

Evolution of larval competitiveness and associated life-history traits in response to host shifts in a seed beetle

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Abstract

Resource competition is frequently strong among parasites that feed within small discrete resource patches, such as seeds or fruits. The properties of a host can influence the behavioural, morphological and life-history traits of associated parasites, including traits that mediate competition within the host. For seed parasites, host size may be an especially important determinant of competitive ability. Using the seed beetle, *Callosobruchus maculatus*, we performed replicated, reciprocal host shifts to examine the role of seed size in determining larval competitiveness and associated traits. Populations ancestrally associated with either a small host (mung bean) or a large one (cowpea) were switched to each other's host for 36 generations. Compared to control lines (those remaining on the ancestral host), lines switched from the small host to the large host evolved greater tolerance of co-occurring larvae within seeds (indicated by an increase in the frequency of small seeds yielding two adults), smaller egg size and higher fecundity. Each change occurred in the direction predicted by the traits of populations already adapted to cowpea. However, we did not observe the expected decline in adult mass following the shift to the larger host. Moreover, lines switched from the large host (cowpea) to the small host (mung bean) did not evolve the predicted increase in larval competitiveness or egg size, but did exhibit the predicted increase in body mass. Our results thus provide mixed support for the hypothesis that host size determines the evolution of competition-related traits of seed beetles. Evolutionary responses to the two host shifts were consistent among replicate lines, but the evolution of larval competition was asymmetric, with larval competitiveness evolving as predicted in one direction of host shift, but not the reverse. Nevertheless, our results indicate that switching hosts is sufficient to produce repeatable and rapid changes in the competition strategy and fitness-related traits of insect populations.

Introduction

Competition for resources among conspecifics is ubiquitous in nature (Gurevitch *et al.*, 1992). For many organisms, intraspecific competition is a primary factor regulating population growth (via negative density dependence; Zhang *et al.*, 2015). Competition also influences the evolution of behavioural and life-history traits that affect an individual's ability to compete when densities are high (Rodrigues *et al.*, 2016). In particular,

when interactions between competitors are frequent, selection can favour genotypes that directly interfere with their competitors, possibly even engaging in agonistic interactions (combat) and killing conspecifics (Brockelman, 1975). Such contests can free up resources for the winning individual, but are often costly. For example, traits that increase success in contests (such as large body size and necessary weaponry) can be energetically expensive (Emlen, 2001). Contest behaviour also increases the risk of injury or even death, and distracts from other activities, such as foraging (Briffa & Sneddon, 2007; Georgiev *et al.*, 2013). We thus expect direct interference via agonistic behaviours to evolve only when resources are so limited that

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the benefits of eliminating competitors (or reducing their access to resources) exceed energetic costs and injury risk.

Resource competition is frequently strong among endophagous insects, that is, insects that feed within small discrete hosts, such as seeds, fruits or other insects (as occurs in parasitoid wasps) (Averill & Prokopy, 1987; Godfray, 1994; Nufio & Papaj, 2001; Pekas *et al.*, 2016). In most cases, juvenile stages cannot move between hosts, and must complete development within the single, natal host chosen by its mother (Messina, 2002; Harvey *et al.*, 2013). Co-occurring larvae are thus likely to encounter each other within a host (Colegrave, 1994). Behavioural responses to this resource competition can vary widely, even between closely related species or among different populations of the same species (Boivin & Van Baaren, 2000). Some species (or populations within species) evolve aggressive contest behaviour within hosts, and others display tolerance or avoidance behaviours (and thus act as scramble competitors). A large literature has developed to understand and predict the conditions that favour the evolution of contest vs. scramble strategies (Mayhew, 1998; Pexton *et al.*, 2009). Several important variables have been identified, including host availability, dispersion and, especially, host size, all of which can influence the frequency of encounters (Godfray, 1987; Joshi & Thompson, 1995; Lane & Mills, 2003; Vamosi *et al.*, 2011). When individual hosts are large relative to the resource needs of a single larva, avoidance or tolerance of competitors may maximize larval fitness, but aggressive behaviour may be favoured in very small hosts, where direct encounters are unavoidable (Smith & Lessells, 1985).

Success in agonistic interactions between larvae can be affected by a variety of traits, such as intrinsic fighting ability or mobility (Mayhew & van Alphen, 1999; Pexton & Mayhew, 2004), but most notable among these is body size, with an advantage to large size. This is often observed, for example, when larger, older larvae outcompete smaller, younger ones (Messina, 1991a; Tena *et al.*, 2008; Zhu *et al.*, 2016; Mackauer, 2017). The body size of juvenile insects is in turn affected by egg size, particularly early in development. Egg size directly determines hatchling size (Ernsting & Isaaks, 1997; Fischer *et al.*, 2002), but also can influence size differentials throughout development because larger hatchlings have a head start on growth (Fox, 1994; Azevedo *et al.*, 1997; Svensson & Sinervo, 2000). We thus expect that an increased frequency of contests will lead to direct selection for larger body size and indirect selection for larger egg size (but see Lalonde, 2005). Because body size and egg size simultaneously influence many aspects of growth and reproduction (e.g. there is a well-established trade-off between egg size and fecundity; Fox & Czesak, 2000; Dani & Kodandaramaiah, 2017), the evolution of larval competition

strategy should affect a suite of developmental and life-history traits (Guedes & Smith, 2008).

Larvae of the seed beetle, *Callosobruchus maculatus* (F.), develop inside legume seeds, and are restricted throughout development to the seed chosen for them by their mother. Populations of this beetle vary substantially in how larvae interact within seeds (Messina, 1991a,b; Toquenaga, 1993). In most populations, larvae are primarily scramble competitors that exhibit minimal aggressive interactions within seeds (Colegrave, 1994; Takano *et al.*, 2001). These larvae tend to avoid interacting with each other within seeds, and actively construct “walls” to decrease the chance that their burrows will intersect (Mano & Toquenaga, 2008a,b). In these populations, females will readily lay additional eggs on occupied seeds (Messina & Mitchell, 1989; Horng, 1997; Guedes & Yack, 2016). In other populations, larvae exhibit aggressive contest interactions within seeds; larvae fight within seeds, so that small- and even medium-sized seeds often yield only one adult (Messina, 1991b; Toquenaga *et al.*, 1994). Females from these populations generally avoid laying additional eggs on seeds that bear previously laid eggs.

Theoretical analyses (Smith & Lessells, 1985; Tuda & Iwasa, 1998), population comparisons (Messina, 1991a; Guedes *et al.*, 2007) and selection experiments (Tuda, 1998; Messina, 2004) have suggested that features of the host species, particularly seed size, account for much of the differences in larval competitiveness among populations. In large seeds, which provide enough resources to support development of multiple larvae, avoidance and tolerance strategies appear to maximize individual fitness, whereas aggressive or contest behaviour is favoured in small seeds, within which co-occurring larvae are bound to come into contact (Smith, 1990). Differences in larval competitiveness tend to covary with other fitness-related traits (Katsuki *et al.*, 2013). For example, females from populations with highly aggressive larvae typically produce fewer but larger eggs relative to female body mass (Messina, 1991a).

In this study, we use reciprocal host shifts to examine the role of seed size in determining the evolution of larval competitiveness and other fitness components in *C. maculatus* populations. Replicate lines of a population associated with a small host, mung bean (*Vigna radiata*) were transferred to a larger host, cowpea (*Vigna unguiculata*). At the same time, lines of a population chronically associated with cowpea were switched to mung bean. Both hosts are highly suitable for *C. maculatus* and present similar nutritional profiles, but cowpea seeds of the ‘California Black-eye’ cultivar provide 3–4 times the mass of mung beans (Messina, 2004; Gunathilake *et al.*, 2016). Messina (2004) previously found that larval competitiveness and body size evolved in the predicted direction after beetle lines were switched from the small mung bean host to the much

larger cowpea. Here, we extend that study by simultaneously performing reciprocal host shifts and by examining the responses of additional fitness-related traits. We compared larval competitiveness, egg-to-adult development time, body size (mass at adult emergence), egg size and lifetime fecundity between lines remaining on the ancestral host and those switched to the novel host. We tested the hypothesis that the larval competition strategy would evolve from a more contest type to a more scramble type in lines switched from mung bean to cowpea, and the reverse would be true for lines switched from cowpea to mung bean. We also examined whether other traits in lines switched to the novel hosts would evolve towards those observed in the populations chronically associated with that host.

Materials and methods

Beetle populations

We used two well-studied laboratory populations of *C. maculatus*. The South Indian (SI) population is adapted to mung bean; larvae are contest competitors, and females avoid laying on egg-laden seeds unless other seeds are unavailable, lay relatively large eggs and exhibit low lifetime fecundity (Messina, 1991a, 2004). The Burkina Faso (BF) population is adapted to the larger cowpeas; larvae are scramble competitors and crowded seeds frequently yield multiple adults. Females are less deterred from egg laying by the presence of previously laid eggs, lay small eggs and exhibit fairly high size-adjusted fecundity (Fox *et al.*, 2004, 2009).

Our laboratory population of the SI population was established in 1979 from infested pods of mung bean and the closely related black gram, *V. mungo*, in Tirunelveli (Mitchell, 1991). It had been maintained on mung bean at large population size for more than 300 generations before the start of this experiment. Its high degree of larval competitiveness was later found to be characteristic of beetle populations in the Tamil Nadu region of India (Mitchell, 1991). The BF population was collected in 1989 from infested pods of cowpea in Ouagadougou. It was maintained on cowpea at large population size for more than 200 generations before the start of this experiment.

Experimental evolution

Each beetle population was split into six replicate lines, three to be reared on mung bean and three to be reared on cowpea. Lines were maintained at ~25 °C, L : D 15 : 9, on ~ 1.05 L of seeds, which is ~800 g of mung bean [~14 000 seeds] or 680 g of cowpea [~3300 seeds], in well-ventilated plastic containers. Every ~4 weeks (just before adults begin to emerge), 80% of seeds were removed from the colony and discarded. The remaining 20% of the infested seeds were retained,

and 1.05 L of clean seeds (measured by volume) was added to the container. Starting a new generation with a sample of infested seeds, rather than a sample of emerged adults, ensured that we were not imposing inadvertent direct selection on development time or indirect selection on other, correlated traits. We did not attempt to control the number of founding adults per generation in each treatment; this would have been difficult because of the large differences in larval competitiveness and female oviposition behaviour in the BF and SI populations (as described above). However, starting each new generation with 20% of the infested seeds from the previous generation corresponded to transferring 2800 mung seeds and 660 cowpea seeds; this corresponds to transferring ~ 4100 and 13 000 larvae per generation in the SI and BF control lines, respectively. We thus maintained reasonably high genetic variability in all treatments. In addition, because all seeds received multiple eggs in all treatments, all lines experienced a highly competitive environment. In short, our protocol was intended to impose quasi-natural selection (Fry, 2003) that mimicked what would occur if each population (with its existing and distinctive set of competition-related traits) colonized the other population's host. Nevertheless, by focusing on performing natural selection in the laboratory, we cannot eliminate the possibility that some of the variation among treatments was due to differences in the amount of genetic variation available to evolve in response to the host shift.

Clean seeds were separated from infested seeds by placing them in adjacent plastic trays within each container. Females were required to disperse between plastic trays within the colony (a distance of only a few centimetres) to move from their emergence seed to the clean seeds. Dispersing adults would lay eggs and then die within a few days after colonizing the clean seeds, whereas the next generation of adults would emerge after a few weeks. We could thus produce discrete generations by forming each new generation without including beetles from the previous generation. Experimental assays were conducted under the same conditions as those used to rear the beetle lines.

Quantifying evolved differences in life history and larval competition

Thirty-six generations after the replicate lines were established, we quantified mean body mass, egg size and lifetime fecundity of beetles reared at low density (one larva per seed). We also measured larval survival to adult emergence and body mass at adult emergence for beetles reared with or without larval competition (at two larvae vs. one larva per seed, respectively). To quantify genetic differences among lines, we needed to remove, as much as possible, sources of nongenetic variation, including whether the parents of test larvae

developed in cowpea vs. mung bean. We therefore maintained all lines at low density on mung bean for one generation before each assay (described below). Because both mung bean and cowpea are excellent hosts in terms of larval survival, development rate, body mass, etc. (Messina, 2004), we chose mung bean as the common pre-assay host; mung bean is more convenient for obtaining isolated, unmated adults (females lay fewer eggs per seed on mung because the seeds are small, and eggs are easier to see because the seeds are green).

To obtain newly emerged, unmated adults, we isolated seeds bearing eggs in 35-mm Petri dishes and collected newly emerged unmated beetles daily. Pairs of virgin beetles (both from the same line) were allowed to mate, and were then placed in 35-mm dishes containing ~40 mung beans (estimated by volume). Females were allowed to lay eggs for ~48 h. Larvae hatching from these eggs were reared to the adult stage at a density of one larva per seed (excess eggs were scraped off before egg hatch, which is usually 5–7 days after eggs are laid). Each seed bearing a single larva was isolated in a dish. Emerging adults were collected within 24 h of emergence and weighed on an electronic balance (total number of weighed beetles = 1880 females and 1958 males, with an average N of 156.7 and 163.2, respectively, per line).

After weighing, each adult was paired with a virgin beetle of the opposite sex and placed in a Petri dish containing seeds of mung bean or cowpea. Beetles commence mating and ovipositing within hours after emergence from seeds. These pairs were divided into three groups; one set served as parents to produce beetles for the larval competition experiment described below, another was used to quantify fecundity, and a third was used to quantify egg size.

Effects of host shifts on fecundity and egg size

To quantify fecundity, pairs (produced as described above) were confined in a Petri dish containing either 100 mung beans or 100 cowpea seeds (one pair per dish). Females were allowed to lay eggs until death, after which we counted the total number of eggs per dish (total $N = 729$ females, with an average of 30.4 females per line*test host combination). We used two test hosts for the fecundity assay in case lines had evolved differences in host acceptance, and thus expressed reduced fecundity on their less-preferred host (Messina *et al.*, 2009).

To quantify egg size, pairs were confined in a Petri dish containing 30 mung beans and were allowed to lay eggs for 24 h (total $N = 348$ females, with an average of 29 per line). Providing 30 seeds ensured that even the BF females would lay only one or a few eggs per seed. Mung bean was used as the test host in this assay because it is easier to observe the edges of the egg against the darker seed coat, and previous work

demonstrated that there is no effect of host species on egg size. We sampled three eggs per dish (i.e. per female) and measured egg length using an ocular micrometer in a 55× dissecting microscope.

Effects of host shifts on competitive outcomes

To quantify the degree of larval competitiveness, a trio of mated females was confined in a 60-mm Petri dish for 24 h with either 20 mung beans or 100 mung beans, and females were allowed to lay eggs for 24 h. We used mung bean rather than cowpea as the test host for assessing larval competitiveness. Earlier work (Messina, 2004) showed that evolved differences in larval competitiveness were particularly easy to detect in mung beans, whose small size leads to strong competition even when there are only two larvae per host. In addition, larval performance on mung bean was already known to be high and similar in the BF and SI populations in the absence of competition (e.g. Fox *et al.*, 2011). Using only mung bean as the test host, we essentially tested whether shifting some BF lines to a much smaller host for many generations altered the way larvae compete in that host, and, at the same time, whether shifting some SI lines to a much larger host led to differences in the way larvae compete in their ancestral, small host.

The 20-seed dishes allowed us to collect seeds with multiple eggs per seed, whereas the 100-seed dishes provided one-egg seeds (seeds to be reared were haphazardly chosen from the dishes; those with more than the target number of eggs had the excess eggs scraped off). Seeds were sorted individually into 35-mm dishes and placed in the incubator, and inspected daily for emerging adults. We quantified survival to adult emergence and measured egg-to-adult development time and body mass at emergence for all beetles that emerged from seeds. In total, we reared larvae from 2533 one-egg seeds (129 dishes; 2457 beetles survived to adult) and from 1472 two-egg seeds (129 dishes; 1836 beetles survived to adult).

Analyses

For body mass, egg size and fecundity, we used analysis of variance (SAS Proc Mixed) to examine the fixed effects of beetle population (BF vs SI), selection host (the host on which beetles were reared for the previous 36 generations) and their interaction. Sex was included for body mass. For the larval competition experiment, we also include larval density (one vs. two per seed) as a fixed effect on the dependent variables of survival, development time and body mass, in the presence or absence of competition. For traits measured on multiple full-sib offspring of a single pair, we used family means rather than individual beetles as data points. For the larval competition experiment, in which each dish received three females, we used the dish means as data

points. In each analysis, line was the experimental unit (i.e. lowest level of independence); we thus included line nested within selection host * population interaction in all models, and used this line effect as the denominator for all *F*-tests other than the line effect itself (details in Tables 1 and 2). We used linear contrasts to test for differences between selection hosts within each population. Linear contrasts are *t*-distributed with five degrees of freedom (number of lines within each population minus 1).

To analyse survival in the presence or absence of competition, we used logistic regression (SAS Proc Glimmix), of the form *Number of Survivors/Number of Eggs* (one ratio per dish) = *Population + Selection Host + Interaction + Line* (nested within the interaction). We perform separate analyses at each larval density, followed by linear contrasts (within densities) to test for evolved differences between lines reared on cowpea vs. mung bean. We do not test for interactions between rearing density and population and/or selection host because the interpretation of such interactions in logistic regression is not analogous to the interpretation of interactions in a general linear model, and thus these interactions are not useful for testing the hypotheses of interest (Ganzach *et al.*, 2000; Mood, 2010). Line was again the lowest level of statistical independence.

Results

Evolution of body mass, egg size and fecundity

As was demonstrated in previous studies, beetles from the mung bean-adapted SI population are larger than those of the cowpea-adapted BF population (Fig. 1). However, 36 generations after being switched from cowpea to mung bean, BF beetles had evolved

increased adult mass; females were ~12.2% heavier and males were 11.5% heavier in the mung bean lines compared to the cowpea lines (averaged across lines; Fig. 1, Table 1). In contrast, the reverse host shift did not affect body mass; the size of SI beetles switched from mung bean to cowpea did not evolve to become smaller (Fig. 1; Table 1). Sexual dimorphism also differs between the two populations, with SI beetles more dimorphic than BF beetles (Fig. 1). However, the effect of the host shift on body mass did not differ between the sexes; that is, there was no significant population*selection host*sex interaction; $F_{1,8} = 0.19$, $P = 0.68$) and thus no evidence that the degree of sexual dimorphism evolved in response to either host shift.

The evolution of egg size following the host shift was similarly asymmetrical, but in the opposite direction. Specifically, SI females in lines switched to cowpea (the larger seed) laid eggs that were 4.3% shorter compared to the eggs of SI females in lines that remained on mung bean (averaged across lines; Fig. 2; Table 1). This is equivalent to a ~12% decrease in egg volume, assuming the egg is approximately half of an oblate spheroid. In contrast, egg size did not differ between BF lines switched to mung bean and those remaining on cowpea.

Along with the evolution of smaller egg size, SI females in the cowpea lines evolved to lay 16% more eggs (averaged across lines and test hosts) compared to SI females in lines remaining on mung bean (Fig. 3; Table 1). Fecundity of BF females also evolved following the shift to a new host, but this was observed only when cowpea was the test host (Fig. 3; Table 1). Specifically, females from BF lines that had been switched to mung bean laid ~10% more eggs (averaged across lines) on cowpea compared to females in lines remaining on cowpea. When mung bean was the test host, there was

Table 1 Analysis of variance for the effects of selection host (cowpea vs. mung bean) and source population [Burkina Faso (BF) or South Indian (SI)] on fitness-related traits of *Callosobruchus maculatus*.

Trait	Source of variation [†]				Linear contrasts	
	Selection host	Population	Population × selection host	Line*	Between selection hosts	
	<i>F</i> (<i>P</i>)	<i>F</i> (<i>P</i>)	<i>F</i> (<i>P</i>)	<i>F</i> (<i>P</i>)	BF <i>t</i> (<i>P</i>)	SI <i>t</i> (<i>P</i>)
Body mass						
Female	22.5 (0.002)	176.7 (< 0.001)	12.7 (0.007)	2.80 (0.005)	-9.87 (< 0.001)	-1.38 (0.17)
Male	11.1 (0.01)	63.1 (< 0.001)	6.02 (0.04)	4.44 (< 0.001)	-8.60 (< 0.001)	-1.32 (0.19)
Fecundity						
On cowpeas	7.07 (0.03)	7.08 (0.03)	87.9 (< 0.001)	0.37 (0.94)	-2.90 (0.004)	5.16 (< 0.001)
On mung beans	4.80 (0.06)	0.60 (0.46)	3.13 (0.11)	1.21 (0.29)	0.34 (0.74)	3.00 (0.003)
Egg Length	14.5 (0.005)	73.2 (< 0.001)	11.9 (0.009)	1.17 (0.32)	-0.28 (0.78)	-5.42 (< 0.001)

*Line is nested within population × host.

[†]For all traits, the degrees of freedom are 1, 8 (numerator, denominator) for selection host, population and population * host interactions. The degrees of freedom for the line effect vary among traits: 8, 624 (female body mass), 8, 620 (male body mass), 8, 270 (fecundity on cowpea), 8, 278 (fecundity on mung bean) and 8, 232 (egg length).

Entries in bold are significant at $P < 0.05$.

Table 2 Logistic regression examining the effects of selection host (cowpea vs. mung bean) and source population [Burkina Faso (BF) or South Indian (SI)] on the survivorship of *Callosobruchus maculatus* larvae reared in mung beans at densities of one or two eggs per seed.

	Source of variation				Linear contrasts	
	Selection host	Population	Population × selection host	Line*	Between selection hosts	
					BF <i>t</i> (<i>P</i>)	SI <i>t</i> (<i>P</i>)
Larval density	χ^2_1 (<i>P</i>)	χ^2_1 (<i>P</i>)	χ^2_1 (<i>P</i>)	χ^2_8 (<i>P</i>)		
One egg per seed	6.54 (0.01)	12.4 (< 0.001)	1.34 (0.25)	10.9 (0.22)	-3.22 (0.002)	-0.86 (0.39)
Two eggs per seed	4.24 (0.04)	123.5 (< 0.001)	9.90 (0.002)	8.88 (0.36)	-0.67 (0.50)	4.41 (< 0.001)

*Line is nested within population * host for all analyses.

Entries in bold are significant at $P < 0.05$.

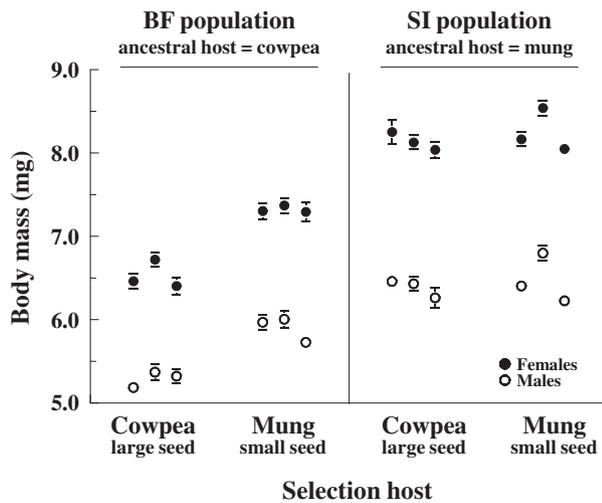


Fig. 1 Body mass (mean \pm SEM) at adult emergence of females (●) and males (○) from beetle lines maintained on the ancestral host or switched to the novel host for 36 generations. All beetles were reared on mung bean in the absence of competition. Means were calculated by first averaging across beetles within a family and then across families within lines.

no apparent effect of selection host on the lifetime fecundity of BF females (Fig. 3).

Evolution of larval competition strategy

In the absence of competition, survival of both SI and BF larvae to adult emergence in mung bean was uniformly high, usually $> 90\%$ (Fig. 4). This confirms that both mung bean and cowpea are excellent hosts for *C. maculatus*, and differ mainly in host size. The only effect of the host shift on survival in the absence of competition was a slight increase in average survival on mung bean in BF lines switched to mung bean compared to those remaining on cowpea (96.6% vs. 92.0%, Table 2).

In contrast, the host shift did affect survival in the presence of a competitor; shifting to a larger host decreased the competitiveness of SI larvae. When two SI larvae co-occurred in a mung bean (the small seed), overall survival to adult emergence was 59.4% in lines

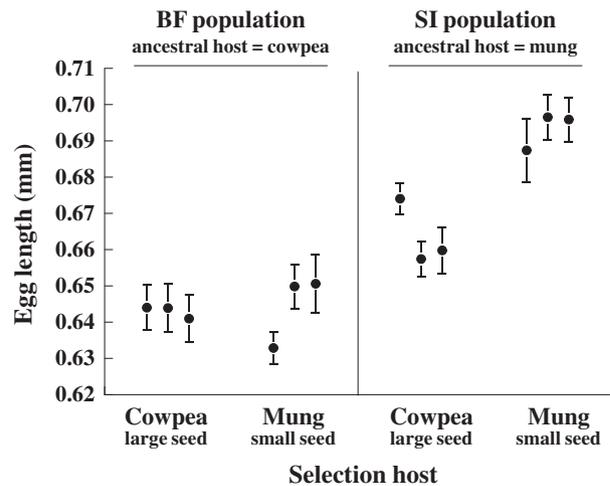


Fig. 2 Egg size (mean \pm SEM) in beetle lines maintained on the ancestral host or switched to the novel host for 36 generations. All egg-laying females were provided 30 mung beans. Means were calculated by first averaging across eggs laid by a single female, then across females within a family and then across families within lines.

switched to cowpea vs. 48.6% in lines remaining on mung bean (Fig. 4; Table 2). Whereas a mung bean bearing two larvae almost never produced two emerging adults in the SI lines remaining on mung bean, seeds bearing two larvae commonly produced two emerging adults (about 20% of seeds; Fig. 4) in the lines that were switched on cowpea. If evolutionary responses to the host shift were symmetrical, we would expect BF larvae from lines switched to mung bean to evolve to be more contest competitive compared to larvae in lines remaining on cowpea. However, shifting from cowpea to mung bean had no effect on the competitiveness of BF larvae; when two larvae shared a host seed, overall survival of BF larvae was 73.8% (averaged across lines) in lines remaining on cowpea vs. 74.3% in lines switched to mung bean for 36 generations (Fig. 4; Table 2).

The evolution of more frequent scramble competition in the SI lines adapting to the larger cowpea host was

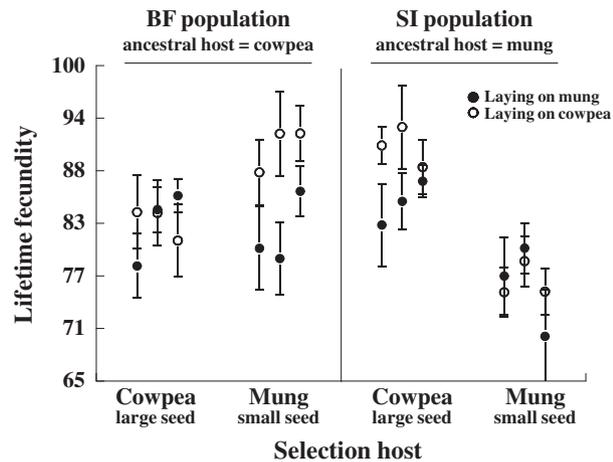


Fig. 3 Lifetime fecundity (mean \pm SEM) of females laying on mung bean (●) or cowpea (○) in beetle lines maintained on the ancestral host or switched to the novel host for 36 generations. Means are calculated by first averaging across females within a family and then across families within lines.

also evident in the differences in adult mass at emergence for beetles reared with vs. without competition (Fig. 5; Table 3). In SI lines remaining on mung bean, we observed no difference in mass between beetles reared at one vs. two larvae per seed (linear contrast; $t = 1.27$, $P = 0.13$). Because one larva nearly always dies when two larvae co-occur in a seed, there are apparently ample resources for the surviving beetle. However, in SI lines switched to cowpea, we did observe a significant difference in adult mass between beetles reared at one vs. two larvae per seed ($t = 10.7$, $P < 0.001$); competition decreased the sizes of emerging adults. In the BF population, competition consistently decreased the average mass of both males and females (Fig. 5). We observed that BF lines switched from cowpea to mung bean evolved to be larger (Fig. 5), but there was no evidence that the difference in mass between BF beetles in the cowpea lines and those in the mung bean lines depended on larval density (there was no significant host \times density interaction; $F_{1,8} = 3.08$, $P = 0.12$).

There was some evidence that development time also evolved in response to the host shifts. In both populations, development time was longer in lines that had been switched to the new host (Table 3; Fig. 6). SI beetles from the cowpea lines took longer to develop compared to SI beetles from lines remaining on mung bean, and BF beetles from the lines switched to mung bean took longer to develop than beetles from lines remaining on cowpea. This increase was generally small, only 2% or 0.6 days (averaged across populations and lines). As expected, larvae that developed with competition within a seed generally took longer to emerge compared to larvae that developed with no competition. However, we found no evidence that the effect of competition on development time depended on selection

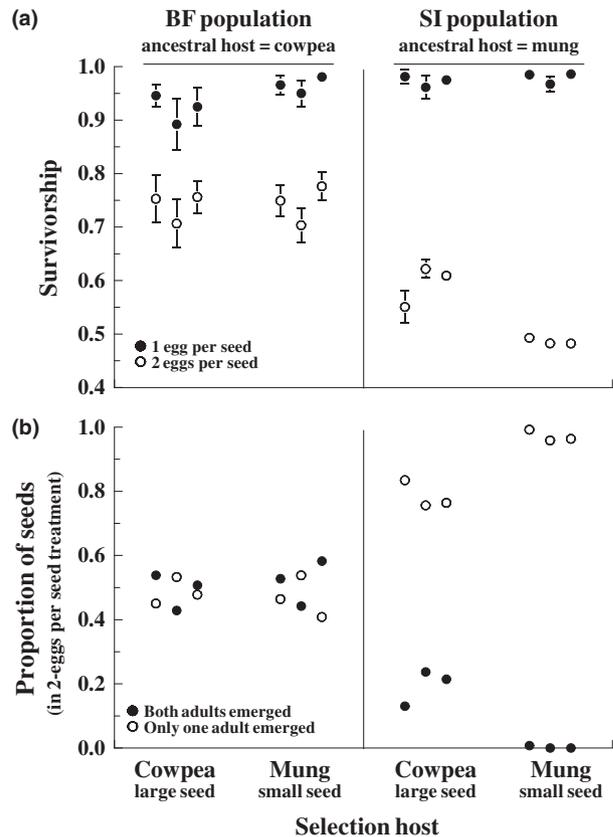


Fig. 4 (a) Survivorship to adult emergence in mung beans containing one larva (●) or two larvae (○). Larvae were from lines maintained on the ancestral host or switched to the novel host for 36 generations. (b) Proportion of seeds receiving two larvae that yielded one (○) or two (●) adults. Proportions do not add to 1 because a small and similar percentage of seeds ($\approx 2\%$) yielded no adults in each treatment. For some means, standard errors are smaller than the points.

host; neither the two-way interaction between larval density and selection host nor the three-way interaction of population \times selection host \times density was significant (Table 3).

Discussion

Variation in the properties of alternative hosts, particularly host size, can lead to the evolution of variation in the fitness-related traits of endophagous insects (Haga & Rossi, 2016; Savković *et al.*, 2016). Variation in seed size among populations of *C. maculatus* has been considered a primary determinant of their larval competition strategy (Smith, 1990; Toquenaga, 1993; Tuda & Iwasa, 1998; Messina, 2004). In this study, we performed reciprocal and replicated host shifts by switching a scramble-competing population of *C. maculatus* to a small host, and a contest-competing population to a large host. After 36 generations, we found that the shift

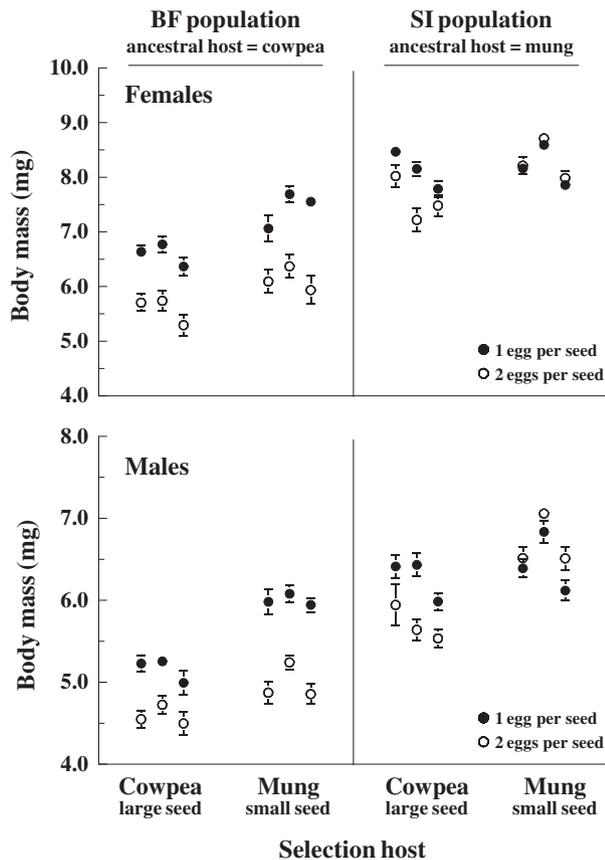


Fig. 5 Body size (mean \pm SEM) at adult emergence from mung beans in the absence of competition (one larva per seed, ●), or in the presence of competition (two larvae per seed, ○). Larvae were from lines maintained on the ancestral host or switched to the novel host for 36 generations. Means are calculated by first averaging across beetles from the same treatment dish and then across families within each line and density combination. For some means, standard errors are smaller than the points.

to a large host caused the contest-competing population (SI) to evolve greater tolerance of conspecifics within seeds (as indicated by the frequency of small mung beans yielding two adults), and to lay more smaller eggs. In contrast, shifting the scramble-competing population (BF) to a smaller host did not lead to changes in larval competitiveness or egg size, but the lines did evolve larger adult mass. These asymmetric responses to host shifts are similar to those previously observed for oviposition behaviour (Fox *et al.*, 2017): SI lines switched to the larger host evolved reduced avoidance of seeds bearing previously laid eggs, but BF lines switched to a smaller host did not evolve greater avoidance of egg-laden seeds.

Responses to changes in seed size were thus asymmetrical, but with the direction of the asymmetry varying among traits. These asymmetric responses are unlikely to reflect population differences in standing

Table 3 Analysis of variance* for the effects of selection host (cowpea vs. mung bean), larval density (one or two larvae per seed) and source population (South Indian (SI) or Burkina Faso (BF)) on body mass and development time in *Callosobruchus maculatus*.

	Body mass <i>F</i> (<i>P</i>)	Development time <i>F</i> (<i>P</i>)
Population	279.3 (< 0.001)	101.0 (< 0.001)
Density	51.6 (< 0.001)	123.4 (< 0.001)
Selection host	50.0 (< 0.001)	0.93 (0.34)
Sex	346.3 (< 0.001)	0.26 (0.61)
Population*Density	22.9 (< 0.001)	8.93 (0.005)
Pop*Host	1.10 (0.30)	22.3 (< 0.001)
Density*Host	1.32 (0.26)	2.74 (0.11)
Pop*Sex	10.4 (0.003)	0.26 (0.61)
Density*Sex	1.80 (0.19)	1.30 (0.26)
Host*Sex	0.10 (0.75)	0.08 (0.78)
Pop*Host*Density	11.6 (0.002)	0.87 (0.36)
Line	3.32 (< 0.001)	1.17 (0.23)

*The full model was $ResponseVariable = Population + Density + SelectionHost + BeetleSex +$ all interactions between these variables, plus *Line* (nested within population * density * host). Three-way and four-way interactions that were nonsignificant for both traits were deleted from the model. The degrees of freedom for all effects (except line) are 1,8 (numerator, denominator).

Entries in bold are significant at $P < 0.05$.

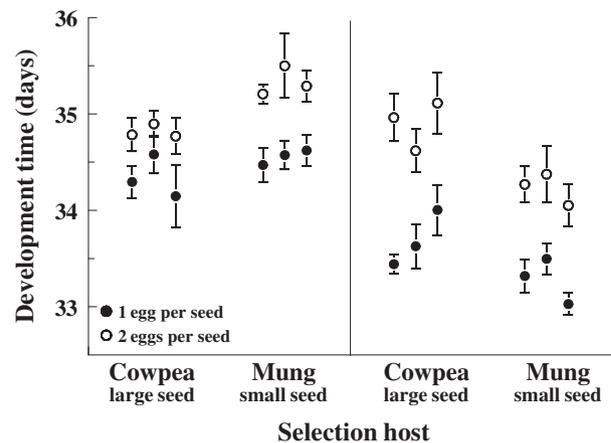


Fig. 6 Egg-to-adult development time (days, mean \pm SEM) in mung beans containing one larva (●) or two larvae (○). See the legend for Fig. 5.

genetic variation. In the populations maintained on their ancestral hosts, heritabilities in the BF and SI populations (estimated by parent–offspring regression) were significantly greater than zero for both female body mass ($h^2 = 0.61 \pm 0.10$ and 0.43 ± 0.11 for BF and SI, respectively) and male body mass ($h^2 = 0.32 \pm 0.12$ and 0.60 ± 0.13). We also observed heritable variation in egg length (h^2 for BF and SI = 0.49 ± 0.11 and 0.71 ± 0.14 , respectively), and fecundity ($h^2 = 0.39 \pm 0.09$ and 0.29 ± 0.13) (see also Price *et al.*, 2017). Yet each of these traits evolved asymmetrically in our experiment.

We did not quantify heritability estimates for larval behaviour, so it is possible that the BF population lacked sufficient genetic variation to cause a change in larval competitiveness. However, previous studies have suggested that even in a scramble-competing species, larvae will engage in fighting behaviour if the walls between larval burrows are experimentally removed (Mano & Toquenaga, 2008b). BF larvae in lines switched to mung bean may have continued to maintain effective burrow walls so that seeds frequently yielded two adults. In contrast, the increased frequency of mung beans yielding two adults in the SI lines switched to cowpea almost certainly reflects a change in larval behaviour; it cannot be explained by a change in mass, as SI lines switched to cowpea did not evolve to become smaller.

Asymmetric responses to host shifts could reflect an asymmetry in the evolutionary stability (or invasibility) of alternative strategies in small vs. large hosts. Among insects whose larvae feed in small, discrete hosts, parasitoids have been particularly well studied with respect to the evolution of aggressive larval behaviour (as in solitary species) vs. tolerant behaviour (as in gregarious species). Early theory suggested that aggressive larval behaviour should always invade a population of tolerant larvae, unless the cost of fighting is very high (Godfray, 1987). Yet tolerance of conspecifics is very common in parasitoids (Brodeur & Boivin, 2004) and seed beetles (Guedes *et al.*, 2007). Various authors have thus tried to identify the conditions that favour tolerance or at least resistance to invasion by more aggressive genotypes. These analyses have shown that female-biased sex ratios and single-sex broods (Rosenheim, 1993), reduced mobility in aggressive genotypes (Pexton & Mayhew, 2001; Brodeur & Boivin, 2004), Allee effects (where the presence of conspecifics improves resource quality; Khafagi & Hegazi, 2008) and reduced host availability (Hoffmeister *et al.*, 2005) all reduce the likelihood that an aggressive genotype will displace the tolerant genotype in a population. Similar theory has been used to examine when we expect to observe interference vs. exploitative strategies in seed beetles (Smith & Lessells, 1985; Toquenaga *et al.*, 1994; Tuda & Iwasa, 1998). Because most *C. maculatus* populations exhibit larval tolerance within seeds (Guedes *et al.*, 2007), and both this study and a previous one (Messina, 2004) have shown that greater tolerance readily evolves in only a few dozen generations, there may be greater constraints associated with evolving an increase in aggression than in its decay (but see Tuda, 1998).

We speculate that the asymmetries in evolutionary responses following the experimental host shifts are largely a consequence of asymmetries in fitness consequences of the host shift. Shifting beetles from a small host to a large one substantially relaxes selection favouring conflict within seeds; because a large seed can support more than one larva, larvae are less likely to

encounter other individuals and less likely to benefit from a contest if they do encounter another individual. The resulting relaxation of selection favouring contests in turn relaxes selection on hatchling size, and thus egg size, which in turn allows the evolution of greater fecundity (which trades-off with egg size; Fox & Czesak, 2000). However, because hatchling larvae are smaller, they need to take longer to reach the adult stage (Fox, 1997; Fox & Savalli, 1998) and thus they exhibit increased development time. The evolution of tolerance thus influences a suite of developmental and life-history traits – smaller eggs, higher fecundity and increased developmental time – all of which evolved in our experimental lines. In contrast, switching from a large to a small host appears to have less influence on selection, and thus produce less trait change, at least in our study population of primarily (or entirely) scramble competitors.

Asymmetric fitness consequences of environmental shifts have been observed in a few previous studies, mostly with microbial populations (e.g. Travisano, 1997; Buckling *et al.*, 2007; Jasmin & Kassen, 2007; Lee *et al.*, 2009; Remold, 2012; but see Angert *et al.*, 2008). Such asymmetries can occur when alleles that increase fitness in one environment reduce it in a different environment, whereas the reverse is not true to the same degree (i.e. antagonistic pleiotropy is asymmetric or conditionally neutral; Anderson *et al.*, 2013). Alternatively, the adaptive landscape for loci affecting larval competition strategy may have multiple fitness peaks (local optima), with some shared or similar among environments and others unique to specific environments. In such cases, whether an environmental shift affects selection depends on the starting phenotype and direction of the shift (Dercole *et al.*, 2002). Regardless of the specific genetic mechanism, we propose that fitness asymmetries are the most likely cause of the asymmetry in evolutionary response observed for larval competitiveness and other traits.

Alternatively, or possibly simultaneously, the asymmetric evolution of competition strategy (and the associated changes in egg size and fecundity) may derive from a mutational asymmetry. If being a contest competitor requires the beetle to execute one or more specific behaviours, loss of these behaviours may simply require a loss-of-function mutation in one of the genes influencing this behaviour. Loss-of-function mutations provide a mechanism by which organisms can adapt to new environments (Hottes *et al.*, 2013), such as when a functioning allele reduces fitness compared to a non-functional allele (Olson, 1999) or when loss of a function permits the evolution of new phenotypes (Goldman-Huertas *et al.*, 2015). In contrast, evolving contest behaviours from an ancestral scramble genotype may require one or more gain-of-function mutations, or the reversion of a previously lost functional allele (MacArthur *et al.*, 2012). It is likely that the *C. maculatus* originates from Africa (Kébé *et al.*, 2017) and that wild

cowpea hosts of *C. maculatus* in Africa bore small seeds (Lush & Wiens, 1980; Lush & Evans, 1981; Coulibaly *et al.*, 2002). It therefore seems likely that the contest-type competition is ancestral, with the evolution of a scramble-type competition following the development of the large, domestic cowpea (Toquenaga *et al.*, 1994).

Taken together, the results from this study and a similar previous one (Messina, 2004) suggest that although there is some predictability to parasite responses to host size, there are also likely to be idiosyncratic or unpredictable responses. For example, Messina (2004) also found that seed beetles transferred from mung bean to cowpea quickly evolved greater larval tolerance within seeds, but the magnitude of the change was larger than that seen here (the reciprocal host shift was not performed). However, that study also observed that adult body size declined by 10–15% following the transfer to the larger host, and suggested that this reduction in adult mass likely contributed to the large increase in the frequency of seeds yielding two adults (Messina, 2004). In contrast, we did not observe a significant reduction in body size in the current study, and there was a more modest increase in the proportion of seeds yielding two emergers. The reason for this discrepancy is unclear, but it may be due to different culturing techniques, population sizes and ambient conditions in the two laboratories, or could depend on even small differences in the amount and type of standing genetic variation at the time selection was imposed. Resolving such discrepancies would be greatly aided by obtaining genomic profiles before and after selection (Gompert & Messina, 2016; Plucain *et al.*, 2016). Model laboratory systems like the *Callosobruchus*/grain legume interaction can be used in this way to complement field studies aimed at assessing the relative predictability of evolutionary change following shifts to novel environments (Losos, 2010; Szendro *et al.*, 2013; Thorpe, 2017).

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Author contribution statement

CWF managed the selection experiment and analysed the data. CWF and FJM co-wrote the manuscript.

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