatus* is characterized by the exploitation of novel food sources due to its rapid host diversification and its capacity for frequent host shifts. This capacity is associated with the adaptive evolution of female oviposition behaviour and larval feeding biology (Fricke and Arnqvist, 2007). Morse and Farrell (2005) examined the associations between host plant and bean seed beetles (*Bruchus rufimanus*) and concluded that seed beetles often exhibit specializations and that the members of one beetle group predominantly use closely related plant taxa belonging to a single host tribe. The adaptation to one novel host may simultaneously affect an insect's performance on other hosts, including hosts that the population may never have encountered. All suitable hosts for *C. maculatus* belong to the subfamily Papilionoideae within the legume family Fabaceae. Within the Papilionoideae, most *C. maculatus* hosts belong to the tribe Phaseoleae, and the most badly infested crops belong to the genus *Vigna* (Tuda *et al.*, 2005). Insects have only rarely been reported to attack lentil (*Lens culinaris* Medikus), which belongs to the tribe Vicieae within the Papilionoideae, and is thus distantly related to the insect's typical hosts (Choi *et al.*, 2004). Research by Messina *et al.* (2009) showed that the egg-laying behaviour of *C. maculatus* is a critical determinant of host range. Females of *C. maculatus* generally accept lentil much less readily than their Phaseoleae hosts. In Tunisia, *C. maculatus* is among the major insect pests that attack stored chickpea resulting in considerable economic losses (Jerraya, 2003; Haouel-Hamdi *et al.*, 2015). Previously, we showed that individual and population demographic traits correlate with the chickpea only because its ancestral host, the cowpea, was not cultivated in the country (Haouel-Hamdi *et al.*, 2017a).

We reared a laboratory colony of *C. maculatus* on chickpea (*Cicer arietinum*) for 45 generations. We then exposed members of this colony to the presence of the ancestral host (cowpea), the novel host (lentil), or the habitual host (chickpea) in order to investigate host shifts and host discrimination behaviour of *C. maculatus* for six generations. During that time, we measured population dynamics, reproductive parameters, demographic traits, and fitness.

MATERIALS AND METHODS

Insect rearing and seed material

The strain of *C. maculatus* used for the experiments was isolated from infested seeds of chickpea and maintained for 45 generations. Ten mature couples of the flightless-form were transferred onto seeds of three hosts: chickpea (*Cicer arietinum*, Amdoun 1 variety), lentil (*Lens culinaris*, Ncir variety), and cowpea (*Vigna unguiculata*, black-eye variety) for six generations.

These stock cultures were maintained in one-litre glass bottles in a growth chamber at 30 ± 5°C, 65 ± 5% relative humidity, and a 12 hour light/12 hour dark photoperiod.

Free host-choice tests

The free host-choice test was performed according to the procedure described by Panzarino *et al.* (2012). A four-way device was used that consisted of the following PVC parts:

- A common release arena made out of a cylindrical box (11 cm diameter, 6 cm depth, capacity 0.7 L). Three circular holes (1 cm diameter), spaced equidistantly, were drilled into the sidewalls of the box, 1 cm from its base.
- Three oviposition arenas made out of smaller cylindrical boxes (5 cm diameter, 3 cm depth, capacity 50 mL) with intact lids. A circular hole (1 cm diameter) was drilled into the sidewall of each box, 3.5 cm from its base.
- Three tubes (6 cm long and 1 cm external diameter) connected the release arena with the oviposition arenas.

Each oviposition arena was filled with 30 seeds of only one of the three hosts. No seeds were placed in the release arena. Fifteen adult female and five adult male *C. maculatus* individuals (all having emerged in the 45-generation culture within the previous 24 hours) were released into the common arena, from where they could freely visit any of the three oviposit arenas. The device was kept in a growth chamber at 30 ± 5°C and 65 ± 5% relative humidity. We removed the adults two days later, before the first larval hatching. We counted the eggs laid on the surfaces of the seeds in each arena. This bioassay was replicated three times with each oviposition arena randomly assigned its host at the start of the tests.

No-host-choice tests

In the no-host-choice tests, the arenas were three one-litres glass bottles, each assigned one of the three hosts. Each bottle received 30 seeds of the host assigned to it. We then released three female and one male adult *C. maculatus* individuals (all having emerged in the 45-generation culture within the previous 24 hours) into each bottle in three replicates. The bottles were kept in a growth chamber at 30 ± 5°C and 65 ± 5% relative humidity; the adults were removed two days later. We then counted the eggs laid on the surfaces of the seeds in each bottle.

Reproductive parameters and demographic traits

We measured the reproductive parameters and demographic traits in both the free- and no-host-choice tests. We determined the longevity of adults (male and female), the total number of eggs laid, the fertility rate and the emergence rate using the methods of Haouel-Hamdi *et al.* (2017b).

Based on methods of Haouel-Hamdi *et al.* (2017a), we also calculated the population growth parameters – namely, mean growth rate, mortality rate of eggs, mortality rate of larvae, sex ratio, net reproductive rate, generation time, and the intrinsic rate of increase.

Statistical analysis

We performed statistical analyses with SPSS v.20.0 statistical software. All values given in this paper represent the mean of three replications and are expressed as the mean ± standard deviation ($\bar{x} \pm SD$). We used the Duncan test to determine significant differences between the mean values ($P \leq 0.05$). For each biological parameter, we subjected the data to two-way analysis of variance, with host-choice test, host, and generation number as the main fixed factors; their interaction terms were also analysed. We separated the means using the Least Significant Difference (LSD) test ($P < 0.05$). Where necessary, we transformed data to common logarithms, square roots, or square-roots (logarithm) to meet the assumptions of normality. Finally, we performed correlation analyses (Pearson's correlation coefficient) between biological parameters (reproductive and demographic traits) and host-choice test, host, and generation number.

RESULTS

Reproductive parameters

Kaplan-Meier survival curves

We used Kaplan-Meier survival curves to plot the survival data (Fig. 1), and used log-rank tests to compare the survival curves of the various parameters (host-choice test, host, and generation). The log-rank tests indicated that there were significant differences in survival of male adults with respect to the parameters 'generation' and 'host' (generation: $\chi^2 = 17.55$, $df = 2$, $P < 0.001$; host: $\chi^2 = 39.35$, $df = 3$, $P < 0.001$). However, the log-rank tests indicated that there were no significant differences in survival of male adults with respect to host-choice tests ($\chi^2 = 0.65$, $df = 1$, $P = 0.42$). Host and generation both had a significant effect on female adult survival (generation: $\chi^2 = 8.54$, $df = 2$, $P = 0.014$; host: $\chi^2 = 41.04$, $df = 3$, $P < 0.001$). Finally, the log-rank tests indicated that there were no significant differences in adult male survival among the host-choice tests ($\chi^2 = 0.97$, $df = 1$, $P = 0.32$).

The longest surviving adults (males and females) occurred in the sixth generation, while those surviving the shortest were seen in the first generation. Survival was intermediate in the third generation. The survivorship curves showed a tendency for higher survival in males reared on chickpea followed by those reared on cowpea, and finally those reared on lentil.

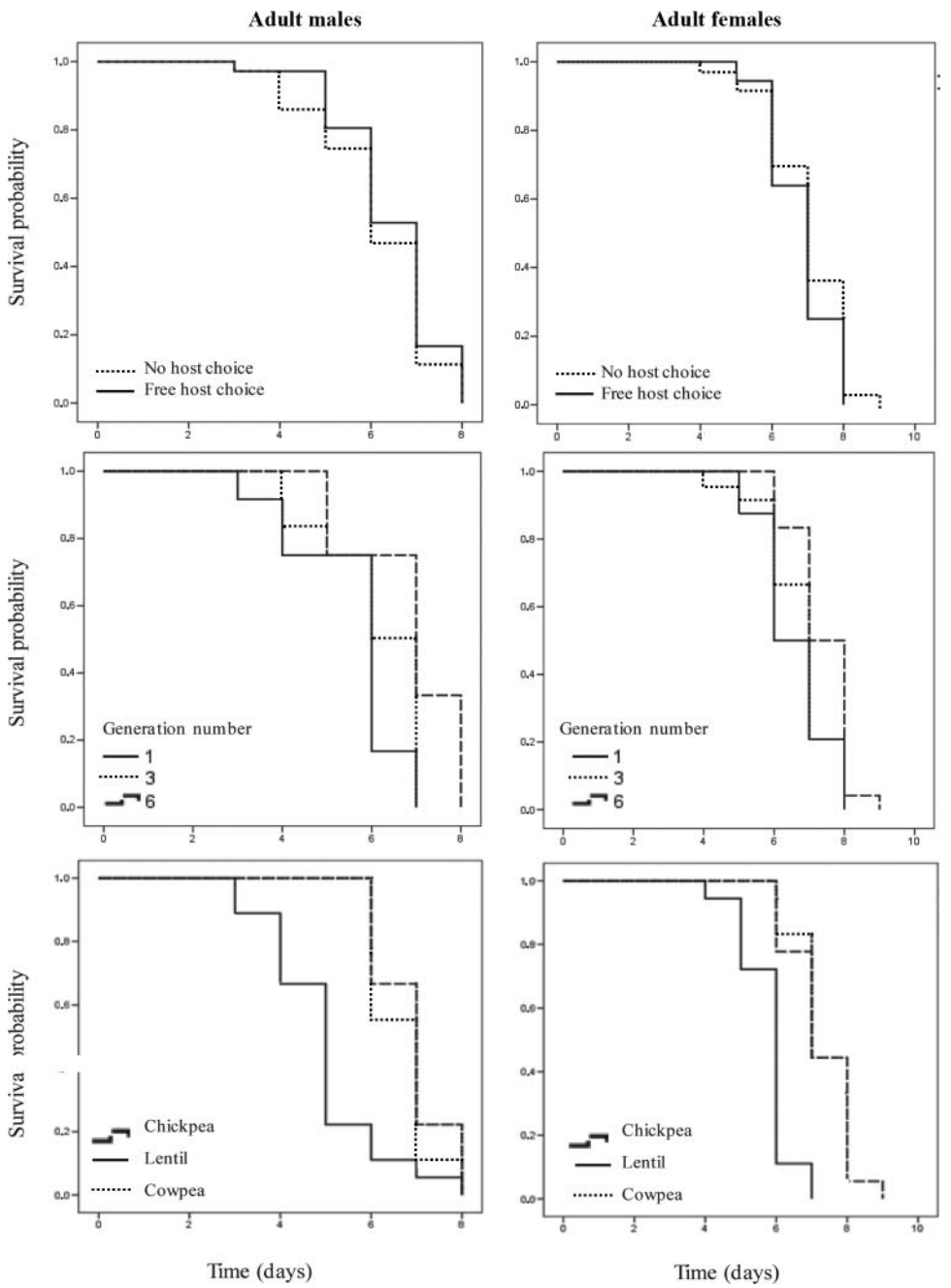

Fig. 1. Kaplan-Meier survivorship curves of adult male and female *C. maculatus* reared on three hosts for six generations under free- and no-host-choice tests.

Table 1. Correlation analyses between the fitness parameters of adult *C. maculatus* and host-choice test, host, and generation

		Host-choice test	Generation	Host	Sex	Weight	Body size
Weight	*r*	0.003	0.17**	−0.15*	−0.39**	1	0.86**
	P	0.97	0.01	0.02	<0.001		<0.001
Body size	*r*	−0.12	0.15*	−0.19**	−0.22**	0.86**	1
	P	0.07	0.02	0.003	<0.001	<0.001	

** $P < 0.01$, * $P < 0.05$.

Adult fitness

Correlation analyses between the fitness parameters of adult *C. maculatus* and host-choice test, host, and generation are illustrated in Table 1. Results show that the adults' fitness exhibited marked variations among generations and hosts (Table 2). A significant and negative correlation was observed between host and the body weight and size of the beetle (weight: $r = -0.15$, $P = 0.02$; size: $r = -0.19$, $P = 0.003$). On the other hand, results showed significant and positive correlations between adult fitness and generation (weight: $r = 0.17$, $P = 0.01$; size: $r = 0.15$, $P = 0.02$) (Table 2).

Total number of eggs laid

Figure 2 shows the total number of eggs laid on three hosts for six generations in the free- and no-host-choice tests. In the first generation, females in the no-host-choice tests laid the largest number of eggs on chickpea, and the smallest number on cowpea. However, in the third and sixth generations, females laid the largest number of eggs on cowpea, and the smallest number on lentil (Fig. 2). This result shows that females did not appear to

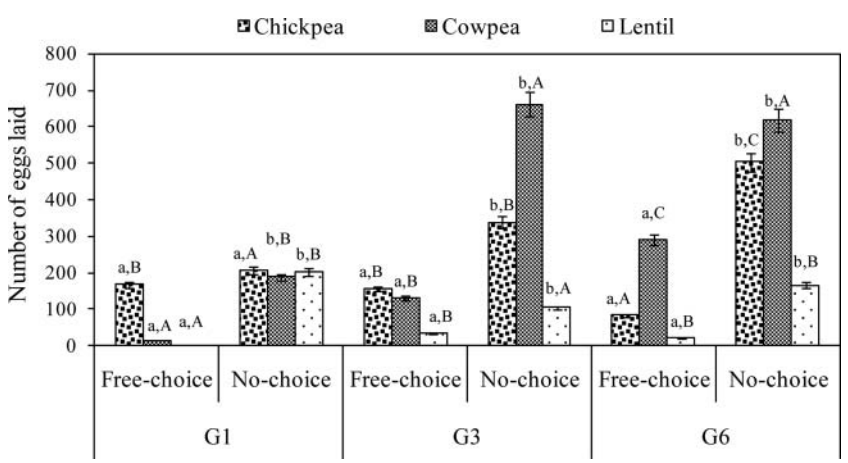

Fig. 2. The total number of eggs laid by a *C. maculatus* female reared on three hosts for six generations in free- or no-host-choice tests. Bars with different letters are significantly different at $P < 0.05$. For each generation and each host, we compared free- and no-host-choice tests (lower-case letters). And for each free- and no-host-choice test and each host, we compared generations (upper-case letters) (Duncan test).

Table 2. Effect of host-choice test, host, and generation on fitness of adult *C. maculatus*

Variables	*df*	*F*	*P*	η^2
Adult weight				
Host-choice test (HCT)	1	0.02	0.90	0.01
Host	2	405.64	**< 0.001**	0.86
Generation	5	33.45	**< 0.001**	0.26
Sex	1	326.59	**< 0.001**	0.63
HCT × Generation	5	4.51	**0.01**	0.05
HCT × Host	2	32.55	**< 0.001**	0.34
HCT × Sex	1	0.51	0.48	0.01
Host × Generation	10	11.66	**< 0.001**	0.27
Sex × Generation	5	1.61	0.20	0.02
Host × Sex	2	40.08	**< 0.001**	0.38
HCT × Generation × Host	10	4.59	**< 0.001**	0.13
HCT × Generation × Sex	5	1.59	0.21	0.02
HCT × Host × Sex	2	0.18	0.91	0.01
Generation × Host × Sex	10	0.76	0.59	0.02
Generation × Host × Sex × HCT	10	0.54	0.78	0.02
Body size				
Host-choice test (HCT)	1	60.39	**< 0.001**	0.24
Host	2	1020.25	**< 0.001**	0.94
Generation	5	55.18	**< 0.001**	0.37
Sex	1	203.29	**< 0.001**	0.51
HCT × Generation	5	25.58	**< 0.001**	0.21
HCT × Host	2	115.46	**< 0.001**	0.64
HCT × Sex	1	1.25	0.27	0.01
Host × Generation	10	18.81	**< 0.001**	0.37
Sex × Generation	5	2.48	0.09	0.03
Host × Sex	2	28.15	**< 0.001**	0.31
HCT × Generation × Host	10	19.37	**< 0.001**	0.38
HCT × Generation × Sex	5	3.19	**0.04**	0.03
HCT × Host × Sex	2	8.02	**< 0.001**	0.11
Generation × Host × Sex	10	0.95	0.46	0.03
Generation × Host × Sex × HCT	10	1.21	0.30	0.04

Note: *P*-values < 0.05 are highlighted in **bold** font.

recognize cowpea as a potential host in the first generation, although by the third generation they had rapidly adapted to the novel host.

Results revealed that in the free host-choice tests, the number of eggs laid by *C. maculatus* on the different hosts ranged from 0 to 166, from 32 to 154, and from 18 to 290 for the first, third, and sixth generation respectively. However, in the no-host-choice tests, the number of eggs laid by *C. maculatus* on the different hosts ranged from 187 to 226, from 102 to 660, and from 164 to 616 for the first, third, and sixth generation respectively.

In both the free- and no-host choice tests, the total number of eggs laid was significantly dependent upon host and generation number (host: $F = 29.84$, $P < 0.001$; generation: $F = 28.49$, $P < 0.001$). In the free-host-choice tests, first-generation females laid significantly

more eggs on chickpea (166 eggs) than on cowpea (12 eggs). By the sixth generation, the number of eggs laid shifted in favour of cowpea (290 eggs) and away from chickpea (81 eggs). Furthermore, the number of eggs laid on lentil (0, 32, and 18 in the first, third, and sixth generation respectively) was always significantly less than that on cowpea and chickpea (Fig. 2).

Fertility and adult emergence rate

Fertility and emergence rates exhibited marked variation in the host-choice tests (Table 3). We observed significant and negative correlation between host-choice test, fertility, and emergence rates (fertility rate: $r = -0.60$, $P < 0.001$; emergence rate: $r = -0.59$, $P < 0.001$). In addition, by the sixth generation, fertility and emergence rates were significantly higher in the free-host-choice tests than in the no-host-choice tests on different hosts (fertility rate: $F = 413.48$, $P < 0.001$; emergence rate: $F = 430.88$, $P < 0.001$). Note, however, that fertility and emergence rates themselves were significantly, positively correlated (Table 3).

Emergence rate

Figure 3 displays the emergence rate data of *C. maculatus* adults on three hosts after six generations. Adults in the free-host-choice tests emerged at a faster rate than those in the no-host-choice tests. The highest emergence rates were on cowpea (78.61% for the first generation, 89.91% for the third generation, and 88.45% for the sixth generation) in the free-host-choice tests. In contrast, in the no-host-choice tests, the highest emergence rates occurred on chickpea (37.66% for the first generation and 37.61% for the sixth generation). The emergence rate on lentil in the no-host-choice tests increased from 5.53% in the first generation to 20.22% in the sixth generation. Thus, the best host was cowpea followed by chickpea, and finally lentil in the free-choice tests. Yet nearly all larvae died before burrowing completely into a seed.

Demographic parameters

The demographic parameters of *C. maculatus* reared on three hosts for six generations in free- and no-host-choice tests are summarized in Table 4. Host-choice test, host, and generation all had a significant effect on mean growth rate, mortality rate of eggs, and generation time (Table 4). However, the intrinsic rate of increase, mortality rate of larvae, and net reproductive rate were not affected by host-choice test (intrinsic rate of increase: $F = 0.05$, $P = 0.83$; mortality rate of larvae: $F = 0.27$, $P = 0.61$; net reproductive rate:

Table 3. Correlation analyses between fertility and emergence rate of *C. maculatus* and host-choice test, host, and generation number

	Host-choice test	Generation	Host	Fertility rate	Emergence rate
Host-choice test	1	0.00	0.00	−0.60**	−0.59**
Generation	0.00	1	0.00	0.04	0.03
Host	0.00	0.00	1	−0.01	−0.04
Fertility rate	−0.60**	0.04	−0.01	1	0.98**
Emergence rate	−0.59**	0.03	−0.04	0.98**	1

** $P < 0.001$.

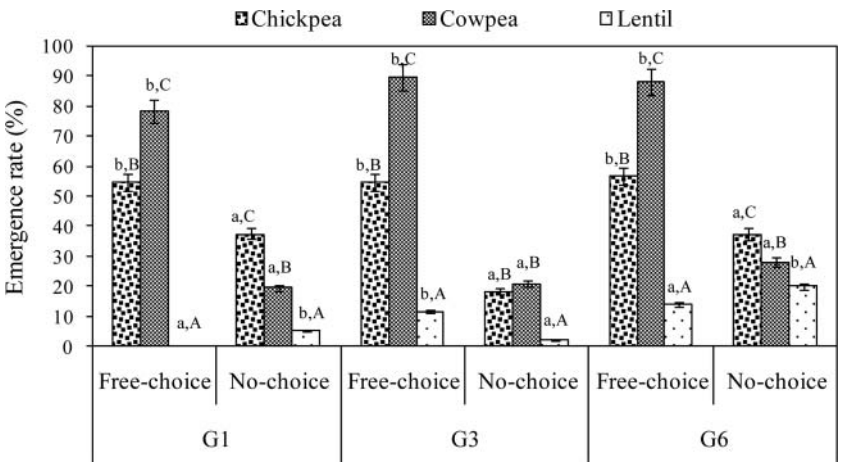

Fig. 3. Emergence rates of *C. maculatus* reared on three hosts for six generations in free- and no-host-choice tests. Bars with different letters are significantly different at $P < 0.05$. For each generation and each host, we compared free- and no-host-choice tests (lower-case letters). And for each free- and no-host-choice test and each host, we compared generations (upper-case letters) (Duncan test).

Table 4. Effect of host-choice test, host, and generation on demographic parameters of *C. maculatus*

Variables	*df*	Mean square	*F*	*P*	η^2
Mean growth rate					
Host-choice test (HCT)	1	3094.75	56.69	**< 0.001**	0.55
Host	2	30696.81	562.27	**< 0.001**	0.97
Generation	5	17461.53	319.84	**< 0.001**	0.93
HCT × Host	2	1625.23	29.77	**< 0.001**	0.66
HCT × Generation	5	515.71	9.45	**< 0.001**	0.29
Host × Generation	10	2392.16	43.82	**< 0.001**	0.85
HCT × Host × Generation	10	1179.43	21.60	**< 0.001**	0.70
Mortality rate of eggs					
Host-choice test (HCT)	1	35191.16	632.68	**< 0.001**	0.93
Host	2	1154.74	20.76	**< 0.001**	0.57
Generation	5	231.76	4.17	**0.02**	0.15
HCT × Host	2	729.76	13.12	**< 0.001**	0.46
HCT × Generation	5	784.73	14.11	**< 0.001**	0.38
Host × Generation	10	691.65	12.44	**< 0.001**	0.62
HCT × Host × Generation	10	681.79	12.26	**< 0.001**	0.57
Sex ratio					
Host-choice test (HCT)	1	188.13	45.95	**< 0.001**	0.50
Host	2	16.91	4.13	**0.01**	0.21
Generation	5	1.23	0.30	0.74	0.01
HCT × Host	2	16.09	3.93	**0.01**	0.20
HCT × Generation	5	1.69	0.41	0.66	0.02
Host × Generation	10	11.99	2.93	**0.02**	0.28
HCT × Host × Generation	10	14.72	3.59	**0.01**	0.28

Table 4. *Continued*

Variables	*df*	Mean square	*F*	*P*	η^2
Intrinsic rate of increase					
Host-choice test (HCT)	1	5.882×10^{-7}	0.05	0.83	0.001
Host	2	0.003	217.42	**<0.001**	0.93
Generation	5	9.882×10^{-5}	8.33	**0.001**	0.27
HCT × Host	2	2.530×10^{-5}	2.13	0.11	0.12
HCT × Generation	5	6.991×10^{-5}	5.89	**0.005**	0.20
Host × Generation	10	5.942×10^{-5}	5.01	**0.001**	0.39
HCT × Host × Generation	10	2.636×10^{-5}	2.22	0.07	0.19
Mortality rate of larvae					
Host-choice test (HCT)	1	0.05	0.27	0.61	0.01
Host	2	16.09	83.24	**<0.001**	0.84
Generation	5	11.39	58.96	**<0.001**	0.72
HCT × Host	2	0.04	0.19	0.89	0.02
HCT × Generation	5	4.79	24.79	**<0.001**	0.52
Host × Generation	10	1.78	9.19	**<0.001**	0.55
HCT × Host × Generation	10	2.55	13.17	**<0.001**	0.59
Generation time					
Host-choice test (HCT)	1	0.002	422.08	**<0.001**	0.90
Host	2	0.003	26.63	**<0.001**	0.64
Generation	5	0.003	28.65	**<0.001**	0.55
HCT × Host	2	0.024	377.67	**<0.001**	0.96
HCT × Generation	5	0.003	44.17	**<0.001**	0.66
Host × Generation	10	0.001	9.18	**<0.001**	0.55
HCT × Host × Generation	10	0.002	34.17	**<0.001**	0.79
Net reproductive rate					
Host-choice test (HCT)	1	0.001	0.02	0.88	<0.001
Host	2	3.01	104.54	**<0.001**	0.87
Generation	5	0.34	11.73	**<0.001**	0.34
HCT × Host	2	0.04	1.22	0.31	0.07
HCT × Generation	5	0.16	5.68	**0.006**	0.20
Host × Generation	10	0.08	2.68	**0.026**	0.26
HCT × Host × Generation	10	0.07	2.25	0.065	0.20

Note: Values $P < 0.05$ are highlighted in **bold** font.

$F = 0.02$, $P = 0.88$), or by the interaction between host-choice test and host (intrinsic rate of increase: $F = 2.13$, $P = 0.11$; mortality rate of larvae: $F = 0.19$, $P = 0.89$; net reproductive rate: $F = 11.73$, $P = 0.31$). Furthermore, the sex ratio varied markedly with host-choice test ($F = 45.95$, $P < 0.001$) and host ($F = 4.13$, $P = 0.01$); in contrast to all the above parameters, sex ratio was not affected by generation ($F = 0.74$, $P = 0.30$), or by the interaction between host-choice test and generation ($F = 0.41$, $P = 0.66$).

Table 4 shows that host-choice test accounted for 93%, 90%, and 55% of the variance in mortality rate of eggs, generation time, and mean growth rate respectively. Also, host

Table 5. Correlation analyses between demographic traits of *C. maculatus* and host-choice test, host, and generation

		MGR	MRE	MRL	SR	R_0	GT	r
Host-choice test	*r*	−0.14	−0.87**	−0.07	0.53**	0.08	0.46**	0.06
	P	0.25	<0.001	0.59	<0.001	0.50	<0.001	0.63
Host	*r*	−0.09	−0.06	0.18	0.15	−0.21	0.08	−0.31**
	P	0.47	0.61	0.14	0.21	0.08	0.51	0.008
Generation	*r*	0.41**	0.01	−0.36**	0.09	−0.10	−0.10	0.01
	P	<0.001	0.95	<0.001	0.45	0.41	0.41	0.91

Note: MGR = mean growth rate, MRE = mortality rate of eggs, MRL = mortality rate of larvae, SR = sex ratio, R_0 = net reproductive rate, GT = generation time, r = intrinsic rate of increase. $^{**}P < 0.01$.

accounted for 57%, 64%, and 97% of the variance. In addition, generation accounted for 15%, 55%, and 93% of the variance in mortality rate of eggs, generation time, and mean growth rate respectively.

Furthermore, the interaction of host-choice-test and host accounted for 66% of the variance in mean growth rate. Also, 85% of the variance in mean growth rate is accounted for by the host × generation interaction, only 29% by the host-choice test × generation interaction, and 70% by the host-choice-test × generation × host interaction.

Thus, some population demographic parameters – mean growth rate, mortality rate of larvae, intrinsic rate of increase, and net reproductive rate – were more dependent on host and generation than on host-choice-test. However, the mortality rate of eggs, sex ratio, and generation time were more reliant on host-choice test than on host and generation.

Correlations

The results of correlation analyses between demographic traits and host-choice test, host, and generation are shown in Table 5. Demographic traits showed high sensibility to the host-choice tests occurring over the six generations. Highly significant and positive correlations were recorded between host-choice test and sex ratio ($r = 0.53$, $P < 0.001$) and generation time ($r = 0.46$, $P < 0.001$). Similarly, there was a highly significant positive correlation between generation and mean growth rate ($r = 0.41$, $P < 0.001$). The results indicated that mean growth rate increases with generation time. There were also highly significant but negative correlations between host-choice test and mean growth rate ($r = -0.87$, $P < 0.001$), between generation and mortality rate of larvae ($r = -0.36$, $P < 0.001$), and between host and intrinsic rate of increase ($r = -0.31$, $P = 0.008$).

DISCUSSION

Insect populations that use different local host plants frequently show divergent egg-laying or feeding preferences on those hosts (Bernays and Chapman, 1994). Because plants represent habitats as well as food, host-plant variation could also affect behaviours not directly related to finding or consuming plants (Carroll *et al.*, 1998). Messina (2004) points out that a microevolutionary change ought to be predictable if replicate populations in a new environment converge towards a population already adapted to that environment. Our results demonstrate that both a juvenile trait and an adult trait (host discrimination) in a

C. maculatus population can be predictably modified because they rapidly shift towards the traits of an ancestral host. Furthermore, *C. maculatus* behaviours in the present study were markedly affected by host-choice test, host, and generation number. In the no-host-choice tests, adult female *C. maculatus* were strongly attracted by the seeds of chickpea and cowpea. However, in the free host-choice tests, they showed a strong preference for the seeds of cowpea. This is in agreement with Rees (2004), who indicated that the bruchids have diverse preferences for different hosts.

Bruchid species exhibit specificity in the choice of the legumes they attack (Rees, 2004). The cowpea weevil *C. maculatus* is primarily a pest of cowpea but has many alternative hosts that are leguminous seeds (Haouel-Hamdi *et al.*, 2017a). Being a field-to-store pest suggests that a dispersing individual is guided by specific cues towards its preferred hosts (Ajayi *et al.*, 2015).

Female oviposition behaviour is known to show heritable genetic variation, and egg-laying preferences in *C. maculatus* seem to be quite evolutionarily labile (Messina, 2004). According to Messina (2004), a new oviposition host is readily accepted after 40 generations. In addition, in *C. maculatus* considerable plasticity remains in traits related to host use even after many years of laboratory culture (Guedes *et al.*, 2003). However, after a host shift, larvae were exposed to a completely new environment that resulted in changes in larval feeding behaviour (Guedes *et al.*, 2003), larval competitive behaviour (Tuda and Iwasa, 1998; Messina, 2004), and adult life-history traits (Messina, 2004). Thus, both in the laboratory and in the wild, host shifts result in adaptations to new food sources (Tuda *et al.*, 2006).

Seeds emit volatile substances and Ignacimuthu *et al.* (2000) confirmed that these chemicals have an attractive effect on *C. maculatus* females. Sankara *et al.* (2010) showed that females of *C. maculatus* are able to recognize odours from their egg-laying substrates and to find their way to the sources of these odours. When these females have the choice between clean air and air containing seed odours, they are significantly more attracted to the smell of seeds.

In this study, we found a rapid evolution of female oviposition behaviour with an increase in novel host acceptance, in line with the findings of Wasserman and Futuyma (1981) and those of Messina and Karren (2003). Most importantly, we observed an acceleration in mean growth rate and an increase in body weight and body size. Thus, the evolution of demographic traits better adapted to the novel host occurred in concert with the increase in population fitness on both cowpea and lentil.

Agosta (2008) found that both the survival of offspring and host seed size were positively correlated with the number of eggs laid on the seed in the free host-choice test. However, in the no-host-choice test, only the survival of offspring and the number of eggs laid showed a significant positive relationship. Seed size and the number of eggs laid on the seed were also significantly correlated.

In the present study, several biological indicators suggested the strong potential for relatively rapid adaptation of *C. maculatus* to the three hosts tested. Other indicators of *C. maculatus* adaptability to cowpea seeds came from the extent of larval survival inside the hosts and the intrinsic rate of increase. Although both parameters were higher on chickpea until the fourth generation, the increase in the intrinsic rate of increase was faster and larger when larvae developed on chickpea rather than cowpea. Furthermore, Sankara *et al.* (2016) showed that larval mortality was important inside bambara seeds, no matter how many eggs were laid. That finding indicates that acceptability is not always correlated with suitability (Giga and Smith, 1987; Shazali, 1989). The chemistry of seeds may be the most important factor determining interactions between the development of bruchids and their host plants (Tuda *et al.*, 2006). Interestingly, Sankara *et al.* (2016) showed that females of *C. maculatus* seem to

remember the volatile signals from cowpea, the original host, even after three years of adaptation or development on *Arachis hypogaea* and *Cajanus cajan*. For instance, if a population has the means to detoxify a particular secondary compound in a novel host, it may be able to exploit closely related hosts that contain similar compounds (Agrawal, 2000). According to Nicole (2002), an insect is specific to a plant if it can first recognize it. Nicole showed that *C. maculatus* was able to recognize its original host cowpea and to deposit its eggs in all tested situations (free choice, semi-choice, and no choice). In addition, Nicole has reported that the transfer of *C. maculatus* to other legumes and its maintenance on these hosts for a relatively long time influences its penchant for each host, but does not alter its ovipositional preference for the host plant.

CONCLUSION

Callosobruchus maculatus is a serious biosecurity threat to Tunisian food legume production and industry. We show that it can shift hosts rapidly, and quickly change its host discrimination behaviour. Evolutionary changes in female oviposition behaviour and in demographic traits of these weevil pests took only three to four generations. Thus, to ensure the food supply, it is prudent to be concerned about the ability of *C. maculatus* to contaminate food. When any agency – governmental or private – introduces a new food legume, it must monitor the process closely.

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