

# Asymmetric evolution of egg laying behavior following reciprocal host shifts by a seed-feeding beetle

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**Abstract** Colonization of new environments can lead to rapid changes in fitness-related traits. For herbivorous insects, switching to a new host plant can be comparable to invading a new habitat. Behavioral, physiological, and life-history traits commonly vary among insect populations associated with different plants, but how host shifts cause trait divergence is often unclear. We investigated whether experimental host shifts would modify a key insect trait, egg-laying behavior, in a seed beetle. Beetle populations associated long-term with either a small-seeded host (mung bean) or a large-seeded host (cowpea) were switched to each other's host. After 36–55 generations, we assayed three aspects of oviposition behavior known to differ between the mung bean- and cowpea-adapted populations. Responses to the host shifts were asymmetrical. Females from lines transferred from mung bean to cowpea produced less uniform distributions of eggs among seeds, were more likely to add an egg to an occupied seed, and were more likely to “dump” eggs when seeds were absent. These lines thus converged toward the cowpea-adapted population. In contrast, the reciprocal host shift had no effect; oviposition behavior was unchanged in lines transferred from cowpea to mung bean. We suggest that these results reflect an asymmetry in the fitness consequences of each host shift, which in turn depended on

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differences in larval competitiveness in the original populations. Interactions among multiple fitness components are likely to make evolutionary responses less predictable in novel environments.

**Keywords** *Callosobruchus maculatus* · Egg dispersion · Experimental evolution · Oviposition behavior · Seed size

## Introduction

Fitness-related traits can vary considerably among populations of the same species, even over small spatial scales (Richardson et al. 2014; Anderson et al. 2015). Such variation is often considered a consequence of local adaptation, i.e., responses to concomitant variation in key environmental variables (Mopper et al. 1995; Joshi et al. 2001; Blanquart et al. 2013). A general requirement for local adaptation is that there is a genotype  $\times$  environment interaction for fitness, so that no single genotype is optimal in each of the habitats occupied by a particular species (Hughes et al. 2017, and references therein). It also presupposes that gene flow is not sufficient to overwhelm local selection, and that the observed phenotypic differences cannot be solely explained by genetic drift (Kawecki and Ebert 2004). Finally, the relevant environmental drivers must show enough temporal constancy to allow the accumulation of locally adapted genotypes.

In natural populations, identifying which habitat differences account for trait divergence can be difficult, because there are often multiple biotic and abiotic factors that vary simultaneously among the habitats occupied by different geographic populations (Futuyma and Bennett 2009). For some organisms, selection experiments (experimental evolution) in controlled environments can provide a complementary method to test hypotheses regarding the causes of intraspecific variation (Fry 2003; Garland and Rose 2009; Kawecki et al. 2012). Experiments can be designed to vary only those factors suspected to be most important in generating population differences. In some cases, selection can be applied reciprocally, whereby divergent populations are placed in selective environments that resemble each other's natural environment (Via et al. 2000; Kawecki and Ebert 2004; Ågren and Schemske 2012). If a particular environmental factor is indeed responsible for the observed population differences, we would expect replicate lines transferred to a novel environment to converge toward populations chronically associated with that environment (Klappert and Reinhold 2005). Reciprocal transfers can also determine whether responses to novel environments are symmetrical, i.e., whether the magnitude of genetic and phenotypic change is similar in each direction. Here, we examine evolutionary responses to reciprocal host-plant shifts in a seed-feeding beetle.

For many herbivorous insects, most or all of the life cycle takes place on the host plant. Hence, different plant species can represent distinct habitats, and even closely related plants may present different profiles of plant chemistry, morphology, and phenology (Forister et al. 2012). Host-plant variation can thus lead to intraspecific variation in insect behavior, morphology, physiology and life histories (Marinosci et al. 2015; Haga and Rossi 2016; Savković et al. 2016). Oviposition behavior is a trait that may be frequently affected by host-plant variation. Because larvae are often sedentary, the quality of the larval environment will be largely determined by maternal egg-laying decisions. This is especially true for endophagous species, whose larvae feed within small, discrete hosts, such as seeds or fruits. Simple variation in host size, for example, can affect female decisions as to whether to lay an egg on a host that already bears eggs, as well as how many eggs to lay

before searching for other hosts. In this study, we investigated whether switching to a smaller or larger host seed modifies egg-laying behavior in the seed-feeding beetle, *Callosobruchus maculatus*. We used two beetle populations that were chronically associated with either a small host or large host, and could thus perform reciprocal host shifts with respect to seed size.

Females of *C. maculatus* lay eggs on the surfaces of legume seeds. Larvae burrow into the underlying seed and must obtain enough resources to complete development within the single, natal host. Geographic populations of *C. maculatus* vary substantially in both egg-laying behavior and larval competitiveness within seeds (Messina and Mitchell 1989; Messina et al. 1991; Horng 1997; Messina and Karren 2003; Fox et al. 2004). In some populations, co-occurring larvae exhibit aggressive “contest” behavior, and seeds consistently yield only one or a few emerging adults. Females in such populations strongly avoid adding eggs to seeds that already bear eggs, and thus produce highly uniform, non-random distributions of eggs among available seeds. In other populations, larvae are primarily scramble competitors and actively avoid each other inside seeds (Messina 1991; Colegrave 1994; Mano and Toquenaga 2008). In these populations, many adults can emerge from a single seed, and females are much more likely to add eggs to occupied seeds (i.e., engage in superparasitism). Population comparisons (Messina and Mitchell 1989; Messina et al. 1991; Horng 1997), a previous selection experiment (Messina and Karren 2003; Messina 2004), and theoretical analyses (Smith and Lessells 1985; Toquenaga et al. 1994; Tuda and Iwasa 1998) have suggested that seed size is the main driver of these population differences. When seeds are large, avoidance and tolerance appear to maximize larval fitness, but aggressive behavior is favored in small seeds, where co-occurring larvae are bound to come into contact. A selection experiment in which beetles adapted to a small-seeded host (mung bean) were switched to a large-seed host (cowpea), found that both maternal egg-laying behavior (Messina and Karren 2003) and larval competition strategy (Messina 2004) evolved in the predicted direction. However, that study evaluated a host shift in only one direction.

We extend these previous studies of the evolution of egg laying behavior by performing reciprocal host shifts, from a large-seeded host to a small-seeded host and vice versa. Replicate lines of a population associated with a small host, mung bean (*Vigna radiata*), were transferred to a large host, cowpea (*Vigna unguiculata*). At the same time, lines of a population chronically associated with cowpea were transferred to mung bean. Although both legume hosts are highly suitable for *C. maculatus*, a typical seed of the ‘California Black-Eye’ cultivar of cowpea (as used in this study) has 3–4 times the mass of a mung bean (Messina 2004; Fox et al. 2010). After 36–55 generations we compared egg-laying behavior between lines that were switched to the new host or remained on the ancestral host. We focused on three aspects of oviposition behavior that systematically differ between populations in a manner predicted to be due to adaptation to seed size: (a) the uniformity of egg dispersions produced by females that were provided clean seeds, (b) the tendency of females to discriminate between clean and egg-laden seeds when presented with the two kinds of seeds in a direct choice test, and (c) the tendency of females to “dump” eggs when no seeds are available (i.e., lay eggs on unsuitable substrates, which causes complete larval mortality) (Messina et al. 2007). For all three traits, we test the explicit hypothesis that switching to a smaller or larger host will cause lines to evolve to resemble populations long associated with that host.

## Methods

### Beetle populations

The South India (SI) population was collected in 1979 from infested seeds of mung and the closely related black gram, *V. mungo*, in Tirunelveli, India (Mitchell 1991). It had been maintained on mung bean at large population size for more than 300 generations before the start of this experiment. Later collections of beetle populations in India revealed that the traits of the SI population (such as uniform egg dispersions and highly competitive larvae) are found in beetle populations throughout the Tamil Nadu region (Mitchell 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea in Ouagadougou, Burkina Faso (Messina 1993), and had been maintained on cowpea at large population size for more than 200 generations before the start of this experiment.

### Natural selection experiment

We split each population (SI and BF) into six replicate lines, three to be reared on mung and three to be reared on cowpea. Although mung bean and cowpea undoubtedly differ in many traits other than seed size, they were used to represent small and large hosts because they are congeneric and are among the most suitable hosts for *C. maculatus*; they are similar in protein, carbohydrate and fat composition (Gunathilake et al. 2016), and larvae on both hosts show similarly high rates of survival and growth across a wide range of temperatures (Stillwell et al. 2007). Lines were maintained at  $\sim 25^\circ\text{C}$ , L:D 15:9, on  $\sim 1.05$  L of seeds ( $\sim 800$  g of mung or 680 g of cowpea, or  $\sim 14,000$  and 3300 seeds, respectively) in well ventilated plastic containers. Every  $\sim 3.5$  weeks, 80% of seeds were removed from the colony and discarded. The remaining 20% of the seeds (all bearing eggs) were retained and 1.05 L of clean seeds (measured by volume) were added. The clean seeds were separated from seeds bearing eggs within each container (they were maintained in separate plastic trays). This step required females to disperse between plastic trays within the colony (a distance of only a few cm) to move from their emergence seed to the clean seeds, and allowed us to produce discrete generations, i.e., form each new generation without including beetles from the previous generation. We conducted all assays in a growth chamber at  $\sim 25^\circ\text{C}$ , L:D 15:9.

### Quantifying evolved differences in egg laying behavior

After 36 generations and again after 50 generations, we scored the uniformity of egg dispersions in all 12 lines (2 source populations  $\times$  2 selection hosts  $\times$  3 replicates per treatment). At 50 generations, we also measured female preference for clean seeds in a paired test. Lastly, we quantified the tendency to dump eggs 55 generations after the start of the experiment.

To estimate genetic differences among lines we needed to remove, as much as possible, sources of non-genetic variation. Because beetles were maintained on mung bean or cowpea, oviposition behavior of females could be influenced by larval rearing host and/or maternal effects (Fox and Savalli 1998; Proffitt et al. 2015). Therefore, we estimated egg dispersion and the preference for clean seeds after all lines were reared at low density (one larva per seed) on mung bean for two generations (for the 36-generation measurement of egg dispersion) or a single generation (for the 50-generation assays).

Each test female was collected within 24 h of emerging from an isolated seed (to ensure they were unmated) and paired with a random male from the same line until they mated (usually within  $\sim 10$  min). If pairs did not mate within  $\sim 15$  min, the male was replaced. If the pair did not mate in the next 15 min period, they were discarded. Mated females were then transferred into test dishes as described below.

## Egg dispersion

After 36 and 50 generations, females were transferred immediately after mating to a 60-mm Petri dish containing either 30 cowpea (half of the females from each line) or 30 mung seeds and allowed to oviposit for 72 h ( $\pm 1$  h). The number of eggs laid on each seed was recorded. After 50 generations, egg dispersion was also measured for females that were provided only 20 seeds and allowed to oviposit for only  $24 \pm 0.5$  h. The 50-generation assay was thus intended to assess the effect of selection host both in the early phase of oviposition and after virtually all eggs had been laid.

Egg dispersion was calculated as the uniformity index ( $U$ ) devised by Messina and Mitchell (1989). This index is derived from the number of “mistakes” that a female makes, where the number of mistakes is the number of eggs that would need to be transferred among seeds to produce the most uniform distribution possible (given a particular number of eggs on a particular number of seeds). This observed number of mistakes is then compared to the expected number of mistakes committed by a female laying eggs randomly, according to a Poisson distribution, on the same seeds.  $U = [(\text{expected mistakes} - \text{observed mistakes})/\text{expected mistakes}]$ . The index usually ranges between 0 and 1, where 0 represents a random distribution and 1 represents a completely uniform distribution. However,  $U$  will be less than 0 if a female clumps her eggs. The index is not biased at low or high fecundities (Messina and Mitchell 1989), but female behavior is influenced by egg densities and random versus uniform distributions are indistinguishable when mean egg number is low (the sampling variance in  $U$  increases with decreasing fecundity). We thus included in our analysis only females that laid on average at least 0.5 eggs per seed, which is 15 eggs over 72 h or 10 eggs over 24 h.

## Preference for clean seeds

As an alternative way to assess the tendency to avoid egg-bearing seeds, we used a paired-design in which newly-mated females were placed in a 30 mm petri dish containing 10 clean and 10 egg-laden cowpeas randomly interspersed in the dish. Egg-laden seeds were created daily by confining a large number of mated females with a large number of cowpea seeds for  $\sim 24$  h, and collecting all seeds bearing a single egg. In contrast to the first assay, in which females encountered a mixture of clean seeds and seeds bearing one of their own eggs, females in this assay were presented clean seeds and seeds bearing an egg ( $< 24$  h old) laid by a different female. Seeds bearing an egg were marked with a black ink dot in half of the preference dishes, and the seeds lacking eggs were marked in the other half of the dishes. Females were allowed to lay eggs for 24 h. Preference for clean seeds was defined as the proportion of eggs laid on clean seeds.

## Egg dumping

Fifty-five generations after the initiation of the selection lines we measured egg dumping behavior. Emerging beetles were collected as described above, albeit at 12 h (rather than 24 h) intervals. Each female was paired with an unmated male and placed into a clean (new) 35 mm Petri dish without any seeds. Females were left in an environmental chamber (27 °C, L:D 16:9) for 6 days, after which we counted the number of eggs per dish.

## Analyses

For egg dispersion ( $U$  scores) and the number of eggs dumped we used general linear models (SAS Proc Glimmix and Mixed) to test for the effect of population (BF vs. SI) and selection host (cowpea vs. mung bean), as well as selection host- $\times$ -population interactions. Each factor was treated as a fixed effect. For  $U$  scores, the model assumed a gamma error distribution; a Gaussian distribution was assumed for the number of eggs dumped (square-root transformed). For egg dispersion, our model also included a term for the test host (cowpea vs. mung bean) and a test host- $\times$ -population interaction. Oviposition preference was analyzed using logistic regression (binomial errors with *EggsLaidOnCleanSeeds/TotalEggs* as the dependent variable). We initially included whether seeds were marked or not as a fixed effect in our analysis of oviposition preference, but there was no evidence that marking seeds affected egg-laying preference of females ( $P = 0.39$ ).

For each analysis, the appropriate experimental units were selection lines rather than individual beetles. We thus included line, nested within the selection host- $\times$ -population interaction, in all models, and used this line effect as the denominator for all  $F$  tests other than the test for the line effect itself (details in Tables 1, 2). We used linear contrasts to test for differences between selection hosts within each population ( $df = 5$  for each contrast).

Egg dumping behavior was analyzed as two separate traits. We first analyzed the proportion of females that dumped at least one egg using logistic regression (binomial errors). We then used ANOVA (as described above) to analyze the number of eggs dumped among those females that dumped at least one egg.

## Results

### Egg dispersion (36 and 50 generations)

Regardless of selection host, females from both populations laid their eggs more uniformly on mung beans than cowpeas (closed circles in both Figs. 1 and 2;  $P < 0.001$  for all analyses in both generation 36 and 50; Table 1).

SI females consistently laid their eggs more uniformly than did BF females, averaged across all lines. However, it was evident by 36 generations that SI females from lines switched to cowpea had evolved to lay eggs less uniformly compared to the SI females in lines that remained on mung bean (Fig. 1a; significant linear contrast in Table 1). If evolutionary responses were symmetrical, we would expect females from BF lines switched to mung bean to lay eggs more uniformly compared to BF females from lines that remained on cowpea. However, egg dispersion did not differ between the BF lines on mung vs. cowpea (Fig. 1a; each linear contrast was not significant; Table 1). Although switching the SI lines to cowpea led to the evolution of less uniform egg dispersions, females from the SI lines on

**Table 1** Analysis of variance for the effects of test host (cowpea or mung bean), population (BF or SI), and selection host (cowpea or mung bean) on the dispersion of eggs (U scores) produced by females given clean seeds for 24 or 72 h

Assay (duration)	Source of variation						Linear contrasts			
	Test host		Population		Selection host		Population × selection host		Selection host × Line <sup>a</sup>	
	F (P)	F (P)	F (P)	F (P)	F (P)	F (P)	F (P)	F (P)	BF t (P) <sup>b</sup>	SI t (P) <sup>b</sup>
36 generations (72 h)	<b>195.4</b> ( <b>&lt;0.001</b> )	<b>150.6</b> ( <b>&lt;0.001</b> )	<b>6.41</b> ( <b>0.04</b> )	<b>6.71</b> ( <b>0.03</b> )	<b>6.15</b> ( <b>0.04</b> )	<b>42.8</b> ( <b>&lt;0.001</b> )	1.77 (0.08)	0.04 (0.97)	<b>-3.59</b> ( <b>0.02</b> )	
50 generations (24 h)	<b>136.6</b> ( <b>&lt;0.001</b> )	<b>58.5</b> ( <b>&lt;0.001</b> )	<b>11.0</b> ( <b>0.01</b> )	<b>7.18</b> ( <b>0.03</b> )	0.45 (0.52)	3.53 (0.10)	1.93 (0.052)	-0.45 (0.67)	<b>-4.23</b> ( <b>0.01</b> )	
(72 h)	<b>452.2</b> ( <b>&lt;0.001</b> )	<b>118.1</b> ( <b>&lt;0.001</b> )	<b>26.9</b> ( <b>0.02</b> )	<b>8.32</b> ( <b>0.02</b> )	0.71 (0.42)	<b>26.6</b> ( <b>&lt;0.001</b> )	<b>4.84</b> ( <b>&lt;0.001</b> )	-1.65 (0.16)	<b>-5.65</b> ( <b>0.002</b> )	

Significant tests are in bold

<sup>a</sup> Line is nested within population-×-selection host, and is the denominator for all F tests other than the line effect itself. *DF* = 1,8 for all effects except replicate line. *DF* for the line effects is 8, 781 (numerator, denominator) at 36 generations, 8, 1018 for the 24-h assay at 50 generations, and 8, 1101 for the 72-h assay at 50 generations

<sup>b</sup> *df* = 5 for all linear contrasts

**Table 2** Effects of population (BF or SI) and selection host (cowpea or mung bean) on female preference for clean seeds (analyzed by logistic regression) and the number of eggs dumped in the absence of seeds (analysis of variance)

Trait	Source of variation				Linear contrasts	
	Population	Selection host	Population × selection host	Line	Selection host	
					BF	SI
Preference for clean seeds <sup>a</sup>	$X^2_1 (P)$ <b>31.9</b> ( <b>&lt;0.001</b> )	$X^2_1 (P)$ 3.52 (0.06)	$X^2_1 (P)$ <b>9.48</b> ( <b>0.002</b> )	$X^2_8 (P)$ 10.3 (0.24)	$t (P)^c$ 0.90 (0.37)	$t (P)^c$ -3.34 ( <b>0.02</b> )
Eggs dumped per female <sup>b</sup>	$F (P)$ <b>3.91</b> ( <b>0.05</b> )	$F (P)$ <b>3.97</b> ( <b>0.05</b> )	$F (P)$ <b>10.8</b> ( <b>0.01</b> )	$F (P)$ 0.68 (0.72)	$t (P)^c$ -0.52 (0.62)	$t (P)^c$ <b>3.23</b> ( <b>0.01</b> )

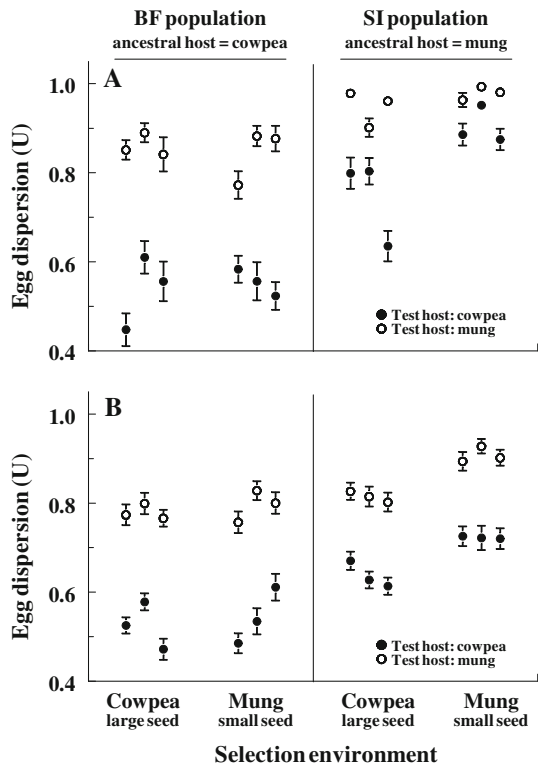
Significant tests are in bold

<sup>a</sup> This experiment used only cowpea seeds, so there is no test-host term

<sup>b</sup> Eggs dumped per female, given that a female dumped  $\geq 1$  egg (females laying 0 eggs were excluded). Data were square root transformed for analysis

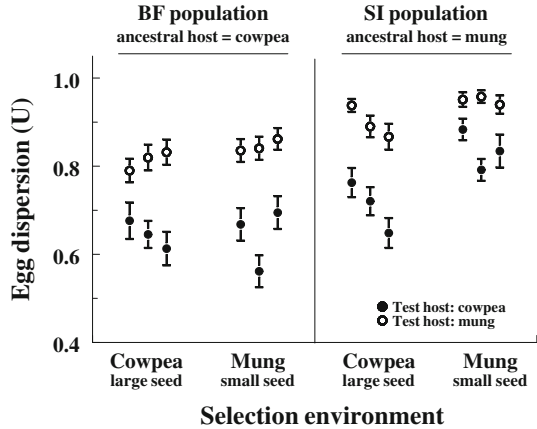
<sup>c</sup>  $df = 5$  for all linear contrasts

**Fig. 1** Long-term assay of egg dispersion ( $U$  scores, mean  $\pm$  SEM) of *C. maculatus* females provided 30 cowpeas (closed circles) or mung beans (open circles) for 72 h. Beetle lines were reared on cowpea or mung bean for the previous 36 (a) or 50 (b) generations.  $N = 3$  replicate lines per treatment





**Fig. 2** Short-term assay of egg dispersion ( $U$  scores, mean  $\pm$  SEM) of *C. maculatus* females provided 30 cowpeas (closed circles) or mung beans (open circles) for 24 h. Beetle lines were reared on cowpea or mung bean for the previous 50 generations.  $N = 3$  replicate lines per treatment



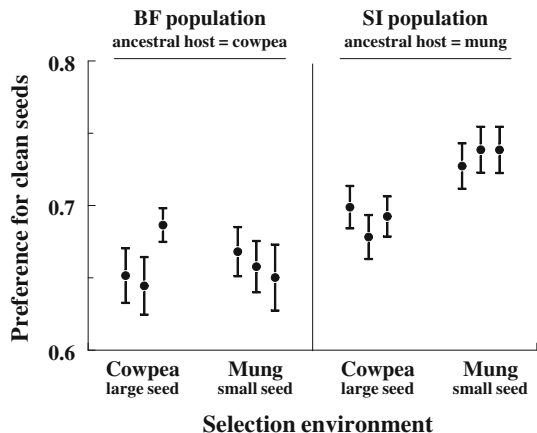
cowpea nevertheless continued to lay their eggs more uniformly than did BF females in lines that remained on cowpea (linear contrast,  $t_5 = -7.8$ ,  $P < 0.001$ ).

This difference between populations in the genetic lability of egg dispersion was also evident after 50 generations. In general, females laid their eggs more uniformly during the short-term assay (24 h on 20 seeds) than during the long-term assay (72 h on 30 seeds) ( $F_{1,8} = 84.9$ ,  $P < 0.001$ ). However, the evolution of egg dispersion was asymmetric in each case; females from the SI lines switched to cowpea evolved to lay eggs less uniformly, but the BF lines switched to mung bean did not evolve to lay their eggs more uniformly (Figs. 1b, 2; linear contrasts in Table 1). Despite a decrease in the uniformity of egg dispersion in the SI lines on cowpea, females from these lines again laid eggs more uniformly than did BF females in the lines that remained on cowpea (linear contrasts,  $t_5 = -3.57$  and  $-5.77$  for  $U$  over 24 and 72 h, respectively;  $P < 0.02$  for each).

**Discrimination between clean and egg-laden seeds (50 generations)**

In arenas containing 10 clean seeds and 10 egg-laden seeds, females laid a larger proportion of their eggs on clean seeds (Fig. 3). Overall, SI beetles preferred clean seeds more

**Fig. 3** The proportion of eggs laid on clean cowpea seeds (mean  $\pm$  SEM) by *C. maculatus* females simultaneously presented 10 clean seeds and 10 egg-laden seeds. The x-axis label is the host that beetles had been reared on for the previous 50 generations.  $N = 3$  replicate lines per treatment



strongly than did BF females. However, SI females from lines switched to cowpea showed a weaker preference for clean seeds compared to SI females from lines that remained on mung bean (Fig. 3; Table 2). In contrast, BF females from lines switched to mung bean did not show an increase in preference for clean seeds compared to females from lines that remained on cowpea. Thus, the asymmetrical responses suggested by the  $U$  scores in the first type of assay were similarly evident in the direct choice tests.

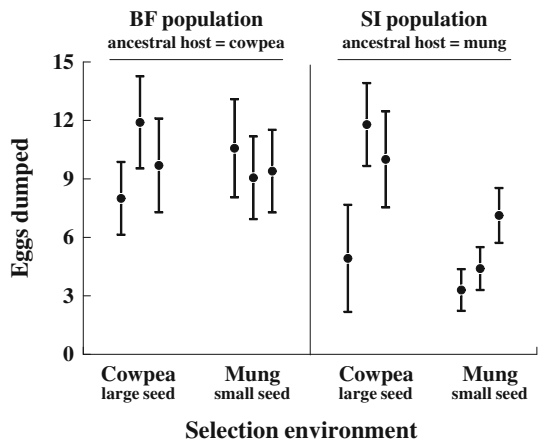
### Egg dumping (55 generations)

There was no evidence that the proportion of females that dump eggs evolved in response to a host shift (logistic regression; Non-significant population- $\times$ -treatment interaction,  $\chi^2_1 = 0.001$ ,  $P = 0.98$ ). However, among females that dumped at least one egg, the effect of the host shifts resembled the pattern described above for egg dispersion and seed discrimination: SI females from lines switched to cowpea dumped significantly more eggs than did SI females from lines remaining on mung bean (Table 2) but shifting to a novel host had no effect on female behavior in the BF lines (females from the cowpea and mung bean lines dumped similar numbers of eggs; Fig. 4; Table 2).

## Discussion

Colonization of novel environments is likely to cause major changes in fitness-related traits. Such changes can be considered predictable if multiple populations independently evolve similar traits in the same environment. Geographic populations of *C. maculatus* have long been known to display considerable variation in fitness-related traits, including oviposition behavior (Credland et al. 1986; Messina and Mitchell 1989; Fox et al. 2004), and this variation may depend in part on differences in the properties of local host plants (Haga and Rossi 2016; Messina and Gompert 2017). Most suitable hosts for *C. maculatus* are members of the genus *Vigna* (Savi), but different *Vigna* species (or even different cultivars within a species) can present a range of seed sizes, nutritional profiles, secondary chemistry, etc. (Tuda et al. 2014). In this study, we performed reciprocal shifts between a small-seeded host, mung bean (*V. radiata*), and a large-seeded host, cowpea (*V.*

**Fig. 4** The number of eggs laid per female (mean  $\pm$  SEM) among females that “dumped” at least one egg in the absence of seeds. Females were from lines reared on either cowpea or mung bean (on the x-axis) for 55 generations.  $N = 3$  replicate lines per treatment



*unguiculata*), two hosts that are highly suitable for *C. maculatus* larvae (Messina 2004) but show a three- to four-fold difference in seed mass. We tested the hypothesis that females in lines switched from a small to a large seed would evolve to be less discriminating in their oviposition behavior, as was previously observed by Messina and Karren (2003), and the reverse would be true for lines switched from a large seed to a small one.

The effects of the two host shifts in this study were asymmetric. Females from SI lines switched from mung bean to cowpea consistently evolved less discriminating oviposition behavior: they distributed their eggs less uniformly among available seeds, showed a greater acceptance of egg-laden seeds, and tended to dump more eggs in the absence of seeds. For each trait, all three replicate lines of the SI switched to cowpea converged toward the BF population that has been chronically associated with cowpea. In contrast, females from BF lines switched from cowpea to mung bean did not become more discriminating; they did not achieve higher *U* scores (i.e., lay their eggs more uniformly), show a greater aversion to egg-laden seeds, or dump fewer eggs. Our results thus confirm an earlier study demonstrating that switching to a larger host—from mung bean to cowpea—can produce predictable changes in fitness-related behaviors (Messina and Karren 2003), but the reverse was not observed.

There are multiple potential explanations for the asymmetric responses to the two host shifts. It is possible that the BF lines simply lacked sufficient standing genetic variation in oviposition behavior, but we consider this explanation unlikely. Previous experiments have revealed significant genetic variation in both egg dispersion and egg dumping in the BF population. For example, two generations of bidirectional artificial selection were sufficient to cause significant divergence between “dumper” and “nondumper” lines (Messina et al. 2007). Moreover, Messina and Fox (2011) observed substantial among-family variation in egg dispersion (*U* scores) in a study that was performed about seven generations after the creation of the selection lines described here. Re-analysis of the data in Messina and Fox (2011) indicates that the variance in *U* among full-sib families was not only significant for both the BF and SI populations, but was actually greater in the BF population. Finally, other traits, such as oviposition preference, body mass, egg size and fecundity, all have non-zero heritabilities in both the BF and SI populations (*unpublished data*). It is also unlikely that genetic drift contributed to creating asymmetric responses. Treatment differences were repeatable among replicate lines and, although effective population sizes were not quantified, the number of adults forming each new generation was consistently large within a line (beetles emerged from at least a few thousand seeds per line) (see also Gompert and Messina 2016).

We propose instead that the asymmetric evolution of egg-laying behavior was more likely the result of an asymmetry in the fitness consequences of superparasitizing seeds. Traits affecting fitness often have different optima under different environmental conditions (Martin and Lenormand 2015), but it is also possible for a trait to be under selection in one environment but not in another, leading to asymmetric evolutionary responses following environmental change. Several studies, mostly with microbial populations (but see Angert et al. 2008), have demonstrated that the fitness consequences of colonizing new environments can be asymmetric (e.g., Travisano 1997; Kassen 2002; Buckling et al. 2007; Jasmin and Kassen 2007; Lee et al. 2009; Remold 2012), analogous to the results of our study. These asymmetries likely occur because alleles that are beneficial in one environment reduce fitness in a second environment, whereas the reverse is not true to the same degree (i.e., the strength of this antagonistic pleiotropy is diminished in the opposite direction, or conditionally neutral; Anderson et al. 2013). For example, populations of *Pseudomonas fluorescens* that evolved in low nutrient media showed reduced fitness when

grown in high nutrient media, but the reverse was not true (Buckling et al. 2007). Alternatively, the adaptive landscape for oviposition preference may have multiple fitness peaks (local optima), with some shared or similar among environments and others unique to specific environments, such that whether an environmental shift affects selection depends on the starting phenotype and direction of the shift (Dercole et al. 2002). The degree of asymmetry in fitness consequences of an environmental shift can be influenced by the specific genetic architecture underlying traits, and thus can vary among populations or clones (Wenger et al. 2011).

In this study, asymmetric fitness consequences of a host shift likely depend on the mode and outcome of competition between co-occurring larvae. Larvae of BF population are scramble competitors, and there is only weak selection against adding another egg to an egg-laden seed, even a small one, as long as larval densities do not become very high. Thus, reducing seed size likely imposes little selection on female behavior. In contrast, increasing seed size reduces the incidence of larval interactions within seeds, substantially relaxing selection against egg-avoidance behavior in a population with contest-type larvae (SI). Superparasitizing large seeds may increase female fecundity without the concomitant increase in larval mortality. Companion experiments (*unpublished data*) examining the evolution of larval competition found results consistent with this hypothesis; we found no evidence that the BF population evolved towards a contest-type competition strategy following a shift to mung bean, but the SI population evolved toward a more scramble-type of competition following the shift to cowpea (see also Messina 2004). We therefore consider it more likely that asymmetry in the fitness consequences of larval co-occurrence is largely responsible for the asymmetry in the evolutionary responses in adult egg-laying behavior.

An alternative hypothesis is that oviposition behavior was not under direct selection in either population, and its evolution in the SI population was merely a response to selection on traits that are genetically correlated with egg-laying behavior. In this case, there could have been an asymmetry in the effect of the two host shifts on the trait or traits that were the target of selection (such as larval behavior), or the genetic correlation could have been present (or stronger) in one host environment (Czesak et al. 2006) and/or one direction of selection (Bohren et al. 1966; Worley and Barrett 2000) but not in the other. Asymmetrical genetic correlations can arise when the effects of some loci are sensitive to the environment whereas the effects of other loci are not, i.e., there is asymmetrical genetic architecture (Czesak et al. 2006). Although asymmetric correlated responses are certainly possible, they seem unlikely to be the primary cause of the observed asymmetry in this study because oviposition behavior has direct fitness consequences for females (avoidance of egg-laden seeds reduces fecundity; Messina 1991). Nevertheless, we do not have adequate data on the genetic correlations among the relevant traits (e.g., behaviors) in larvae and adults of *C. maculatus* to rule out this possibility.

Experimental evolution studies can help identify the likely causes of local adaptation and test the extent to which evolutionary change is predictable. The results of our experimental evolution study are consistent with population comparisons suggesting that populations of *C. maculatus* predictably and repeatedly evolve to be less discriminatory in their egg laying behavior when adapting to large-seeded hosts, but that adaptation to a small-seeded host does not necessarily lead to the evolution of more discriminatory oviposition behavior. Of course, the evolution of traits such as oviposition behavior and larval competitiveness can be influenced by a variety of other factors in natural populations, such as host chemistry and nutritional quality, population structure (which can affect the frequency of larval co-occurrence, Colegrave 1997) or natural enemies (whose access

to larvae can also depend on host size, Tuda and Iwasa 1998). Nevertheless, experimental host shifts can (in the case of the SI population) produce evolutionary responses that are predicted by the patterns observed among geographic populations on different hosts.

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**Author's contribution** CWF managed the selection experiment, quantified egg dispersion at 36 generations, and analyzed the data. RZ quantified egg dispersion (two experiments) at 50 generations and commented on the manuscript. JBD quantified egg dumping and commented on the manuscript. CWF and FJM co-wrote the manuscript.

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