SIBLING EGG CANNIBALISM BY NEONATES OF THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*

by

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Abstract

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by

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Cannibalism reduces competition and provides nutritional benefits. However, when cannibalism involves kin, the benefits obtained must balance inclusive fitness losses, and cannibals should be under selection to avoid killing close relatives. Since cannibalism reduces competition, it may also be higher in populations with greater population density. Neonates of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, readily consume eggs within their natal clutch, which is a combination of full and half siblings, before assessing resource availability, suggesting an evolutionary response to potential resource limitation that should vary with competition between populations. CPB is also a crop pest that rapidly develops resistance to pesticides, but pesticide resistance can result in fitness costs in the absence of pesticide exposure; these costs may be mediated by cannibalism. To explore the fitness consequences of cannibalism, I fed neonates with only potato foliage or with eggs and potato foliage and measured growth and development rates. I used individuals from pesticide-susceptible and pesticide-resistant populations to test for costs of resistance and whether there is an interaction between the benefits of cannibalism and resistance. To determine whether neonates avoid killing relatives, I tested whether hatchlings recognize kin, whether they prefer inviable to viable eggs, and whether egg development is a cue for viability. To explore geographic variation in cannibalism, I studied three CPB populations and a population of *L. undecimlineata* on their native host plants in Mexico to assess differences in competition and cannibalism propensity. Cannibals gained mass and developed more quickly than noncannibals. When mortality risk is high, this decreased development time can reduce the mortality risk sufficiently to balance the inclusive fitness loss of eating a half sibling. There were costs of pesticide resistance, but the benefits of cannibalism reduced many of these costs. Neonates preferred eating eggs from another population, but they did not distinguish among eggs from their own population based on relatedness. They did, however, preferentially consume inviable eggs but did not use egg development as a cue for viability. Cannibalism rates were usually higher in the populations with the highest egg densities, although interspecific comparisons did not show the same pattern.

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CHAPTER 1: Fitness consequences of sibling egg cannibalism by neonates of the Colorado potato beetle, *Leptinotarsa decemlineata*[1]

Egg cannibalism is a widely observed behaviour in nature that provides nutritional benefits without the costs of subduing prey. However, when egg cannibalism involves sibling eggs, the benefits obtained must balance inclusive fitness losses. Neonates of the Colorado potato beetle, *Leptinotarsa decemlineata*, readily consume other eggs within their natal clutch. To explore the fitness consequences of this behaviour, neonates were fed no, one, or two eggs upon hatching, along with their normal diet of potato foliage. Daily mass, the time to each moult, adult volume and mass, and fecundity were measured for each individual, along with mortality rates. Cannibals gained mass more quickly and reached each developmental stage one day earlier than noncannibals. No benefits at the adult stage (other than earlier emergence) were observed, and there were no sex differences in the benefits of cannibalism. Mortality was not reduced by cannibalism under lab conditions. A simulation demonstrated that when the mortality risk is high, decreased development time due to cannibalism can reduce this risk sufficiently to balance the inclusive fitness loss of eating a single half-sibling. Additional benefits of cannibalism, such as reduced competition, need to be investigated to fully explain cannibalism of multiple sibling eggs.

Keywords: cannibalism, fitness benefits, inclusive fitness, kin selection, *Leptinotarsa decemlineata*, Colorado potato beetle

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Cannibalism is widespread, raising the question of its benefits and costs (Fox 1975; Polis 1981; Elgar & Crespi 1992; Pfennig 1997). The benefits are both immediate (a meal) and lasting (fewer competitors). Faster development and reduction of competitors can have compounding benefits. Thus, cannibalism can often be very advantageous for young individuals. An early conspecific meal can translate into increased fitness through faster growth and development, higher survival and larger size, all of which can ultimately result in higher lifetime fecundity (Fox 1975; Polis 1981). Cannibalism may be especially beneficial for females, further increasing adult size (Michaud & Grant 2004) or fecundity (Ho & Dawson 1966). Costs of cannibalism include the risk of injury (if a potential meal fights back) and disease transmission (Polis 1981; Pfennig 1997).

Experimental studies on the benefits of cannibalism on active life stages have given mixed results. In some species of ladybird beetle, larvae fed other larvae in addition to a normal diet have reduced development time and lower mortality compared to noncannibals (Snyder et al. 2000), but in other species, cannibals have higher mortality, longer development and lower mass than individuals that consume a diet without larvae (Michaud 2003). Larval cannibalism is insufficient to compensate for a poor base diet (Snyder et al. 2000). Larvae reared only on conspecific larvae take longer to develop and weigh less as adults, if they are able to complete development at all, compared to noncannibals on a normal diet (Michaud 2003). For six predaceous mite species, juvenile development times are longer on a strictly cannibalistic diet than on normal diets, if they are able to complete development at all; only two of the studied species are able to lay eggs on a cannibalistic diet as adults, and they lay fewer eggs than those on a normal diet (Schausberger & Croft 2000). Damselfly larvae that cannibalize many other larvae can gain more mass than those that cannibalize only one, but they do not develop more quickly (Fincke 2011). Spadefoot toad tadpoles fed dead conspecific tadpoles grew faster than tadpoles feeding on naturally available resources (Bragg 1956), but poison frog tadpoles that cannibalize another live tadpole do not grow larger than noncannibals (Brown et al. 2008). Cannibal morphs of the tiger salamander, *Ambystoma tigrinum mavortium*, grow larger and reach metamorphosis earlier than typical morphs, but cannibals are rare, indicating high costs associated with the morph (Ghioca & Smith 2008).

The costs of cannibalism can be greatly reduced by consuming eggs, which are essentially defenceless and have had less time to acquire pathogens than hatchlings. Consuming eggs within the natal clutch reduces search costs (Dixon 1959). Eggs may also provide greater nutritional benefits than juveniles; in three species of ladybird beetles, larvae have equal or higher survival and shorter development times on a diet of conspecific eggs compared to a diet of conspecific larvae (Michaud 2003).

In carnivorous species, egg cannibalism can enhance predatory abilities. Ladybird beetle neonates are able to survive longer with a single egg meal than with no food (Elnagdy et al. 2011), giving them more chances to successfully capture their first prey and increasing survival in nature. Egg cannibals are more successful in capturing difficult prey in one ladybird species, and those that are not successful can still moult to a larger instar if provided two or three eggs (Noriyuki et al. 2011). Cannibalism can also reduce development time when eggs are supplemental to a normal diet (Michaud & Grant 2004), and even when eggs are the sole diet, at least to the second instar (Gagné et al. 2002; Omkar & Gupta 2006).

For herbivorous species, egg cannibalism by neonates can provide a crucial source of protein, further adding to the benefits of cannibalism (Polis 1981). Eickwort (1973) reported a reduction in time to reach the second instar resulting from egg cannibalism in a milkweed leaf beetle, *Labidomera clivicollis*. Breden & Wade (1989) demonstrated in another leaf beetle that neonates that were allowed to eat eggs upon hatching gained more mass than noncannibals, in addition to having lower mortality in the field. Mertz & Robertson (1970) found that egg cannibalism by larvae of the flour beetle, *Tribolium castaneum*, decreases their development time to pupation and also shortens pupal duration.

While egg cannibalism reduces the general costs of cannibalism in many respects, intraclutch cannibalism incurs inclusive fitness costs. By kin selection theory, if the consumed individual has an otherwise equal probability of surviving as the cannibal, consuming a half-sibling must increase a cannibal's fitness by at least 25%, and a full-sibling's death must be compensated by at least a 50% increase in cannibal fitness in order for cannibalism to be favoured (Hamilton 1964; Mock & Parker 1997). However, the required fitness gains are comparably reduced as the hatching probability of the cannibalized egg declines (Eickwort 1973); this reduced hatching probability could be due to egg infertility or to being consumed by a predator or another cannibal (Perry & Roitberg 2005). Egg cannibalism by hatchlings may be especially favoured because mortality rates for hatchlings are so high, requiring only a small absolute benefit to achieve the necessary relative benefit (Stevens 1992).

One species in which cannibalism is unstudied but is likely to be beneficial is the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), an economically important crop pest. In this species, females mate with multiple males and lay clutches that are a mix of full- and half-siblings. Females lay all eggs in a clutch in a single day and do not add eggs to a completed clutch. There has been no research on whether females can recognize their own offspring, but they do not provide maternal care, so selection for kin recognition may be weak, if present at all. Eggs hatch after 4 or 5 days, and hatchlings begin consuming sibling eggs within as little as 1 h, generally before they consume their own chorion or available foliage (K. Collie, personal observation). When unhatched eggs are available, neonates eat an average of two and up to five eggs within 14 h of hatching, and typically disperse from the egg mass in about 1 day (K. Collie, personal observation). However, they seem unable to distinguish between full- and half-sibling eggs; neonates given either full- or half-sibling eggs do not wait longer to eat the full-sibling eggs compared to half-sibling eggs, and while they eat slightly fewer full-sibling eggs, the difference is not significant (K. Collie, unpublished data).

Many studies have demonstrated significant benefits of cannibalism in various species, especially with regards to reduced development time (see above), but attempts to estimate the quantitative fitness benefit resulting from this reduced development time are extremely rare, although Eickwort (1973) demonstrated how it could be calculated from larval mortality rates. In this study, we examine the nutritional benefits (accelerated growth or development, increased size or fecundity, or reduced mortality) that *L. decemlineata* neonates gain by cannibalizing eggs. We then use a simulation expanding upon Eickwort's (1973) method to estimate the resultant fitness benefits under actual field conditions (Harcourt 1971) in the context of kin selection theory.

Methods

Animals

Leptinotarsa decemlineata used in this experiment were from a laboratory colony derived from adults collected from potato fields in Riverhead, NY, U.S.A., in 2008 and 2009. The experiment began on 3 June 2009 and ended on 6 August 2009. All individuals were returned to the stock colonies at the end of the study.

Insect Rearing

Prior to the experiment, beetles were housed in large wire-mesh cages (71 × 46 × 61 cm). The cages were kept in a warm room (~25 °C) with a 16:8 h light:dark cycle. Adults were housed together in groups of up to 200 individuals per cage. Cages were provisioned with potted whole potato plants, and pots were replaced or watered as needed.

In both the laboratory and the field, multiple females may lay clutches on the same leaf, especially when population density is high (K. Collie, personal observation), but females almost never lay a clutch in contact with other clutches. In the rare instances when this occurred in the laboratory, clutches laid by different females could be distinguished by differences in development time and egg colour (which ranges from yellow to orange but is consistent within female); such overlapping clutches were not used in this study. Egg clutches were collected daily; leaves with clutches were removed from the plants and any leaves with multiple clutches were divided between clutches. All clutches were placed in petri dishes in a Percival® model I-36 VL incubator (Percival Scientific, Inc., Perry, IA, U.S.A.) at 25 °C and a 16:8 h light:dark cycle.

Experimental Procedure

Clutches collected from the laboratory colony were checked daily for maturation, evidenced by pigmentation spots (K. Collie, personal observation). Well-pigmented eggs were checked hourly for hatching. Within 30 min of hatching, neonates were moved by paintbrush into individual 325 ml Nalgene® Utility Boxes (ThermoScientific, Rochester, NY) modified with mesh windows for ventilation. Neonates were either placed directly on a potato leaf clipping with no available eggs, or placed directly on either one or two eggs on the leaf; younger eggs from another clutch were used to allow the neonates the opportunity to consume the eggs before they hatched. Use of potentially unrelated eggs probably did not change neonate response to the egg (K. Collie, unpublished data) and should not change any observed benefits of eating an egg.

Leaves were inserted into floral water picks to maintain leaf quality. Egg provisioning was decided randomly for each larva, with the constraint that larvae from each hatching clutch were divided as evenly as possible between the treatment groups. In total, 86 neonates from 10 clutches (2–29 individuals per clutch) were used.

Larvae provisioned with eggs were checked after 24 h to verify consumption of the egg(s), and egg remains were removed. Only seven of the 60 individuals given eggs failed to eat all provided eggs, typically because the eggs fell off the leaf or hatched; these individuals were analysed based on the number of eggs actually consumed. The remainder of the experiment was blind to initial egg provisioning, so all individuals were treated indiscriminately with regards to treatment group.

Larval growth and development rate

All individual boxes were kept in the same incubator under standard conditions (25 °C, LD 16:8 h) for the entirety of the experiment. Leaf clippings were changed daily to ensure optimal conditions. Beginning 24 h after initial set-up, we determined the mass of each larva to the nearest 0.1 mg using a Mettler Toledo® model AB104-S analytical balance (Mettler-Toledo, Inc., Columbus, OH, U.S.A.); mass and instar were determined daily thereafter (excepting 1 day each week).

When larvae reached the fourth instar, their boxes were filled halfway with moistened Pro-Mix® BX Mycorrhizae™ soil (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) for pupation. Once larvae were no longer visible above the soil, they were recorded as having entered pupation, and their leaf clippings were removed. Pupating beetles were not disturbed or weighed until they emerged from the soil as adults. Pupal duration was recorded as the number of days between burrowing and emergence. Pupation normally takes approximately 2 weeks;

individuals that did not emerge after 4 weeks were considered dead. The soil was sifted to check for cadavers, and no live pupae were ever found 4 weeks after pupation began.

Adult size

Upon emerging, adults were sexed, weighed and provided with a new leaf clipping. Adult length and elytra width at the midpoint were measured with a Mitutoyo® model CD-6″C Digitamatic calliper (Mitutoyo Corporation, Kawasaki, Japan) 24 h after emergence, when their exoskeletons had hardened. By treating beetles as a cylinder, we estimated volume as follows: $\text{length} \times (0.5 \times \text{width})^2 \times \pi$. Adults were weighed and had their leaf clipping changed daily.

Fecundity

Adults were kept separately for 5 days, until they reached sexual maturity, and then paired with an individual of the opposite sex from a different natal clutch, irrespective of treatment. Pair boxes were checked daily for eggs, which were collected and counted under a dissecting microscope. Fecundity was thus measured for 2 weeks after the first clutch was laid, a measure highly correlated with lifetime fecundity (M. B. Baker, unpublished data). Twice as many males emerged as females, so excess males not included in pairs were simply weighed for 19 days. Fecundity was calculated as the mean number of eggs laid per reproductive day (including days with zero eggs after the first clutch was laid but excluding the day of mortality, if applicable).

Mortality

In terms of fitness consequences for the individual, all mortality prior to reproduction is equivalent; only survival to reproduction and reproductive longevity impact lifetime reproductive success. For the purposes of this study, mortality was scored simply as whether an individual survived to reproductive maturity (5 days post-adult eclosion). Because of time constraints, we were unable to keep adults beyond the 2-week egg-laying period until actual mortality. Rather than measuring adult longevity, we therefore categorized reproductively mature adults as having a short reproductive period (less than 2 weeks for adults dying prior to this time) or a long reproductive period (at least 2 weeks).

Statistical Analyses

All statistical transformations and tests were done in JMP v.8.0.2 (SAS Institute, Inc., Cary, NC, U.S.A.).

To determine the effect of egg consumption on overall growth, we calculated growth slopes for each individual. Daily masses were logarithmically transformed, $\text{mass}' = \ln(\text{mass (mg)} + 1)$, to correct for increasing variance in mass with increasing age and to make the data a better fit to a straight line; the transformed masses were then regressed on age for each individual. After 10 days the regression plots either stopped as larvae pupated, or levelled off as larvae stopped growing preceding pupation, so all later days were removed from regression calculations. The final regressions of transformed mass on days 1–10 generated a growth slope for each individual; individuals that died as larvae were excluded from this analysis.

Larvae rarely moulted while being observed and occasionally moulted on days that observations were not made, so the age at which an individual reached each moult (to the second through fourth instars, pupa and adult) was estimated for each individual by logarithmically regressing life stage as an ordinal factor on the age at each observation, using maximum likelihood to estimate the slope and intercepts of instar probability curves for each individual. These values were used to calculate the midpoint of each curve as the best estimate of the time to each moult. When individuals were actually observed moulting, the observed time was used in place of the estimate. The time spent in each life stage was also calculated from these age estimates.

All measures were fitted separately to mixed models with egg consumption as a categorical fixed effect, to allow for the possibility of nonadditive effects between treatment levels, and natal clutch as a random effect. Because of unbalanced replication with random effects, all mixed models including clutch were tested using restricted maximum likelihood (REML). Clutch was occasionally estimated as having a negative variance, probably because its effect was very small (SAS Institute), and in these cases it was removed from the model, which was then tested by ANOVA.

If there is an effect of cannibalism on development time, it may be due solely to an increase in growth rate, with larger larvae moulting earlier; alternatively, there may be an effect of cannibalism on development time beyond what can be explained by an increased growth rate. To determine whether there was an effect of cannibalism on development time independent of growth rate, the time to each instar and the time spent in each larval instar were also fitted to full models controlling for the effect of growth slope as well as egg consumption.

Additional models for each measure included sex as an effect to determine whether there were sex differences in the benefits of cannibalism, but these models could only include individuals surviving to adulthood. For emerging mass, we used an additional model that also included body volume to test for effects on density (Moya-Laraño et al. 2008). Body volume was also included in an additional model for maximum adult mass, but length and width, from which body volume was calculated, are measures of structural body condition that do not vary with changing nutrition levels (Moya-Laraño et al. 2008); abdomen depth, which does vary with nutrition levels in this species, was not measured in this study. Body volume in the model for maximum mass thus corrects for structural body condition but should not be interpreted as giving a true estimate of effects on density. An additional model was also fitted for fecundity including maximum mass in addition to egg consumption.

We used nominal logistic regression models based on likelihood regression tests (LRT) to determine whether egg consumption affected binary mortality in the absence of predation and starvation. Pupal survival was fitted to a model including egg consumption and prepupal mass. Survival to reproductive maturity was fitted to a model with egg consumption and growth slope as the predictor effects; in this model, we included slope for all individuals, including those that failed to survive to pupation. A final model included only individuals that survived to reproduction and fitted survival to the end of the experiment (i.e. 2 weeks beyond reproductive maturity) to egg consumption and sex.

Simulation

We developed a simulation from the suggestions of Eickwort (1973) to quantify the net fitness benefit resulting from any decreased development time due to cannibalism, taking into account the inclusive fitness cost of cannibal–victim relatedness. This model adds to Eickwort's equation a discount in accord with the probability of the victim egg hatching without this instance of cannibalism and directly incorporates the base mortality risk of the cannibal. If the mortality risk for a given life stage is known and that mortality risk is assumed to be constant throughout that life stage, the increase in the probability of survival due to reduced development time can be used to calculate the minimum benefit necessary to balance inclusive fitness losses:

$$\frac{t_c}{t_n} \leq \frac{\ln[rP(\text{hatch})+1]}{\ln P(\text{survival})}+1 \quad , \qquad (1)$$

where P(survival) is the probability of an individual surviving a given life stage, r is the coefficient of relatedness between the egg and the cannibal, *P*(hatch) is the probability of the egg hatching in the absence of this instance of cannibalism and takes into account any cause of mortality, including consumption by another cannibal, t_c is the time taken by a cannibal to develop through the life stage, and t_n is the time taken by a noncannibal to develop through the same life stage. This equation plots the relative decrease in development time required to balance inclusive fitness losses due to sibling cannibalism based on the underlying risk of larval mortality. This model is general to any developmental period for any cannibal species. For this study, separate curves were calculated for full- and half-sibling eggs ($r = 0.5$ and 0.25, respectively) using two different probabilities of egg survival if not cannibalized: 57%, the lowest egg survival rate from field data collected by Harcourt (1971), which includes infertility and cannibalism, and 100%, equating cannibal and victim initial fitness and mimicking neonate-on-neonate cannibalism for general comparison. The results of the model were compared to the range of field mortality rates observed by Harcourt (1971) and to the ratio of mean cannibal and noncannibal development times from this experiment, with confidence intervals around this ratio estimated by bootstrapping with 10 000 resamples.

Results

Experimental Results

Growth rate

Egg consumption increased the rate at which larvae gained mass, hereafter referred to as ‘growth rate’ ($P = 0.007$; Table 1, Fig. 1). A Tukey honestly significant difference (HSD) test ($\alpha = 0.05$) distinguished noncannibals (zero eggs eaten) from individuals eating one egg and those eating two eggs; the latter two groups were not significantly different. For those individuals surviving to adulthood, the effect of egg consumption (increased growth) remained ($P = 0.014$; Table 2), but there was no sex difference ($P = 0.16$; Table 2).

Development time

Cannibalism decreased the time taken to reach the second instar when comparing all individuals ($P = 0.0002$; Fig. 2, Table 1) or only those surviving to adulthood ($P = 0.002$; Table 2); there was no sex difference in development time ($P = 0.2$; Table 2). Growth rate was negatively correlated with the time to reach the second instar ($P = 0.003$; Table 3). Even when controlling for the increased growth rates of cannibals, they still reached the second instar more rapidly than noncannibals ($P = 0.002$; Table 3). There was also an interaction between egg consumption and growth rates ($P = 0.0001$; Table 3); the negative correlation between growth rates and development time held for individuals consuming zero or one egg, but individuals consuming two eggs developed quickly regardless of growth rate. This interaction disappeared for those individuals surviving to adulthood ($P > 0.3$), and the effect of egg consumption was only marginal when controlled for sex and growth rate ($P = 0.097$), but results were otherwise similar to those for all individuals (Table 4).

There was no effect of egg consumption on the duration of any life stage after the first instar when comparing all individuals ($P > 0.3$; Table 1), even when controlling for growth rate ($P > 0.14$; Table 3). Growth rate was negatively correlated with the duration of the second and fourth instars ($P < 0.016$; Table 3), but not with the duration of the third instar ($P = 0.11$; Table 3). There was an interaction between growth rate and egg consumption on the time spent in the fourth instar ($P = 0.017$), with a consistently short fourth instar regardless of growth rate for individuals eating zero eggs. When comparing only those individuals surviving to adulthood (Table 2), those eating one egg spent marginally longer in the third instar ($P = 0.052$), while those eating two eggs spent marginally longer in the fourth instar ($P = 0.087$). There were no sex differences in the duration of any larval instar, but males spent marginally longer in pupation ($P = 0.096$). When controlling for sex and growth rate in those individuals surviving to adulthood (Table 4), the increased time spent in the third instar by individuals eating one egg became significant ($P = 0.017$). Individuals eating two eggs still spent marginally longer in the fourth instar ($P = 0.076$), and there was an interaction between sex and growth rate ($P = 0.003$), with little effect of growth rate for females.

Although there was no effect of cannibalism on the duration of any instar other than the first, that initial reduction was sustained throughout development, so that cannibals reached each remaining larval moult earlier than noncannibals ($P < 0.003$; Fig. 2, Table 1), and individuals eating two eggs pupated earlier than those eating zero eggs ($P = 0.018$; Fig. 2, Table 1). Cannibals likewise emerged as adults prior to noncannibals ($P = 0.009$; Fig. 2, Table 1). For individuals surviving to adulthood (Table 2), egg consumption still decreased the time to each life stage ($P < 0.03$). There was no sex difference in development time to any life stage ($P > 0.13$), except that females took marginally longer to reach the fourth instar compared to males ($P = 0.091$). Growth rate was strongly negatively correlated with the time to each life stage ($P < 0.0001$; Table 3). Even when controlling for the increased growth rate due to cannibalism, egg consumption still decreased the time to reach the third and fourth instar ($P < 0.03$), but not the time to pupation or adulthood ($P > 0.1$). There was an interaction between egg consumption and growth rate for the time to the fourth instar ($P = 0.049$), with the strongest negative effect of growth rate for those eating zero eggs, so that development times converged for the different egg-consumption treatments as growth rates increased. There was also an interaction between sex and growth rate on the time to adulthood, with males experiencing a greater negative effect of growth rate on the time to adulthood ($P = 0.045$; Table 4).

Prepupal mass

Egg consumption did not affect final prepupal mass ($P = 0.77$; Table 1). However, females entered pupation with greater mass than did males ($P = 0.013$; Table 2).

Adult volume and mass

There was no effect of egg consumption on emerging body volume ($P = 0.37$; Table 1), but females emerged with a larger volume than males ($P = 0.027$; Table 2). Emerging mass did not vary with egg consumption ($P = 0.77$; Table 1), but females weighed marginally more than males upon emergence as adults ($P = 0.093$; Table 2). When controlling for body volume (Table 5), there was still no effect of egg consumption on emerging mass ($P = 0.60$), and the sex difference disappeared ($P = 0.72$).

Egg consumption also had no effect on maximum adult mass ($P = 0.89$; Table 1), but females achieved a much higher maximum mass than males ($P < 0.0001$; Table 2). Even when controlling for emerging body volume (Table 5), there was no effect of egg consumption on mass ($P = 0.28$), but females remained much heavier than males when controlling for structural size ($P = 0.0004$).

Fecundity

There was no effect of egg consumption on daily fecundity ($P = 0.45$; Table 1). However, only 18 females could be included in this analysis, resulting in low power. The observed difference between females eating no eggs and those eating two eggs was 10.5 more eggs laid daily by those eating no eggs, with a 95% confidence interval from 28.1 more eggs laid by females eating no eggs to seven more eggs laid by those eating two eggs. Maximum mass was positively correlated with fecundity (ANOVA: $F_{1,14} = 16.85$, $P = 0.001$); individuals consuming two eggs produced marginally fewer eggs than expected for their maximum mass ($F_{2,14} = 3.54$, $P = 0.057$).

Mortality

Egg consumption did not affect the probability of surviving pupation (LRT: $\chi^2_2 = 0.86$, $P = 0.65$), but the probability of surviving increased with prepupal mass (LRT: $\chi^2_1 = 5.29$, $P = 0.021$). There was also no effect of egg consumption on the probability of surviving to reproduction (LRT: $\chi^2_2 = 0.27$, $P = 0.88$), but the probability of surviving increased with increasing growth rate (LRT: $\chi^2_1 = 16.85$, $P < 0.0001$). Neither was there an effect of egg consumption on the probability of reproductive adults surviving at least 2 weeks (LRT: $\chi^2_2 = 0.31$, $P = 0.82$); there was no sex difference in adult survival (LRT: $\chi^2_1 = 2.47$, $P = 0.12$), although a 2:1 sex ratio in favour of males suggests that females might have experienced higher larval or pupal mortality, assuming an equal sex ratio at the egg stage.

Simulation

The general model demonstrates the development rates of cannibals relative to noncannibals necessary to compensate for inclusive fitness losses for a given survival rate (Fig. 3). The first instar was the only life stage significantly shortened by cannibalism, so only this stage was considered. The survival rate for first instars observed by Harcourt (1971) ranged from 63.7 to 100%; under the most dangerous conditions (36.3% mortality), a cannibal first-instar duration up to 0.70 times the normal duration could reduce mortality sufficiently to balance inclusive fitness losses of consuming a half-sibling. The ratio of the least square means of pooled cannibals (2.78 days) to noncannibals (3.83 days) was 0.726, with a 95% confidence interval of 0.610 to 0.857. The reduced development time of cannibals could therefore compensate for inclusive fitness losses of consuming a half-sibling under these conditions, although larval survival would have to be below 53% (less than observed field survival rates) for cannibalism to compensate for consuming a full-sibling.

Discussion

This experiment demonstrated multiple direct benefits of egg consumption. Cannibalism of a single egg by a neonate within 24 h of hatching significantly increased larval growth rates and decreased the average time taken to reach the second instar (and all following life stages) by 1 day or more. Increased growth rates resulted in reduced development time, but there was an effect of egg consumption on development time beyond the effect of increased growth rates for all larval instars, although this additional effect faded by the time individuals reached pupation. There was not a greater benefit of cannibalism for females, in contrast to that found in some other species (Michaud & Grant 2004), nor was there an increase in fecundity resulting from cannibalism, as seen in flour beetles (Ho & Dawson 1966).

Interestingly, the duration of larval periods observed in this study closely match those observed in the field by Tamaki & Butt (1978), with the exception that noncannibals in this study took much longer to reach the second instar than observed in field populations. This difference might be an indication that natural cannibalism rates are high enough to drive average development times in the field towards those observed for cannibals in this study. A high level of field cannibalism is also supported by Harcourt's (1971) observation that cannibalism accounted for more total mortality (from egg to adulthood) than interspecific predation in natural populations.

The benefits observed in this study are similar to those found in studies of egg cannibalism in other species. Reduced development time, especially in the time spent in the first instar, is a common result of cannibalism (e.g. Mertz & Robertson 1970; Eickwort 1973; Snyder et al. 2000; Gagné et al. 2002; Michaud & Grant 2004; Omkar & Gupta 2006). Increased mass or growth rate in cannibals has also been frequently observed (e.g. Breden & Wade 1989; Gagné et al. 2002; Michaud 2003; Kudo & Nakahira 2004; Michaud & Grant 2004; Omkar & Gupta 2006; Fincke 2011).

Although reports of benefits of cannibalism are common, attempts to quantify the fitness effects of these benefits are rare. Our model demonstrates that a decrease in the time taken to reach the second instar can increase fitness enough to select for cannibalism of sibling eggs under some field conditions, but the average decrease observed in this experiment, which could reduce larval mortality by up to 13%, was not great enough to compensate for inclusive fitness losses of consuming a full-sibling. However, the model is conservative in that it leaves out all other potential benefits of cannibalism, including the higher growth rate observed in this experiment. The increased rate of growth for cannibals would make them larger than noncannibals of the same age. Larger larvae should be better able to avoid predation than their smaller conspecifics (Matlock 2005). This benefit would be gained at all larval stages and so would have cumulative effects on decreasing mortality risk. Alternatively, reaching larger instars earlier might make larvae more attractive to parasitoids that lay their eggs in larvae and hatch out during host pupation, resulting in mortality, but the largest instar is also most effective at preventing parasitization (López et al. 1997). Larger larvae were also more likely to survive pupation under these laboratory conditions, an effect that could be amplified under harsher field conditions. While cannibalism did not decrease mortality under the conditions of this study, egg consumption has been shown to increase time to mortality in this species under food-limited conditions (K. Collie, unpublished data), which may further increase the fitness of cannibals under field conditions, in which starvation is a major cause of mortality (Harcourt 1971). In ladybird beetles, egg cannibals had reduced mortality compared to noncannibals on low-quality diets (Michaud & Grant 2004) or starvation conditions (Elnagdy et al. 2011).

Increased larval growth rates did not translate into larger or more fecund adults, as slower-growing noncannibals, by the end of their prolonged larval development, reached the same prepupal mass as faster-developing cannibals. However, the development time advantage of cannibalism did carry through all the way into adulthood and even accumulated to a 1.75-day advantage. Reaching reproductive maturity nearly 2 days earlier could allow female cannibals to lay an additional clutch or two over noncannibals. Under the field conditions of Harcourt (1971), a single average clutch of 40 eggs could translate into 3–20 additional adult offspring. Because the current experiment did not follow adults to senescence, it is unknown whether cannibals remain reproductive as long as noncannibals and actually achieve this additional clutch. However, actual early clutches have greater fitness payoffs than potential later clutches that the adults may not live long enough to lay. Furthermore, earlier emergence allows these adults greater access to resources before fields are defoliated; more high-quality food allows females to gain more mass, which itself increases fecundity. Earlier clutches also provide offspring with access to these resources, increasing their survival and allowing more offspring to complete development before the seasonal food source ends entirely, often abruptly by vine-kill. Finally, in populations with more than two generations per year, early reproduction by the first generation of a season may produce more offspring that are themselves able to reproduce before short days induce a diapause switch (de Kort 1990).

Another factor that could raise the net fitness benefits of cannibalism above what the simulation here predicts is the possibility that larvae selectively consume infertile or dead eggs (K. Collie, K. Mejia & A. Fremont, unpublished data). Such a preference would allow neonates to gain all the benefits of egg consumption without incurring any of the costs of consuming kin. Even imperfect discrimination would lower the benefit threshold required to balance inclusive fitness losses on average.

Females may also facilitate such cannibalism by laying a greater proportion of infertile eggs when oviposition plant quality is poor (K. Collie, unpublished data), which may indicate that females, which otherwise do not provide maternal care, actually provide a food source and use cannibalism to increase survival of offspring under poor conditions. Females could also increase hatching asynchrony to provide greater access to eggs. Cannibalism rates do increase with less synchronous hatching in ladybird beetles (Michaud & Grant 2004) and is predicted to evolve as a female strategy to increase offspring fitness when larval survival is low (Perry & Roitberg 2005). However, females facilitation of cannibalism is only favoured when offspring survival is less than 50% and cannibalism doubles the survival rate of cannibals consuming siblings (Perry & Roitberg 2005). Such a benefit is twice as high as that required for the cannibal to gain inclusive fitness by consuming a full-sibling; under normal field mortality rates and for the benefits observed in this experiment, maternal facilitation of cannibalism would not be favoured on average, although females may be able to respond plastically to the particular conditions that their offspring will face, increasing or decreasing hatching synchrony and infertile eggs based on oviposition plant quality.

The observed decrease in development time could compensate for the inclusive fitness losses of consuming a half-sibling, and the additional benefits of cannibalism, increased growth and potentially increased lifetime fecundity, may be sufficient to compensate for the loss of eating a full-sibling and explain the persistence of this behaviour. The accelerated development into adulthood can also reinforce the persistence of cannibalism by decreasing generation time and generating assortative mating, as early-emerging cannibals would reproduce with each other while noncannibals were still reaching sexual maturity. These effects would be dependent on genetic variation in regards to cannibalism propensity, which has not been conclusively demonstrated in this species, although there are family differences in cannibalism rates, and cannibalism may respond to selection (K. Collie, unpublished data); genetic variation in cannibalism has been demonstrated in other species (Polis 1981; Elgar & Crespi 1992).

While the results of this experiment give insight into the fitness benefits obtained by cannibalizing an egg, even a sibling egg, they cannot explain why neonates often cannibalize multiple sibling eggs. There was no observed direct fitness benefit of consuming a second egg, while multiple consumptions additively decreased inclusive fitness. If additional eggs are consumed without providing additional nutritional benefits, it may indicate that the benefits received come in other forms, such as reduced competition for the cannibal and its surviving kin (Dong & Polis 1992; Pfennig 1997). Competition reduction is a significant benefit of cannibalism in cane toad, *Rhinella marina*, tadpoles (Crossland et al. 2011). In *L. decemlineata*, larvae often remain on the natal plant, even if it is completely defoliated (K. Collie, personal observation), and a population can decimate an entire field, so scarcity and starvation are real threats; Harcourt (1971) observed larval starvation alone to result in over 50% mortality in one population. If there is a real possibility of all members of a clutch starving before development is complete, consuming multiple siblings can indeed be selected, as it both decreases the time spent in a nondispersive stage and reduces competition, leaving more resources available to complete development (Mock & Parker 1997). Inclusive fitness may even be gained, as the additional resources similarly benefit all surviving siblings (West et al. 2002).

Females, which could pre-emptively reduce local competition by laying fewer eggs, may instead be favoured to lay larger clutches as insurance against infertility or predation (Mock & Parker 1997). Egg cannibalism can later act to reduce clutch size to a level that can be supported by available resources; if more (or higher-quality) offspring survive after such culling than would if all viable offspring hatched to compete over limited resources, then both maternal and offspring interests can be served by cannibalism (Mock & Parker 1997). Cannibalism occurs before hatchlings leave the clutch, so they cannot assess resource availability, but females, which may be better able to judge how many quality offspring the oviposition plant can support, could adjust hatching asynchrony to allow for an ‘optimal’ level of cannibalism (Perry & Roitberg 2005). However, such female manipulation has not been documented in this species and, for now, remains theoretical speculation.

Ultimately, the benefits found in this experiment are only a piece of the puzzle explaining why egg cannibalism by siblings is so pervasive in this and other species. Further research examining the relationship between larval size and predation and parasitism risks is needed to determine the fitness effect of increased growth rates. Additionally, the effects of competition on survival (and later fecundity) must be more fully understood to explain why multiple sibling eggs are so readily consumed. Much remains to be explored, but the magnitude of the effects, seen and potential, from just a single egg meal begins to shed light on the observed high rates of sibling cannibalism.

Table 1. Effect of egg consumption on growth rate, development time, prepupal mass, adult size and fecundity in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	Egg no.	LS means ±SE	R^2	Egg effect F (*df*, *df*)	 P	Clutch effect
Growth rate*	0	0.41±0.02^a	0.34	5.35 (2, 57.0)	0.007	25.3
	1	0.48±0.02^b				
	2	0.48±0.02^b				
Age at 2nd instar (days)	0	3.8± 0.3^a	0.413	9.60 (2, 71.59)	0.0002	22.00
	1	2.9± 0.3^b				
	2	2.7± 0.3^b				
Age at 3rd instar (days)	0	6.5± 0.3^a	0.307	8.69 (2, 68.19)	0.0004	12.51
	1	5.2± 0.3^b				
	2	5.3± 0.3^b				
Age at 4th instar (days)	0	9.3± 0.3^a	0.248	6.22 (2, 66.31)	0.0033	9.31
	1	8.2± 0.3^b				
	2	8.0± 0.4^b				
Age at pupation (days)	0	12.7±0.5^a	0.367	4.30 (2, 57.07)	0.0183	30.28
	1	11.7±0.5^{ab}				
	2	11.4±0.5^b				
Age at adulthood (days)	0	25.6±0.5^a	0.176	5.21 (2, 49)	0.009	
	1	23.8±0.4^b				
	2	23.9±0.5^b				
Duration 2nd instar (days)			0.121	0.66 (2, 69.53)	0.52	7.29
Duration 3rd instar (days)			0.155	1.22 (2, 66.56)	0.30	9.84
Duration 4th instar (days)			0.380	0.52 (2, 57.19)	0.60	37.2
Duration pupation (days)			0.026	0.66 (2, 49)	0.52	
Pre-pupal mass (mg)			0.008	0.27 (2, 63)	0.52	
Body volume (mm^3)			0.040	1.01 (2, 48)	0.37	
Emerging mass (mg)			0.191	0.27 (2, 45.8)	0.77	14.75
Maximum adult mass (mg)			0.005	0.12 (2, 49)	0.89	
Fecundity (eggs/day)			0.100	0.85 (2, 15)	0.45	

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA. For all tests with a significant egg consumption effect, least square (LS) means ± SE are given for each egg consumption treatment; different superscripts were determined to be significantly different by a Tukey HSD test ($\alpha = 0.05$).

*Growth rate is the slope of the regression of transformed mass, mass' = ln(mass + 1), on age.

Table 2. Effect of egg consumption and sex on growth rate, development time, prepupal mass and adult size in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	R^2	**Egg effect**		**Sex effect**		**Sex**	**LS means ±SE**	**Clutch effect**
		***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***			
Growth rate*	0.35	4.72 (2, 43.5)	0.014	2.02 (1, 45.0)	0.16			19.8
Age at 2nd instar (days)	0.39	7.43 (2, 45.8)	0.002	1.68 (1, 46.6)	0.20			11.7
Age at 3rd instar (days)	0.28	8.09 (2, 48)	0.0009	2.35 (1, 48)	0.13			
Age at 4th instar (days)	0.29	5.70 (2, 46.6)	0.006	2.98 (1, 47.1)	0.091	F	9.0±0.4	3.6
						M	8.2±0.3	
Age at pupation (days)	0.35	3.84 (2, 44.1)	0.029	1.60 (1, 45.4)	0.21			20.8
Age at adulthood (days)	0.18	5.08 (2, 46)	0.010	0.04 (1, 46)	0.85			
Duration 2nd instar (days)	0.13	0.82 (2, 46.2)	0.45	0.30 (1, 46.9)	0.59			7.3
Duration 3rd instar (days)	0.28	3.17 (2, 44.2)	0.052	0.03 (1, 45.6)	0.86			16.4
Duration 4th instar (days)	0.49	2.59 (2, 42.1)	0.087	0.62 (1, 43.2)	0.43			47.4
Duration pupation (days)	0.08	0.76 (2, 46)	0.47	2.89 (1, 46)	0.096	F	12.3±0.2	
						M	12.8±0.2	
Pre-pupal mass (mg)	0.13	0.25 (2, 48)	0.78	6.66 (1, 48)	0.013	F	172.0±5.7	
						M	153.6±4.3	
Body volume (mm^3)	0.14	1.12 (2, 47)	0.33	5.22 (1, 47)	0.027	F	246.7±7.8	
						M	224.1±6.0	
Emerging mass (mg)	0.26	0.31 (2, 44.3)	0.73	2.95 (1, 45.6)	0.093	F	114.1±3.8	17.9
						M	107.3±3.4	
Maximum mass (mg)	0.30	0.09 (2, 48)	0.92	20.38 (1, 48)	<0.0001	F	171.2±6.2	
						M	136.4±4.7	

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA. For all tests with at least a marginally significant sex effect, least square (LS) means ± SE are given for each sex.

*Growth rate is the slope of the regression of transformed mass, mass’ = ln(mass + 1), on age.

Table 3. Effect of egg consumption and growth rate on development time in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	R^2	*F* (*df*, *df*)	*P*	Growth rate on development time regression			Clutch effect
				Egg no.	Intercept	Slope	
Age at 2nd instar (days)	0.60						29.0
Egg effect		6.67 (2, 67.6)	0.002	0	6.89	-7.54	
Slope effect		9.32 (1, 71.5)	0.003	1	4.89	-4.37	
Egg*growth rate effect		10.10 (2, 68.0)	0.0001	2	1.89	1.95	
Age at 3rd instar (days)	0.83						
Egg effect		3.84 (2, 67.0)	0.026				
Growth rate effect		201.6 (1, 69.6)	<0.0001				
Age at 4th instar (days)	0.92						26.9
Egg effect		3.80 (2, 60.8)	0.028	0	16.94	-15.56	
Growth rate effect		208.4 (1, 66.3)	<0.0001	1	14.77	-13.75	
Egg*growth rate effect		3.16 (2, 61.4)	0.049	2	14.81	-14.54	
Age at pupation (days)	0.88						18.0
Egg effect		2.31 (2, 56.9)	0.11	0	20.75	-19.35	
Growth rate effect		111.5 (1, 58.2)	<0.0001	1	24.00	-25.79	
Egg*growth rate effect		2.64 (2, 57.8)	0.08	2	18.60	-15.10	
Age at adulthood (days)	0.73						
Egg effect		1.15 (2, 48)	0.32				
Growth rate effect		97.45 (1, 48)	<0.0001				
Duration 2nd instar (days)	0.37						11.8
Egg effect		0.11 (2, 68.7)	0.89				
Growth rate effect		23.75 (1, 70.4)	<0.0001				
Duration 3rd instar (days)	0.21						12.9
Egg effect		2.06 (2, 66.1)	0.14				
Growth rate effect		2.68 (1, 68.4)	0.11				
Duration 4th instar (days)	0.47						34.1
Egg effect		1.06 (2, 55.3)	0.35	0	3.40	0.00	
Growth rate effect		6.09 (1, 59.9)	0.016	1	8.48	-10.51	
Egg*growth rate effect		4.39 (2, 55.8)	0.017	2	5.67	-4.25	

Table 3 cont.

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA. For all tests with at least a marginally significant egg consumption by growth rate interaction, the intercept and slope are given for the regression of development time on growth rate for each egg consumption treatment.

Table 4. Effect of egg consumption, sex and growth rate on development time in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	R^2	*F* (*df*, *df*)	*P*	Growth rate on development time regression			Clutch
				Egg no./sex	**Intercept**	**Slope**	**effect**
Age at 2nd instar (days)	0.64						22.0
Egg effect		2.47 (2, 43.9)	0.097				
Sex effect		0.27 (1, 45.6)	0.60				
Growth rate effect		23.70 (1, 47.0)	<0.0001				
Age at 3rd instar (days)	0.86						1.3
Egg effect		3.19 (2, 40.0)	0.052				
Sex effect		0.89 (1, 45.7)	0.35				
Growth rate effect		136.3 (1, 41.4)	<0.0001				
Age at 4th instar (days)	0.96						34.2
Egg effect		5.42 (2, 39.8)	0.008	0	16.70	-17.48	
Sex effect		1.88 (1, 40.7)	0.18	1	11.08	-6.00	
Growth rate effect		66.0 (1, 41.1)	<0.0001	2	15.91	-16.85	
Egg*growth rate effect		3.77 (2, 39.3)	0.032	F	15.36	-15.00	
Sex*growth rate effect		3.55 (1, 42.0)	0.067	M	13.93	-11.89	
Age at pupation (days)	0.92						20.5
Egg effect		0.30 (2, 42.8)	0.74				
Sex effect		0.04 (1, 45.2)	0.85				
Growth rate effect		279.5 (1, 47.0)	<0.0001				
Age at adulthood (days)	0.76						
Egg effect		0.83 (2, 46)	0.44	F	32.28	-17.59	
Sex effect		2.17 (1, 46)	0.15	M	36.58	-25.95	
Growth rate effect		98.1 (1, 46)	<0.0001				
Sex*growth rate effect		4.24 (1, 46)	0.045				

Table 4 cont.

	R^2	*F* (*df*, *df*)	*P*	Growth rate on development time regression			Clutch effect
				Egg no./sex	Intercept	Slope	
Duration 2nd instar (days)	0.46						
Egg effect		0.74 (2, 44)	0.0002	0	5.40	-6.37	
Sex effect		0.03 (1, 44)	0.87	1	10.96	-17.45	
Growth rate effect		3.05 (1, 44)	0.088	2	-0.11	5.66	
Egg*growth rate effect		3.14 (2, 44)	0.053	F	6.83	-9.09	
Sex*growth rate effect		3.21 (1, 44)	0.080	M	4.00	-3.02	
Duration 3rd instar (days)	0.34						18.9
Egg effect		4.45 (2, 43.7)	0.017				
Sex effect		0.02 (1, 45.6)	0.89				
Growth rate effect		2.72 (1, 47.0)	0.11				
Duration 4th instar (days)	0.61						53.0
Egg effect		2.75 (2, 40.3)	0.076				
Sex effect		0.85 (1, 41.8)	0.36				
Growth rate effect		3.88 (1, 41.9)	0.056	F	2.78	1.34	
Sex*growth rate effect		10.32 (1, 42.1)	0.003	M	6.59	-6.58	

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA. For all tests with at least a marginally significant interaction of egg consumption or sex with growth rate, the intercept and slope are given for the regression of development time on growth rate for each egg consumption treatment or sex.

Table 5. Effect of egg consumption, sex and emerging body volume on adult mass in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	R^2	***F* (*df*, *df*)**	***P***	**Sex**	**LS means ±SE**	**Clutch effect**
Emerging mass (mg)	0.59					17.9
Egg Effect		0.52 (2, 42.5)	0.60	F	110.3±3.0	
Sex Effect		0.13 (1, 44.1)	0.72	M	109.1±2.8	
Body Volume Effect		32.62 (1, 43.9)	<0.0001			
Maximum adult mass (mg)	0.61					
Egg Effect		1.31 (2, 46)	0.28	F	164.1±4.9	
Sex Effect		14.30 (1, 46)	0.0004	M	140.4±3.7	
Body Volume Effect		34.64 (1, 46)	<0.0001			

The test for emerging mass incorporated clutch as a random effect and was analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. The clutch effect could not be estimated for maximum adult mass, which was analysed by ANOVA. Least square (LS) means ± SE are given for each sex.

Figure 1. Effect of egg consumption on growth in Colorado potato beetles, *Leptinotarsa decemlineata*. Solid lines represent the reverse-transformed average growth curves for each number of eggs consumed; dashed lines represent their 95% confidence intervals. Curves for one egg and two eggs are almost completely overlapping, obscuring the curve for one egg. Growth slopes were calculated by regressing log-transformed mass on age for each individual.

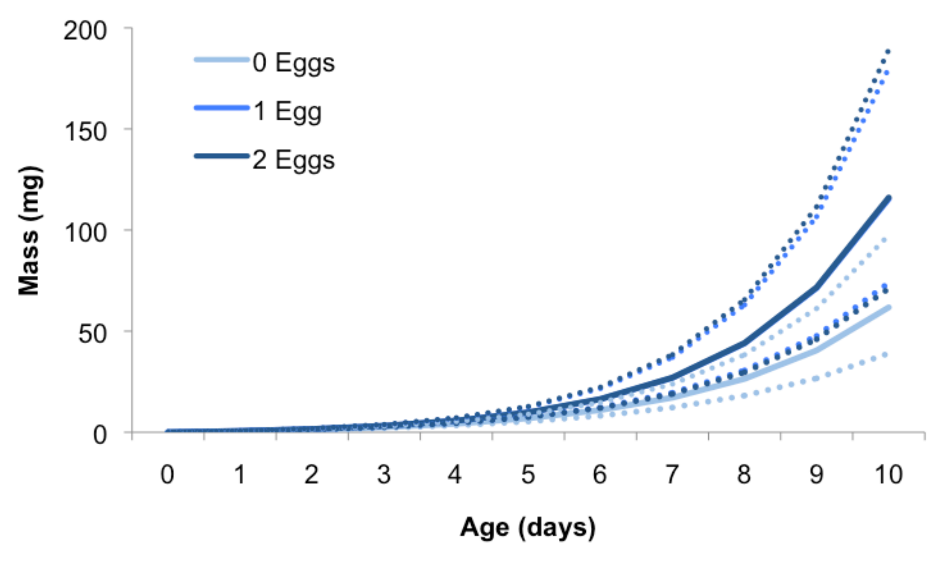


Figure 2. Effect of egg consumption on development time in Colorado potato beetles, *Leptinotarsa decemlineata*. Horizontal bars represent the duration of each developmental period. Error bars are ± SE for each developmental period.

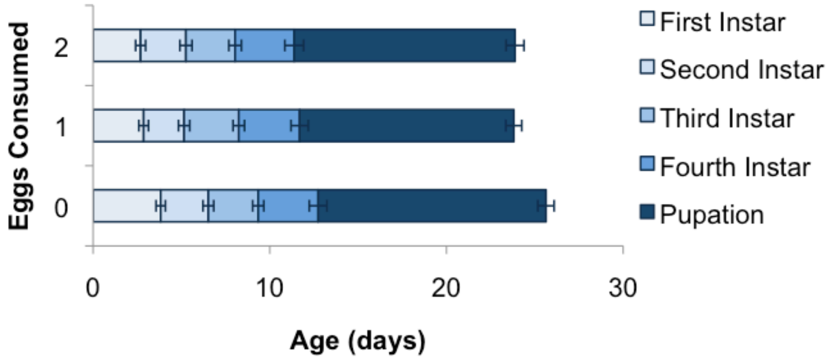
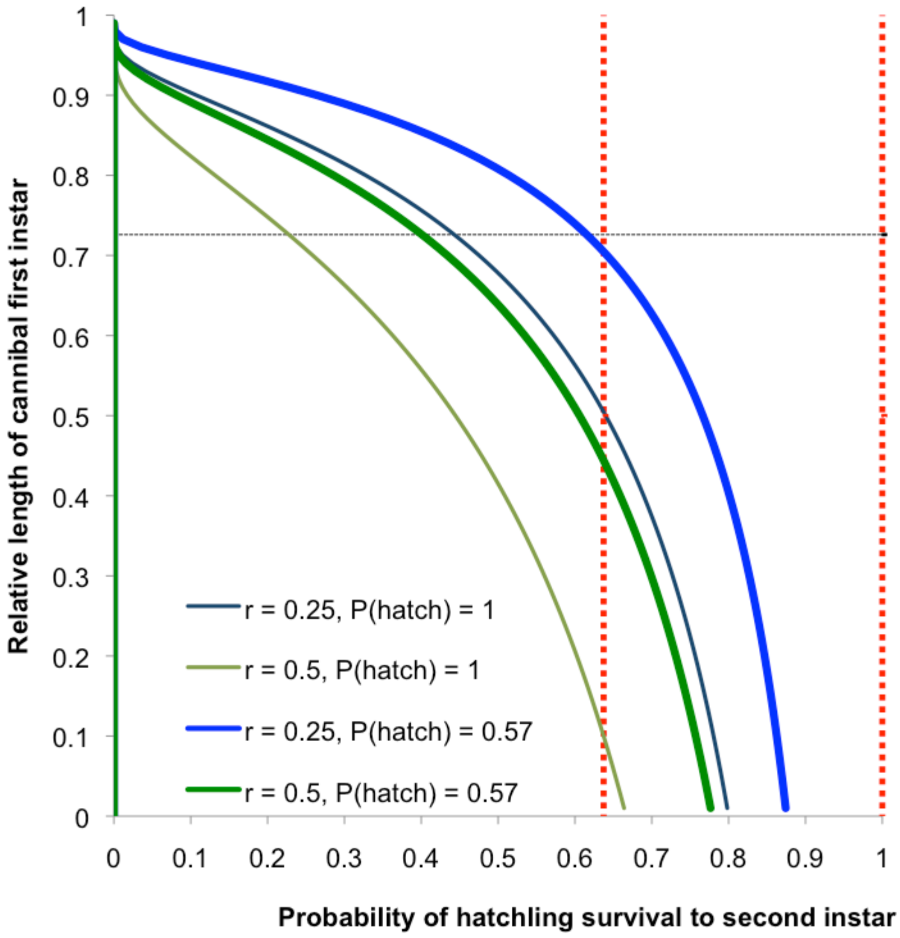


Figure 3. Model of survival benefits of cannibalism in Colorado potato beetles, *Leptinotarsa decemlineata*, due to reduced development time. Curves are fitted to equation (1) (see text). The *Y* axis represents t_c/t_n, the ratio of the duration of the first instar for cannibals and noncannibals. Curves represent the minimum development-time reduction required for egg cannibalism to outweigh the inclusive fitness loss of consuming a sibling, for a given survival rate. Separate curves are fitted for cannibalism of half siblings ($r = 0.25$) and full siblings ($r = 0.5$), for *P*(hatch) = 1, simulating neonate-on-neonate cannibalism, and for *P*(hatch) = 0.57, the lowest hatch rate observed in the field (Harcourt 1971). The vertical lines bracket the range of observed survival rates of first instars in the field (Harcourt, 1971). The horizontal line is the ratio from this experiment of the least square mean first-instar durations of pooled cannibals to noncannibals; while this line does not intersect any curve within the range of observed field survival rates, its 95% bootstrap confidence interval includes the curve for eating a half-sibling with *P*(hatch) = 0.57.



CHAPTER 2: Sibling egg cannibalism reduces costs of resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae)

Pesticide resistance often results in fitness costs in the absence of pesticide exposure, reducing the relative fitness of resistance alleles in pesticide-free refuges and slowing resistance evolution when such refuges are present. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) typically develops resistance to pesticides very quickly, so costs of resistance can be important in resistance management. I measured larval mass and development stage daily, as well as pre-pupal mass, adult size, emerging and maximum mass, fecundity, and mortality for individuals from populations that were either susceptible or resistant to the pesticide imidacloprid to test for costs of resistance. Egg cannibalism by resistant neonates can reduce development time, so I tested whether these benefits are also seen in susceptible larvae or if there is an interaction between the benefits of cannibalism and pesticide resistance. Imidacloprid-susceptible larvae gained mass more quickly, reached some larval molts and adulthood earlier, emerged as slightly larger adults, and became heavier females compared to resistant beetles. Cannibalism increased growth rates and reduced development time for individuals from both populations, but the benefits were greater for the resistant population, reducing or eliminating many of the costs of resistance. By reducing these costs for resistant larvae, prevalent cannibalism may reduce the effectiveness of refuges and should be incorporated into models of resistance evolution.

Keywords: cannibalism, Colorado potato beetle, costs of resistance, *Leptinotarsa decemlineata*, resistance evolution

Melander (1914) was the first to demonstrate that insects could develop a resistance to pesticides that could then be passed on to their offspring. Pesticide resistance in pest species is now a major problem—of the more than $10 billion spent annually on pesticides in the United States, $1.5 billion is estimated to be due to higher application rates required by increased resistance, and an estimated 10 percent of crops are still lost to resistant pests annually, resulting in an additional $1.5 billion in yield loss (Pimentel 2005).

Refuge strategies are often used to slow the rate of evolution of pesticide resistance by providing untreated safe havens for susceptible individuals, which can mate with nearby resistant populations, maintaining more susceptible alleles in the population (Caprio 1998, Gould 1998). Such strategies are most effective if susceptible individuals have higher fitness in the absence of pesticide exposure than resistant individuals; high costs of resistance in refuges can slow or even reverse the evolution of pesticide resistance (Carrière and Tabashnik 2001). Indeed, refuges coupled with high costs of resistance have been credited with preventing the development of resistance to transgenic crops expressing toxins from the bacterium *Bacillus thuringiensis* for at least seven years (Tabashnik et al. 2003).

These costs may result because resources are directed toward maintaining resistance and are, therefore, diverted from other fitness-enhancing traits (Carrière et al. 1994) or because they directly affect behavior (Chevillon et al. 1997). For example, obliquebanded leafrollers, *Choristoneura rosaceana*, from pesticide-resistant populations have reduced larval and pupal mass and longer development time to pupation than individuals from susceptible populations, and these costs increase with increasing resistance levels (Carrière et al. 1994). Similar costs of resistance, as well as smaller forewing size in resistant adults, are observed in the codling moth, *Cydia pomonella* (Konopka et al. 2012). In pink bollworms, *Perctinophora gossypiella*, individuals from resistant strains do not have reduced pupal mass or lengthened development time, but they do have reduced survival on untreated plants compared to individuals from susceptible strains, though the cause of this lower survival is unknown (Carrière et al. 2001). One resistance gene in mosquitoes, *Culex pipiens*, incurs a consistent overwintering cost, while another resistance gene with a different mode of action seems to incur an overwintering cost that is environment specific (Chevillon et al. 1997).

However, costs of resistance are expected to decline over time as modifiers or less costly alleles arise (Raymond et al. 2001). Indeed, in the case of *C. pipiens*, a modifier has arisen at the first locus that reduces its cost (Raymond et al. 2001), and a more recent resistant allele at the second locus does not seem to incur an overwintering cost at all (Chevillon et al. 1997). In some cases, resistance seems to evolve without any costs from the beginning (e.g. Lopes et al. 2008).

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is an especially problematic pest species because of its history of rapidly evolving resistance to all known classes of pesticide (reviewed by Alyokhin et al. 2008). This invasive species shifted from wild hosts such as buffalo bur, *Solanum rostratum*, to domestic potato, *S. tuberosum*, along with other solanaceous crops, allowing it to spread from its native range in central Mexico across North America and Europe (Casagrande 1987) and into Asia (Liu et al. 2012). One of the most widely used pesticides against CPB is imidacloprid, a neonicotinoid, but resistance to this pesticide has been established for over a decade (Zhao et al. 2000) and has also resulted in cross-resistance to other neonicotinoids (Alyokhin et al. 2007). In this species, imidacloprid resistance does come with various costs, including reduced sprint speed, reduced fecundity, lower hatch rates (although this cost may have been lost), and delayed time to egg hatching (Baker et al. 2007). Resistance also results in reduced overwintering survival (Baker and Porter 2008).

It is unknown whether imidacloprid resistance also results in reduced mass or delayed larval development in this species. I have shown cannibalism to significantly increase growth rates and decrease larval development time (Collie et al. 2013). Thus, even if pesticide resistance does result in reduced mass or delayed larval development, cannibalism may serve as a compensatory mechanism to overcome these developmental costs of resistance.

In this study, an extension of the study on a resistant population (Collie et al. 2013), I provided newly hatched neonates from resistant and susceptible populations either eggs and foliage or foliage only. Larval growth and development, adult size and fecundity, and mortality were observed to determine whether there were development costs associated with pesticide resistance and whether cannibalism increased growth and development rates equally for both populations or if there was an interaction between cannibalism and resistance. I predicted that, if resistance does incur development costs, cannibalism should be more beneficial (i.e., speed development more) for resistant than susceptible larvae.

Materials and Methods

This experiment included two laboratory colonies—one derived from an imidacloprid-resistant population in potato fields in Riverhead, NY, and one derived from an imidacloprid-susceptible population housed in a United States Department of Agriculture greenhouse-reared population in Maryland; this population was originally collected from fields in Beltsville, MD. The experiment began on 3 June 2009 and ended on 19 August 2009.

Complete methods for creating cannibalistic and non-cannibalistic treatments are described by Collie et al. (2013) (see Chpt. 1). Clutches were collected daily from the separate lab colonies. Upon hatching, neonates were either placed directly on potato foliage or on one or two eggs on potato foliage; while neonates may consume up to five eggs in a day, a maximum of two eggs were used in this experiment to ensure that most neonates would consume all provided eggs. In total, 86 neonates from 10 clutches (2-29 individuals per clutch) were used from the NY population; 145 neonates from 12 clutches (3-38 individuals per clutch) were used from the MD population.

Larvae provisioned with eggs were checked after 24 hours to verify consumption of the egg(s); only 13 of the 161 individuals given eggs failed to eat all provided eggs (7 of 60 for NY and 6 of 101 for MD). These individuals were analyzed according to the number of eggs actually eaten. Egg remains were removed from the cages, and the remainder of the experiment was blind to the initial provisioning.

Larvae were weighed and their developmental stage (first through fourth larval stadia, pupa, or adult) recorded daily (excepting one day each week). When larvae reached the fourth stadium, their boxes were filled halfway with moistened soil for pupation. The larvae were recorded as having entered pupation when they were no longer visible above the soil. While larvae may not immediately molt to pupae upon entering the soil (personal observation), exact time of pupation cannot be known without disturbing them, so burrowing was used as a standard estimate of pupation. Pupae were not weighed while they were in the soil. Duration of pupation was recorded as the number of days between burrowing and emergence. Individuals that did not emerge after four weeks were considered dead.

Adults were sexed and weighed when they emerged from the soil. Adult length and width were measured 24 hours later, after their exoskeletons had completed hardening. Adults were kept in individuals cages for five days, until they reached sexual maturity, and then paired with an individual of the opposite sex from the same population but a different natal clutch. Eggs were collected from each pair and counted daily for two weeks after the first clutch was laid. Nearly twice as many males emerged as females; excess males were not included in pairs but were weighed for 19 days.

Mortality was scored as a binary trait—survival to or mortality prior to reproductive maturity (five days post-adult eclosion). Adults that reached maturity, were also recorded as having either a short reproductive period (less than two weeks; the adult died during the experiment) or a long reproductive period (at least two weeks; the adult survived to the end of the experiment).

All statistical tests were performed in JMP® 8.0.2 (SAS Institute, Inc., Cary, NC). Transformations are described by Collie et al. (2013).

Collie et al. (2013) found no differences between NY neonates consuming one egg or two eggs, so both groups were pooled into a single cannibal category. All measures were fit separately to mixed multiple regression models with egg consumption (eating eggs or not), resistance, and their interaction as fixed effects, and natal clutch as a random effect. All nonsignificant interactions were removed from models. Due to unbalanced replication of random effects, all mixed models including clutch were tested by restricted maximum likelihood (REML). When clutch was estimated as having a negative variance because its effect was very small (SAS Institute Inc. 2009), it was removed from the model, which was then tested by ANOVA. For those individuals surviving to adulthood, additional models for each measure included sex and its interactions with egg consumption and resistance, to determine whether there were sex differences in the impacts of cannibalism. For emerging mass and maximum mass, additional models included body volume. Including initial adult volume in the model for emerging mass tests for effects on density (Moya-Laraño et al. 2008); volume, as calculated from length and width, is a measure of structural body condition (SBC) that does not change with adult nutrition levels (Moya-Laraño et al. 2008), it serves as a correction for SBC but should not be interpreted as giving a true estimate of effects on density with maximum mass. For fecundity, an additional model included maximum mass, in addition to egg consumption and resistance.

Nominal logistic regression models based on *G* tests were used to determine whether egg consumption or resistance affected binary mortality in the absence of predation and starvation. Survival to reproductive maturity was fit to a model with cannibalism and resistance as the predictor effects. A second model included only individuals that survived to reproduction and fit survival to the end of the experiment (i.e., two weeks beyond reproductive maturity) to egg consumption, resistance, and sex.

Results

Statistical results of the effect of cannibalism and resistance on growth rate, development time, pre-pupal mass, and daily fecundity are given in Table 1. Growth rates for all resistant and susceptible cannibals and noncannibals surviving to pupation are shown in Figure 1. Susceptible larvae gained mass marginally faster than resistant larvae ($P = 0.082$). There was a main effect of cannibalism, with cannibals gaining mass faster than noncannibals ($P = 0.030$). There was a marginally significant interaction between egg consumption and resistance on the growth rate ($P = 0.075$), such that cannibalism had an effect for resistant larvae but not susceptible larvae. There was no effect of cannibalism on the final larval mass prior to pupation ($P = 0.67$), nor was there a difference between susceptible and resistance larvae ($P = 0.20$).

Development times in and to each molt for all resistant and susceptible cannibals and noncannibals surviving each life stage are shown in Figure 2. Cannibals reached the first molt earlier than noncannibals ($P < 0.0001$). There was no effect of resistance on the time taken to reach the first molt ($P = 0.13$), but there was an interaction between cannibalism and resistance ($P = 0.009$), with a greater effect of cannibalism for resistant than susceptible larvae. Cannibals also spent less time in the second stadium ($P = 0.027$). Although they did not spend less time in the other larval stadia ($P > 0.15$), they did reach all other larval molts earlier ($P < 0.004$). There was no effect of resistance on the time spent in any single stadium ($P > 0.37$), but susceptible individuals took marginally less time to reach the second molt ($P = 0.084$) and significantly less time to reach the third molt ($P = 0.049$). However, there was no resistance effect on the time to pupation ($P = 0.17$).

All analyses testing for sex differences, including only individuals that survived to adulthood, were complicated because all female susceptible noncannibals failed to reach adulthood, so no sex by resistance by cannibalism interactions could be tested. Statistical results of these analyses are expounded by comparing available subgroups (e.g. male resistant cannibals) within each comparison. Statistical results of the effect of sex, cannibalism, and resistance and their interactions on growth rate, development time, pre-pupal, emerging, and maximum mass, and body volume are given in Table 2.

Growth rates for male and female cannibals and noncannibals are shown in Figure 3. Female resistant noncannibals (FRN) gained mass more slowly than female susceptible (FSC) and male resistant cannibals (MRC). Overall, there was still a strong effect of cannibalism ($P = 0.0004$), but resistance was no longer a significant effect ($P = 0.25$). Males gained mass marginally faster than females ($P = 0.082$), and there was an interaction between sex and cannibalism ($P = 0.048$), with cannibalism increasing the growth rate of females but not of males. There were no differences in pre-pupal mass between any subgroup, but overall, females entered pupation with more mass than males (LS mean ± SEM, 173.8 ± 6.0 mg for females, 158.8 ± 5.5 mg for males; $P = 0.026$). There was no effect of cannibalism ($P = 0.79$) or resistance ($P = 0.53$).

Development times for all subgroups are shown in Figure 4. MRC reached the first molt faster than all resistant noncannibals; MRN took longer than all female cannibals. There was no difference between any subgroups in the time spent in the second stadium. FRN took longer to reach the second molt than all cannibals; susceptible cannibals of both sexes also reached the second molt faster than MRN. There were no differences between the subgroups for the time spent in any other stadia, but FRN took longer to reach the third molt than all other subgroups except MRN. FRN also took longer to reach pupation than MRC and FSC.

Overall, there was no sex difference ($P = 0.12$) or resistance effect ($P = 0.26$), but cannibals reached the first molt earlier than noncannibals ($P < 0.0001$). Cannibals spent less time in the second stadium ($P = 0.008$) and also spent less time in the third stadium ($P = 0.045$). Cannibals reached all molts earlier than noncannibals ($P < 0.0002$). Susceptible larvae reached the second molt marginally earlier than resistant larvae (LS mean ± SEM, 5.3 ± 0.2 vs. 5.8 ± 0.2 days, respectively; $P = 0.054$), but there were no other effects of resistance on the time to any molt or in any stadium ($P > 0.13$). Although there was no sex difference in the time spent in any stadium ($P > 0.2$), females took marginally longer to reach the second molt (LS mean ± SEM, 5.8 ± 0.2 days for females vs. 5.3 ± 0.2 days for males; $P = 0.077$) and significantly longer to reach the third molt ($P = 0.004$), but there was no sex difference in the time to pupation ($P = 0.36$). There was an interaction between sex and cannibalism on the time to reach the third molt ($P = 0.032$), with an effect of cannibalism for females but not for males. There was a marginal interaction between resistance and cannibalism for the time spent in the fourth stadium (*P* = 0.058), with cannibalism reducing development time for susceptible larvae.

There were no differences between groups in pupal duration, but MSC emerged as adults earlier than all resistant noncannibals. Overall, there was no effect of cannibalism on the time spent in pupation (*P* = 0.24), nor was there a resistance effect (*P* = 0.56), but females spent marginally longer in pupation than males (*P* = 0.070). There was also an interaction between resistance and sex (*P* = 0.002), with a sex difference for susceptible but not resistant pupae. Cannibals emerged earlier as adults than noncannibals (*P* = 0.002) and susceptible adults emerged earlier than resistant adults (*P* = 0.007), but there was no sex difference in emergence time (*P* = 0.18).

FSC had a larger body volume than resistant males. Overall, there was no effect of cannibalism on the body volume of emerging adults (*P* = 0.16), but susceptible adults were marginally larger than resistant adults (LS mean ± SEM, 263.8 ± 9.1 mm^3 for susceptible, 234.7 ± 6.8 mm^3 for resistant; *P* = 0.067). Females were larger than males (LS mean ± SEM, 262.3 ± 7.7 mm^3 for females, 236.2 ± 7.0 mm^3 for males; *P* = 0.007).

There were no subgroup differences in emerging mass. Overall, there was no effect of cannibalism on emerging mass (*P* = 0.72), nor was there an effect of resistance (*P* = 0.10), but females were heavier at emergence than males (LS mean ± SEM, 121.4 ± 3.9 mg for females, 111.9 ± 3.7 mg for males; *P* = 0.022). Emerging mass was positively correlated with body volume (Table 3; *P* < 0.0001). When controlling for volume to determine the effects on density, there were no differences between subgroups. Overall, there was no effect of cannibalism (*P* = 0.13), nor was there an effect of resistance (*P* = 0.16), or a sex difference (*P* = 0.97). There were no interactions between sex and either cannibalism or body volume (*P* > 0.34), but there was an interaction between cannibalism and body volume (*P* = 0.041), with emerging mass increasing more with body volume for cannibals than for noncannibals. However, this interaction was sex-dependent (*P* = 0.038), present in females but not in males.

FRC reached a greater maximum mass than MRC, FRN were larger than resistant males, and FSC were larger than all male groups. Overall, there was no effect of cannibalism on the maximum adult mass (*P* = 0.74), nor was there an effect of resistance (*P* = 0.40), but females reached a greater mass than males (LS mean ± SEM, 183.6 ± 6.5 mg for females, 133.4 ± 6.1 mg for males; $P < 0.0001$). There was also a marginal interaction between resistance and sex (*P* = 0.062), such that susceptible females were heavier than resistant females (LS mean ± SEM, 194.8 ± 10.0 and 172.4 ± 7.9 mg, respectively), but there was no such difference in male mass (LS mean ± SEM, 131.1 ± 10.0 mg for susceptible, 135.7 ± 7.1 for resistant).

Maximum adult mass was positively correlated with body volume at emergence (Table 3; $P < 0.0001$). When controlling for this structural body condition, FRN reached a larger maximum mass than male noncannibals and MRC; FSC reached a greater mass than all male groups. Overall, there was still no effect of cannibalism (*P* = 0.34), but there was a marginal interaction between cannibalism and initial body volume (*P* = 0.090), with cannibal maximum mass increasing more with body volume than noncannibal mass. There was no effect of resistance (*P* = 0.54), but females were still heavier than expected compared to males based on their initial adult volume (LS mean ± SEM, 176.9 ± 3.9 mg for females, 143.1 ± 3.9 mg for males; $P < 0.0001$). The marginal interaction between sex and resistance also remained (LS mean ± SEM, 183.1 ± 6.2 mg for susceptible females and 170.8 ± 4.5 mg for resistant females, compared to 140.9 ± 6.4 and 145.4 ± 4.4 mg for susceptible and resistant males, respectively; *P* = 0.090).

There were no differences between subgroups in daily fecundity. Overall, there was no effect of cannibalism on fecundity (Table 1; $P = 0.74$), nor was there an effect of resistance ($P = 0.44$). Maximum mass was positively correlated with daily fecundity (REML: $F = 10.92$; df = 1, 24.3; $P = 0.003$). When taking maximum mass into account, there were still no subgroup differences, nor was there an effect of cannibalism (REML: $F = 0.02$; df = 1, 15.7; $P = 0.90$) or of resistance (REML: $F = 0.12$; df = 1, 12.1; $P = 0.74$). There was a marginal interaction between resistance and maximum mass (REML: $F = 2.96$; df = 1, 24.3; $P = 0.098$), with daily fecundity increasing more with maximum mass for resistant than susceptible females.

There was no effect of cannibalism on the likelihood of surviving to reproduction (*G* test: $G = 0.02$; df = 1; $P = 0.90$), but resistant individuals were more likely to survive (*G* test: $G = 27.51$; df = 1; $P < 0.0001$). For susceptible individuals, there was an interaction between cannibalism and sex on survival to adulthood ($\chi^2 = 9.48$; df = 1; $P = 0.002$)—no female susceptible noncannibals reached adulthood (although 11 male susceptible noncannibals did), but 13 female susceptible cannibals were able to reach adulthood (compared to 11 male susceptible cannibals). There was no effect of cannibalism on the likelihood of a reproductive adult surviving to have a long reproductive period (*G* test: $G = 0.19$; df = 1; $P = 0.67$), nor was there an effect of resistance (*G* test: $G = 0.72$; df = 1; $P = 0.40$), but females were more likely to die before the end of the experiment (*G* test: $G = 4.50$; df = 1; $P = 0.034$).

Discussion

I found multiple costs of resistance that have not previously been described in the Colorado potato beetle. Susceptible larvae gained mass more quickly than resistant larvae and took less time to reach the second and third molts. Susceptible adults emerged earlier, with slightly greater volume, than resistant adults. Susceptible females also reached a higher maximum mass than resistant females. I did not find an effect of resistance on fecundity, in contrast to the fecundity cost found by Baker et al. (2007). However, only 30 females survived to reproduction, resulting in a wide confidence interval (95% CI: 26.0 more susceptible eggs to 12.1 more resistant eggs daily).

Several of the development time benefits of cannibalism observed in the resistant population (Collie et al. 2013) were general to both populations—cannibalism reduced the time spent in the first and second stadia and reduced the time to each molt for both populations. While the chemical composition of CPB eggs is unknown, insects at various life stages have higher lipid and protein content than plant leaves of several species (Whitman et al. 1994), which may play an important role in growth and development. The pronounced reduction in development time, especially in the in early stadia, found in this study is in accord with other studies examining the benefits of egg cannibalism (e.g. Eickwort 1973, Gagné et al. 2002, Michaud and Grant 2004, Omkar and Gupta 2006); in some species, cannibalism decreases the duration of later stages (Mertz and Robertson 1970). Similarly, ladybird larvae reared on an intermediate-quality diet developed to the second molt more quickly if their diet was supplemented with dead conspecific larvae (Snyder et al. 2000). However, cannibals do not always develop more quickly (Ho and Dawson 1966, Schausberger and Croft 2000, Fincke 2011), and the effect of cannibalism on development time may depend on the quality of the base diet (Snyder et al. 2000). The greater increase in larval mass for cannibals is also observed in other insects (Breden and Wade 1989, Gagné et al. 2002, Kudo and Nakahira 2004, Omkar and Gupta 2006). In some species, cannibals are larger or heavier adults (Fincke 2011), while in others, they are not (Michaud 2003).

While both populations benefited from cannibalism, there were greater benefits of cannibalism for the resistant population. Cannibalism increased the growth rate of resistant larvae but not susceptible larvae, raising the growth rate of resistant cannibals to match the higher growth rate of susceptible larvae. The decrease in time spent in the first stadium resulting from cannibalism was also greater for resistant larvae than the slight but nonsignifcant reduction for susceptible larvae. In each case, the small or absent effect of cannibalism for susceptible larvae may indicate that they are near a ceiling of maximum development rate, which cannibalism allows resistant larvae to approach. In each developmental stage, resistant cannibals were able to reach or even surpass the development rate of susceptible noncannibals. Thus, cannibalism nearly or completely compensates for the developmental costs of resistance in this species.

Cannibalism has been shown and suggested to compensate for costs in other systems as well. In a study on costs associated with daily handling in flour beetle larvae, Mertz and Robertson (1970) found that cannibalism more than compensated for the development delay resulting from handling. Chilcutt (2006) also credited cannibalism in *Helicoverpa zea* larvae with compensating for costs of Bt exposure. Although he did not directly demonstrate such reduction of costs, larvae taken from Bt corn exhibited higher cannibalism rates than larvae from non-Bt corn; cannibalism marginally increased survival to pupation for larvae from both Bt and non-Bt corn, and Bt-exposed larvae had the same survival rate and development times as unexposed larvae. In my study, resistant neonates were no more likely to eat available eggs than susceptible individuals, but I was able to directly demonstrate the effect of cannibalism in compensating for costs of resistance.

The effect of cannibalism in reducing costs was also seen in developmental differences between the sexes. Male larvae gained mass faster than females, and cannibalism increased the growth rate of females but not of males. Females took longer to reach the second and third molts, and cannibalism reduced the time to reach the third molt more for females than for males. Nearly twice as many males emerged as adults as females, indicating higher female mortality, assuming an equal sex ratio at the egg stage. Female susceptible beetles also seemed to be more sensitive than males to trace amounts of pesticide, since no female susceptible noncannibals were able to survive to adulthood. Females also had higher adult mortality than males. Although cannibalism did not reduce overall mortality, it had a greater effect for females than males, at least for the susceptible population, since female susceptible cannibals emerged at the same rate as male cannibals and noncannibals. Females may be experiencing higher costs than males and may gain greater benefits from cannibalism because they are more resource-sensitive, needing to gain greater body mass to produce eggs, which itself incurs higher physiological costs (Michaud and Grant 2004).

Resistant individuals were more likely to survive to reproduction due to a high larval mortality of susceptible individuals. I observed what appeared to be pesticide intoxication in many of the susceptible larvae but not in the resistant larvae, suggesting that the larvae may have had inadvertent contact with trace amounts of pesticide present in our lab, possibly residual on equipment used to maintain or handle the larvae. Under these conditions, the costs of resistance that were observed are likely to be conservative estimates, as the susceptible individuals may have been somewhat compromised.

The inability of females in this study to survive in a challenging environment in the absence of cannibalism is similar to the results found by Snyder et al. (2000) in a ladybird beetle.

Larvae reared on a low-quality aphid diet were able to survive to the third instar but were unable to reach adulthood, while 50% of larvae on the same aphid diet supplemented with dead conspecific larvae were able to reach adulthood.

The developmental costs observed in this study would reduce the relative fitness of resistant beetles in refuges. Under the harshest field conditions observed by Harcourt (1971), the increased development time for resistant beetles could add up to a 10.5 percent mortality cost (assuming a constant daily mortality risk), without taking into account the higher growth rate of susceptible larvae or other, previously described costs (Baker et al. 2007, Baker and Porter 2008). However, egg cannibalism by resistant Colorado potato beetle neonates essentially eliminates the developmental costs of resistance, which can have implications for resistant management. Cannibalism is prevalent, almost always occurring when unhatched eggs are available (personal observation), so resistant neonates may often be able to raise their probability of survival to the level of susceptible individuals, reducing the effectiveness of refuges.

Additionally, although there was no overall effect of egg cannibalism on survival, cannibalism may have increased the ability of at least susceptible female larvae to survive trace pesticide exposure. Further research needs to be done to determine whether cannibalism can also improve the survival of larvae on more heavily treated plants. Horner and Dively (2003) observed that Bt-intoxicated *H. zea* larvae were less aggressive than larvae not exposed to Bt, which could reduce cannibalism by susceptible larvae in treated corn and make them more vulnerable to being cannibalized themselves by resistant larvae. They suggested that this advantage for resistant larvae would be compounded by their use of susceptible larvae as an additional “refuge” from Bt.

CPB neonates cannibalize eggs prior to consuming leaf material, so in addition to compensating for the costs of resistance on untreated plants, egg cannibalism can provide a food source that allows larvae to grow before they are exposed to pesticide on treated plants. Thus, cannibalism may enhance the survival of resistant or heterozygous beetles both in the presence and absence of pesticide, speeding resistance evolution. Models of resistance evolution should therefore take into account the observed effects of cannibalism in reducing the costs of resistance in refuges, as well as the potential use of eggs to increase the survival of resistant and heterozygous neonates in treated fields.

Table 1. Effects of cannibalism and resistance on growth rate, the age at which larvae reach each molt and the duration of each larval stadium, prepupal mass, and daily fecundity in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	Cannibalism		**Resistance**		**Cannibalism*resistance**		**Clutch**
	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	**%**
Growth rate	4.86 (1, 95.8)	0.030	3.61 (1, 11.7)	0.082	3.23 (1, 95.8)	0.075	
Age at first molt	30.10 (1, 159.1)	<0.0001	2.48 (1, 22.9)	0.13	7.02 (1, 159.1)	0.009	24.7
Age at second molt	30.74 (1, 123.2)	<0.0001	3.49 (1, 13.2)	0.084			14.2
Age at third molt	18.05 (1, 109.0)	<0.0001	4.71 (1, 13.3)	0.049			11.7
Age at pupation	13.49 (1, 95.5)	0.0004	2.14 (1, 13.1)	0.17			24.1
Duration stadium II	5.03 (1, 124.0)	0.027	0.88 (1, 12.7)	0.37			9.5
Duration stadium III	2.09 (1, 108.3)	0.15	0.18 (1, 12.2)	0.68			11.3
Duration stadium IV	0.24 (1, 94.1)	0.62	0.06 (1, 11.1)	0.81			24.1
Prepupal mass	0.18 (1, 99.0)	0.67	1.82 (1, 15.1)	0.20			10.1
Daily fecundity	0.12 (1, 17.5)	0.74	0.65 (1, 11.0)	0.44			52.7

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Growth rate, for which clutch could not be estimated, was analysed by ANOVA.

Table 2. Effects of cannibalism, resistance, and sex on growth rate, development time, prepupal mass, body volume, and emerging and maximum mass in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	Cannibalism		**Resistance**		**Sex**		**Clutch**	**Interactions**	
	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	**%**	***F* (*df*, *df*)**	***P***
								Sex*cannibalism	
Growth rate	13.52 (1, 77.5)	0.0004	1.60 (1, 5.8)	0.25	3.10 (1, 81.7)	0.082	8.0	4.05 (1, 79.8)	0.048
Age at first molt	24.13 (1, 80.3)	<0.0001	1.41 (1, 10.1)	0.26	2.46 (1, 82.8)	0.12	11.1		
Age at second molt	34.42 (1, 83)	<0.0001	5.23 (1, 83)	0.054	3.21 (1, 83)	0.077		**Sex*cannibalism**	
Age at third molt	24.25 (1, 81.2)	<0.0001	2.54 (1, 4.1)	0.18	8.89 (1, 81.7)	0.004	2.3	4.78 (1, 82.0)	0.032
Age at pupation	15.43 (1, 78.6)	0.0002	2.78 (1, 9.4)	0.13	0.84 (1, 83.0)	0.36	15.1		
Duration stadium II	7.41 (1, 79.4)	0.008	0.26 (1, 11.3)	0.62	0.77 (1, 83.0)	0.38	15.3		
Duration stadium III	4.14 (1, 82.1)	0.045	2.64 (1, 12.1)	0.13	0.79 (1, 81.9)	0.38	5.9	**Cannibalism* resistance**	
Duration stadium IV	1.02 (1, 76.9)	0.32	0.11 (1, 11.7)	0.75	1.67 (1, 79.8)	0.20	24.9	3.69 (1, 75.6)	0.058
Prepupal mass	0.07 (1, 80.7)	0.79	0.41 (1, 15.8)	0.53	5.18 (1, 83.0)	0.026	14.2		
Age at adult	10.12 (1, 83)	0.002	7.58 (1, 83)	0.007	1.79 (1, 83)	0.18		**Sex*resistance**	
Duration pupation	1.39 (1, 82)	0.24	0.34 (1, 82)	0.56	3.37 (1, 82)	0.070		10.38 (1, 82)	0.002
Body volume	2.03 (1, 78.8)	0.16	6.57 (1, 3.7)	0.067	7.86 (1, 73.4)	0.007	0.9		
Emerging mass	0.13 (1, 79.3)	0.72	3.06 (1, 14.8)	0.10	5.43 (1, 82.6)	0.022	20.1	**Sex*resistance**	
Maximum mass	0.11 (1, 77.0)	0.74	0.76 (1, 11.2)	0.40	49.5 (1, 81.8)	<0.0001	19.2	3.58 (1, 82.0)	0.062

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA.

Table 3. Effects of cannibalism, resistance, sex, and body volume on emerging and maximum mass in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	Emerging mass			**Maximum mass**		
	***F* (*df*, *df*)**	***P***	**%**	***F* (*df*, *df*)**	***P***	**%**
Cannibalism	2.30 (1, 68.4)	0.13		0.92 (1, 74.5)	0.34	
Resistance	2.20 (1, 16.6)	0.16		0.43 (1, 4.4)	0.54	
Sex	0.001 (1, 71.6)	0.97		44.35 (1, 48.8)	<0.0001	
Volume	47.0 (1, 72.7)	<0.0001		52.44 (1, 76.0)	<0.0001	
Resistance*sex				2.98 (1, 56.7)	0.090	
Cannibalism*sex	0.29 (1, 69.2)	0.59				
Cannibal*volume	4.32 (1, 72.6)	0.041		2.95 (1, 75.9)	0.090	
Sex*volume	0.91 (1, 73.5)	0.34				
Cannibalism*sex*volume	4.47 (1, 71.4)	0.038				
Clutch			21.2			3.6

All tests incorporating clutch as a random effect were analysed by restricted maximum likelihood (REML); clutch effect is given as the percentage of the random variation explained by clutch. Factors for which clutch could not be estimated were analysed by ANOVA.

Figure 1. The effect of cannibalism and resistance on growth rate in Colorado potato beetles, *Leptinotarsa decemlineata*. Solid lines represent the reverse-transformed average growth curves for cannibals and noncannibals of each resistance type; dashed lines represent their 95% confidence intervals. Statistical tests are presented in Table 1. Slopes were calculated by regressing log-transformed mass on age for each individual.

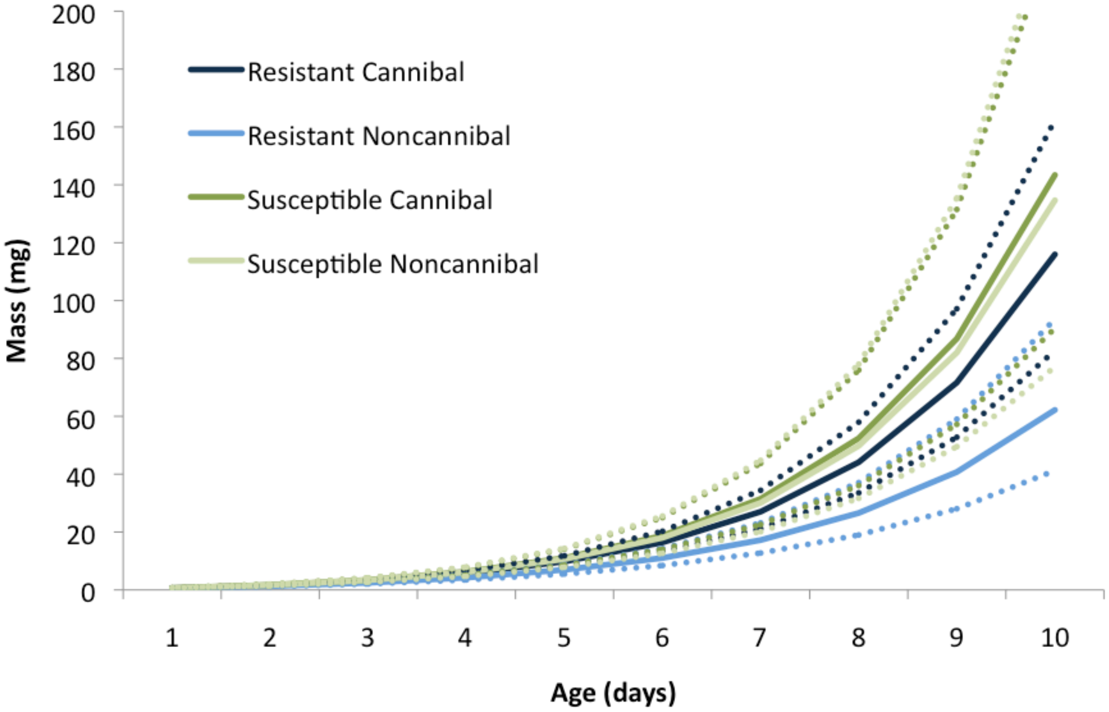


Figure 2. The effect of cannibalism and resistance on development time in Colorado potato beetles, *Leptinotarsa decemlineata*. Horizontal bars represent the least square mean time spent in each developmental period, with each developmental period including data from all individuals that completed that stage. Error bars are ± SE for the time to complete each developmental period; statistical tests are presented in Table 1.

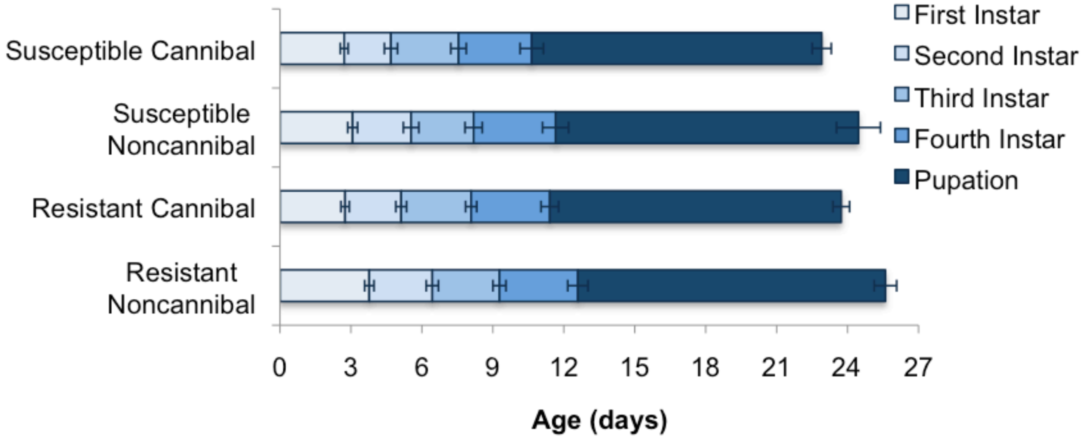


Figure 3. The effect of cannibalism and sex on growth in Colorado potato beetles, *Leptinotarsa decemlineata*. Solid lines represent the reverse-transformed average growth curves for each number of eggs consumed; dashed lines represent their 95% confidence intervals. Slopes were calculated by regressing log-transformed mass on age for each individual.

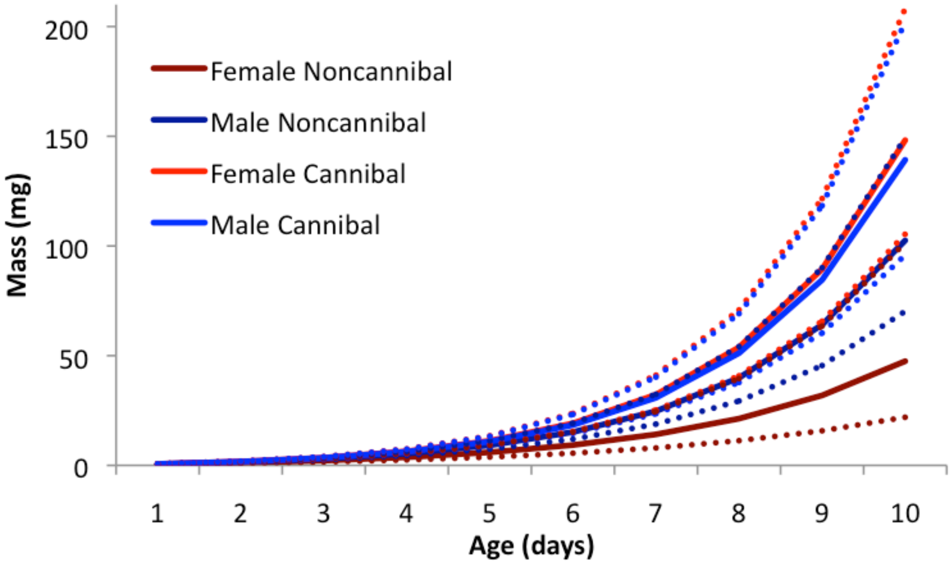
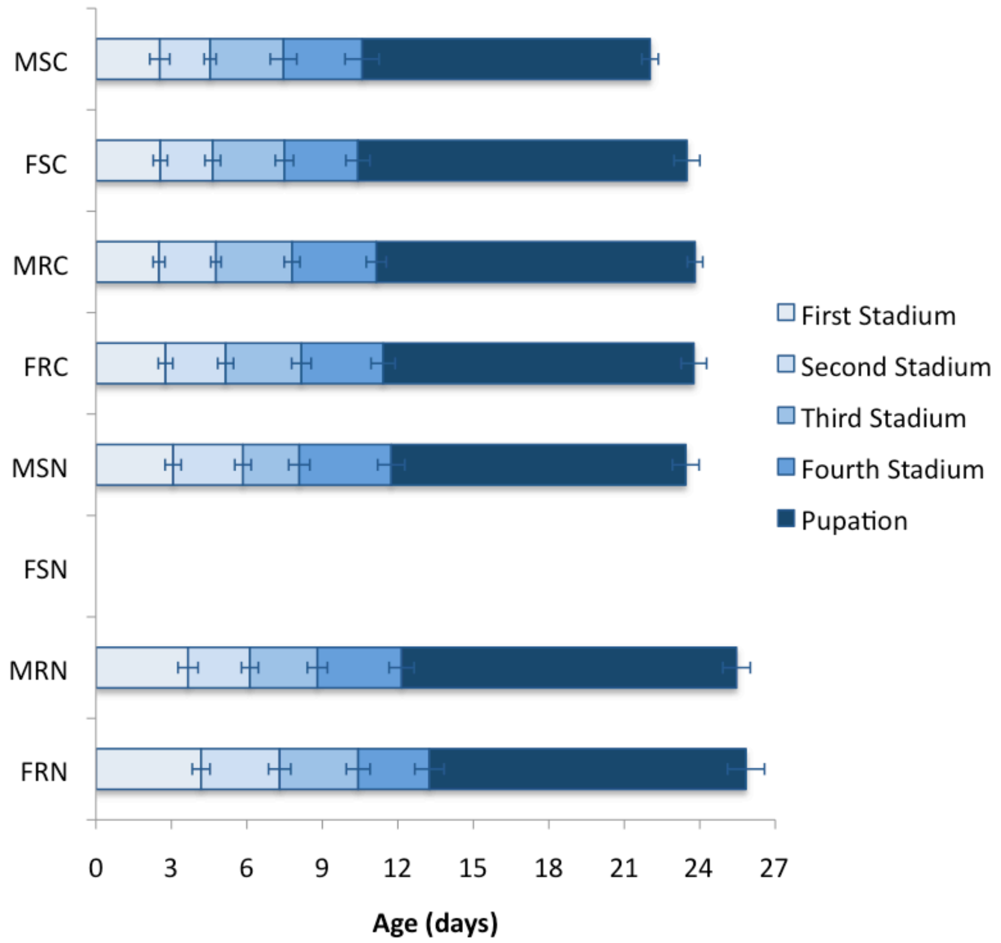


Figure 4. The effect of cannibalism, resistance, and sex on development time in Colorado potato beetles, *Leptinotarsa decemlineata*. Horizontal bars represent the time spent in each developmental period, with only data from individuals that reached adulthood included. Error bars are ± SE for the time to complete each developmental period; statistical tests are presented in Table 2.



CHAPTER 3: **Mechanisms to reduce inclusive fitness costs in the Colorado potato beetle, *Leptinotarsa decemlineata***

Cannibalism can provide reduction of competition and nutritional benefits that are especially advantageous for young individuals, but pre-dispersive juveniles are also likely to encounter and potentially cannibalize kin. Young cannibals may therefore be under selection to avoid killing relatives. Neonates of the Colorado potato beetle, *Leptinotarsa decemlineata*, often consume eggs in their natal clutch, which is a combination of full and half siblings. Hatchlings were provided full-sibling, paternal half-sibling, maternal half-sibling, or nonkin eggs from their own or another population, and latency to cannibalize and the number of eggs eaten were measured. Neonates began eating sooner and ate more eggs when given eggs from another population, but they did not distinguish among eggs from their own population based on relatedness. Neonates were also given either fertile or unfertilized eggs or a choice between living and dead eggs. Neonates rejected unfertilized eggs less often, began eating them sooner, and ate more unfertilized than fertile eggs. When given a choice, they more often ate a dead egg first and ate more dead than live eggs. To determine whether egg development is a cue for egg viability, neonates were provided eggs of different ages or given a choice between normal and developmentally-delayed eggs. There was no effect of egg age or development on egg consumption. Neonates may not be able to distinguish eggs based on relatedness, but they do reduce the costs of killing siblings by preferentially consuming inviable eggs, although egg development is not used as a cue for viability.

Keywords: cannibalism, Colorado potato beetle, egg discrimination, kin recognition, kin selection, *Leptinotarsa decemlineata*

Cannibalism is a normal part of the life history of many species (Fox 1975; Polis 1981; Elgar and Crespi 1992) and may be an important means of gaining nutrients that are scarce in plant material, especially for herbivorous insects (Whitman et al. 1994). The nutritional benefit gained from cannibalism can increase growth rates and decrease development time for immature individuals (Polis 1981). Intraspecific predation also reduces competition, which can lead to similar developmental benefits for young cannibals (Crossland et al. 2011). Juveniles also have the highest likelihood of pre-reproductive mortality, and so small absolute benefits can have large relative fitness benefits (Eickwort 1973; Fox 1975). Cannibalism may therefore be particularly advantageous during early life stages (Stevens 1992). However, pre-dispersive juveniles may be more likely to encounter their own kin, increasing the risk of inclusive fitness loss if cannibalism occurs (Pfennig 1997).

Cannibals should preferentially consume less-related individuals to maximize their inclusive fitness (Hamilton 1964; Waldman 1988); such a preference can also reduce the competitive pressure on their closer relatives, further increasing inclusive fitness (West et al. 2002). Differential consumption is dependent on the ability to discriminate between kin, an ability that is observed in many, but certainly not all, species. Kin recognition may be based on learned associations and familiarity (indirect) or by matching traits of an individual’s phenotype to one’s own phenotype (direct) (Waldman 1988).

Many vertebrate species are able to discriminate kin, often by learned odour cues. Many fish species can distinguish siblings or offspring from non-kin within the same population (Olsén 1999). For example, male sticklebacks, which exhibit paternal care, are able to determine the proportion of eggs within their clutch fathered by themselves (rather than by other males). Males often initially accept clutches even with many foreign eggs but later totally cannibalize clutches in which they have a low share of paternity, suggesting that paternal cues released by the eggs may become more detectable as embryos mature (Mehlis et al. 2010). However, male spottail darters, *Etheostoma squamiceps*, do not preferentially cannibalize foster eggs over sired eggs (DeWoody et al. 2001; Bandoli 2006). Sharptooth catfish, *Clarias gariepinus*, do not show higher cannibalism rates in mixed-progeny groups compared to full sibling groups (Baras and d'Almeida 2001). Fire salamanders, *Salamandra infraimmaculata*, show increasing aggression toward conspecifics with increasing genetic divergence along a gradient from siblings to larvae from a distant population (Markman et al. 2009), and female smooth newts, *Lissotriton vulgaris*, preferentially cannibalize non-offspring eggs (Tóth et al. 2011). However, Indian bullfrog tadpoles, *Hoplobatrachus tigerinus*, do not preferentially associate with siblings or non-siblings (Rajput et al. 2011). Neonate smooth snakes, *Coronella austriaca*, discriminate between sibling and non-kin odours, responding to the latter in a similar way as they do odours of prey species (Pernetta et al. 2009).

Kin recognition is also varied in invertebrate species. Females avoid cannibalizing their own offspring in crayfish (Mathews 2011), spiders (Anthony 2003), and ladybird beetles (Agarwala and Dixon 1993), but not in waterstriders (Cárcamo and Spence 1994) or ants (Bourke 1994). In the latter case, *Leptothorax acervorum* ant queens are unable to distinguish between their own eggs and those laid by other queens within their colony, although they are able to distinguish eggs from another colony (Bourke 1994). In this species, multiple queens lay eggs in a single heap, so that eggs from different queens are in physical contact, suggesting the possibility that egg recognition is based on surface chemicals that get intermingled between eggs, preventing within-colony recognition (Bourke 1994).

Sibling cannibalism avoidance is observed in spiders (Evans 1999; Bilde and Lubin 2001; Beavis et al. 2007), caterpillars (De Nardin and de Araújo 2011), earwigs (Dobler and Kölliker 2010), and ladybirds (Agarwala and Dixon 1993; Joseph et al. 1999; Michaud 2003). In many of these cases, kin cannibalism avoidance could be due to reduced cannibalism on familiar individuals, although caterpillars (De Nardin and de Araújo 2011) and some ladybirds (Agarwala and Dixon 1993; Joseph et al. 1999) preferentially consume nonkin over unfamiliar kin. However, failure to discriminate siblings from nonkin is also seen in land snails (Baur 1992), crab spiders (Morse 2011), seed bugs (Anderson and Solbreck 1992), caterpillars (Boots 2000), ants (Blatrix and Jaisson 2002), and ladybird beetles (Agarwala and Dixon 1993; Michaud 2003).

In the absence of kin discrimination, several other mechanisms have been proposed to ameliorate inclusive fitness costs of kin cannibalism. For example, spatial (Edgar 1971) or temporal (Crossland et al. 2011) segregation of siblings can reduce encounters between cannibals and their kin. Cannibalism most often involves a larger individual preying upon a smaller one (Polis 1981), and so hatching synchrony (Michaud and Grant 2004) or homogeneity in offspring size (Bry and Gillet 1980) may also reduce cannibalism on kin. For example, larvae of the mosquito *Trichoprosopon digitatum* do not have lower cannibalism rates when confined with clutchmates than with other larvae of the same age, but cannibalism in this species is size-dependent, so cannibalism rates between larvae of the same size are very low (Sherratt and Church 1994). In this case, reluctance to consume larvae of the same size would have the effect of kin avoidance, as smaller larvae are very unlikely to be (viable) kin (Sherratt and Church 1994). Alternatively, in species where individuals are most likely to cannibalize during early developmental stages, hatching asynchrony may reduce cannibalism (Schausberger and

Hoffmann 2008). Potential cannibals may also utilize a “temporal switch,” changing their behaviour at times when kin are likely to be present (Elwood 1992; Eggert and Müller 2000).

These alternative mechanisms to avoid killing kin in the absence of kin recognition often have the effect of preventing cannibalism altogether until siblings disperse. Because cannibalism may be most advantageous in young individuals, these cannibalism-avoidance mechanisms can incur a direct fitness cost. A less restrictive mechanism potentially available to egg cannibals, regardless of their ability to recognize kin, would be the ability to distinguish and preferentially consume inviable eggs within the natal clutch. If neonates consume inviable eggs rather than viable ones, they can obtain all the benefits of egg consumption without incurring any inclusive fitness losses. Such a preference is often assumed in theories regarding the evolution and benefits of trophic eggs**,** which females may lay to provision offspring or to reduce parent-offspring conflict by laying cheaper or more appetizing sterile eggs (Crespi 1992; Perry and Roitberg 2006). However, I know of only one study explicitly testing for such a preference in species without true trophic eggs; in the seed bug, *Spilostethus pandurus*, hatchlings prefer eating inviable eggs to viable eggs (Anderson and Solbreck 1992).

One species in which kin selection could be shaping cannibalism behaviour is the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), an herbivorous pest insect. After mating with many different males, females lay clutches with up to 90 eggs (population averages range from 12 to 39 eggs; Chpt. 4; Appendix E), that contain a combination of full and half siblings. Neonates readily consume unhatched eggs in their natal clutch, sometimes within one hour after hatching (personal observation), indicating a lack of temporal switch to avoid cannibalizing siblings. After the first egg in a clutch hatches, the remaining eggs hatch within 2 to 15.7 hours to complete hatching (Appendix E), and disperse from the clutch approximately 24 hours later, typically after all eggs and chorion are consumed (personal observation), so synchronous hatching does not seem to prevent sibling cannibalism in this species. Larvae in this species do not consume other larvae, so the distribution and relative size of individuals do not play a role in mediating cannibalism. The benefits that a neonate obtains from consuming a single egg can be sufficient to compensate for the inclusive fitness losses of killing a half sibling under field conditions, although it is uncertain whether they are great enough to compensate for the death of a full sibling (Collie et al. 2013). Therefore, kin selection should favour neonates that can distinguish between full- and half-sibling eggs.

The cues used in recognition will shape the predictions regarding kin recognition. If recognition occurs in *L. decemlineata* and is based on surface chemicals produced by the mother, as seems to be the case in ants (Bourke 1994), then full and maternal half siblings may be indistinguishable, while any eggs produced by another female (including paternal half siblings) could be treated as non-kin. However, if embryos express paternal cues, as seems to be the case in stickleback (Mehlis et al. 2010), then larvae may be able to distinguish maternal half siblings from full siblings and paternal half siblings from entirely unrelated eggs. Familiarity is used as a recognition cue in many species but often trumps discrimination based on actual kinship and so must be controlled in studies to examine innate recognition abilities (Waldman 1988). In this species, full and half siblings within the natal clutch would be equally familiar, so kin recognition based on familiarity would not allow discrimination at this scale, requiring other cues for neonates to minimize inclusive fitness losses. Such cues could instead be based on phenotype matching (thus also extending to unfamiliar eggs with similar phenotypes).

Additionally, while this species does not have distinctive trophic eggs, clutches often contain some eggs that are not viable, as indicated by a lack of pigmentation spots that develop as a viable egg matures (personal observation), so that neonates may often be faced with a choice between inviable eggs and viable siblings, generating selection for individuals that can distinguish between the two. If such discrimination does exist, one possible mechanism is lack of egg development, which should result in similar discrimination between mature and immature eggs.

In this study, I test whether *L. decemlineata* neonates can distinguish among eggs on the basis of relatedness, independent of familiarity. Familiarity cannot be used to discriminate between full and half siblings and, for comparisons between siblings and non-siblings, could be confounded with kinship if comparing eggs from the natal clutch to eggs from another female, preventing me from determining whether neonates are able to discriminate based on kinship itself. Therefore, only unfamiliar eggs are used in all tests. I also explore whether neonates show a preference for viable or inviable eggs, and whether egg development is used as a cue, by examining their consumption when presented with either unfertilized or viable eggs of various degrees of development, as well as their response when given a choice between viable and killed eggs or between normally-developing and cold-delayed eggs. Each of these experiments will be addressed separately.

Methods

Experiment 1: Kin Recognition

Animals

L. decemlineata used in these experiments were from two source populations. One population was derived from clutches from a United States Department of Agriculture greenhouse-reared population in Maryland; this population was originally collected from fields in Beltsville, MD. The second population was founded with adults collected from potato fields in Riverhead, NY.

Insect rearing

Beetles for all experiments were housed in metal cages (71 cm x 46 cm x 61 cm), with separate cages for each population source. All cages had wire mesh walls for ventilation and were kept in a warm room (approximately 25° C) with a 16:8 hr light:dark cycle. Beetle larvae were housed together on potted whole potato plants in groups of up to 200 individuals per cage. New plants were added to cages and pots were watered as needed; larvae burrowed into the potted soil for pupation, so pots were not removed from larval cages until all adults had emerged. Newly emerged adults were removed from the larval cages and sexed every one to two days, prior to sexual maturation. These virgin adults were then housed by population and sex in 325 mL Nalgene® Utility Boxes (ThermoScientific, Rochester, NY) modified with mesh windows for ventilation, with up to 5 adults per box. Boxes were provisioned with potato leaf clippings inserted into floral water picks, which were replaced daily. At the start of the experiment, virgin females were moved into individual utility boxes, with a single female per box, and similarly provisioned. All boxes were maintained in a Percival® model I-36 VL incubator (Percival Scientific, Inc., Perry, IA) at 25° C and a 16:8 hr light:dark cycle.

Experimental procedure

In the first trial, a single male was paired sequentially to two females from the same population. Thus, any eggs laid by a female were full siblings, while eggs laid by the second female mated to the same male were paternal half siblings to the offspring of the first female. Two such groups were established from the MD population, as well as one group from the NY population, also adding the possibility of non-kin from within and between populations.

In the second trial, maternal half siblings were produced based on the findings of Roderick et al. (2003) that previously-mated females then mated exclusively to a single different male will lay eggs fertilized almost entirely (99±1%) by the new male by the third clutch. For the present study, two females were mated alternately to two males. Males were switched every 9–20 days for 12 weeks. All eggs laid in the third or later clutches were considered full siblings to each other, while those offspring were considered maternal half siblings to any offspring produced before the males were switched or to the third clutch after the males were switched again. The first two clutches laid by each female after switching the males were discarded.

In both trials, focal individuals were mature eggs, which were each surrounded with 5 immature eggs from a different clutch. Trials took place on a potato leaf clipping inserted into a floral pick. In the first trial, the eggs in relation to the focal individual were full siblings, paternal half siblings, non-kin from the same population, or nonkin from a different population; 23 neonates were tested. In the second trial, the eggs were either full siblings or maternal half siblings to the neonate; 17 neonates were tested. All trials were recorded under a Sony® model HDR-HC9 MiniDV HD Handycam® camcorder (Sony Corporation, Tokyo, Japan) using BTV Pro Carbon® 5.4.1 time-lapse software (Ben Bird, www.bensoftware.com). The videos were later analyzed, blind to relatedness, for the total number of eggs consumed within 14 hours and, when eggs were consumed, the latency from hatching to cannibalize the first egg.

Statistical analysis

All statistical tests were performed in JMP® 8.0.2 (SAS Institute, Inc., Cary, NC).

For the first trial, the effect of kinship on the number of eggs consumed within 14 hours was fitted to a multiple regression model including kinship and population as fixed effects and mother as a random effect; because of unbalanced replication with random effects, the mixed model was tested using restricted maximum likelihood (REML). The effect of kinship on latency to cannibalize, including only those individuals eating at least one egg, was tested by two-way ANOVA including kinship and population. Due to polygamy and the possibility of inbreeding within the source colonies, which would have the effect of homogenizing relatedness between kin and "non-kin" from the same population (Markman et al. 2009), additional contrasts were conducted between responses to eggs from the different population and pooled treatments from the same population. For the second trial, all individuals were from the same population, so the effect of kinship on the number of eggs consumed and latency to cannibalize were analyzed by *t* tests.

Experiment 2: Neonate Preference for Viable or Inviable Eggs

Sterile eggs vs. fertile eggs

Females were collected from the laboratory populations upon emergence as adults and housed in utility cages in the incubator under identical conditions as previously described (see *Insect rearing* above). Sexually mature females who have been kept from mating begin to lay unfertilized eggs (personal observation). While females in the field would normally have access to mates prior to oviposition, even mated females lay some sterile eggs (personal observation). Sterile eggs laid by virgin females were used in this experiment to mimic such natural sterile eggs to ensure that the eggs were indeed inviable. These eggs were collected daily from unmated females and kept in the incubator. Fertile egg clutches were also collected daily from adult laboratory colonies containing males and females and were likewise stored in the incubator. All fertile eggs hatched by 6 days, so no sterile eggs of this age or older were used in trials.

The effect of viability on the number of eggs consumed within 7 hours was analyzed by a *t* test, as was the effect of egg fertility on latency to cannibalize, including only those individuals eating an egg.

Dead (frozen) eggs vs. live eggs

Eggs and beetles came from a laboratory colony derived from a standard laboratory strain (French Agricultural Research, Inc., Lamberton, MN) and from the laboratory colony derived from beetles collected from potato fields in Riverhead, NY.

Fertile egg clutches of both MN-lab and NY-field populations were collected. Each clutch used in the experiment was separated in half; each half was placed in a separate labelled petri dish. One half was frozen (-20° C) overnight to kill the eggs and then thawed in the incubator at 25° C. The other half remained in the incubator the entire time.

Newly hatched larvae were taken from different clutches than the eggs with which they were tested, so all eggs were unfamiliar to the neonates. Each larva was placed in the centre of four eggs—two dead eggs and two live eggs from the same source clutch—on filter paper marked to indicate the status of each egg. Larvae on eggs were checked every hour for 6 hours, and again the following day (20 to 24 hours after set-up) for egg consumption. A total of 97 neonates were tested—55 from the NY field population and 42 from the MN lab population.

In this choice experiment, the status of the first egg eaten (alive or dead) was examined by a Chi-square test for each population. A paired *t* test on the difference between the number of frozen and live eggs eaten by each individual in the first day was also performed for each population.

Eggs of different ages

The experimental setup was nested within the *Sterile eggs vs. fertile eggs* methods above. Daily egg collection resulted in eggs of known age, from newly laid (< 24 hours) to 5 days. Egg age was treated as an ordinal variable. ANOVAs were used to test whether egg age affected the number of eggs consumed within 7 hours, including all individuals given fertile eggs, or the latency to cannibalize, including only those individuals eating a fertile egg.

Delayed vs. non-delayed eggs

The methods for this experiment were very similar to those described in *Dead (frozen) vs. live eggs* methods above. The key difference was that clutches were divided between the standard incubator (25° C) and a cold incubator (12° C) for 24 hours, followed by a return to the standard incubator. Cold has the effect of delaying development without resulting in mortality for this incubation period (Appendix F).

Newly hatched larvae were taken from different clutches than the test eggs, so the eggs were all unfamiliar. Each larva was placed in the centre of four eggs—two delayed eggs and two non-delayed eggs from the same source clutch—on filter paper marked to indicate the status of each egg. Larvae on eggs were recorded using a Dino-Lite® AM-2011 digital microscope (AnMo Electronics Corp., Hsinchu, Taiwan) for 6 hours. Thirty neonates were tested. A paired *t* test on the difference between the number of delayed and non-delayed eggs eaten by each individual within 6 hours was performed.

Results

Experiment 1: Kin Recognition

In the first trial, there was no effect of population on the number of eggs consumed (REML: $F_{1,\,2.9} = 2.90$, $P = 0.19$). When controlling for mother, there was an effect of kinship on the number of eggs consumed (Fig. 1a; REML: $F_{3,\,15.9} = 3.53$, $P = 0.039$), with neonates eating an average of 1.8±1.7 (Tukey 95% CI) more eggs from a different population than half-sibling eggs. However, there was no significant difference between the number of full- and paternal half-sibling eggs eaten (Tukey Honestly Significant Difference [HSD], $\alpha = 0.05$). When contrasting responses to eggs from the same or the different population, neonates ate more eggs from the different population (REML: $F_{1,\,15.4} = 5.61$, $P = 0.031$). There was no effect of population on latency to cannibalize (ANOVA: $F_{1,\,15} = 0.07$, $P = 0.80$), nor was there an effect of kinship (Fig. 1b; ANOVA: $F_{3,\,15} = 1.57$, $P = 0.24$). However, the observed latencies were in the predicted order, with neonates waiting longer, but not significantly so, to consume full siblings than paternal half siblings (123.2±314.0min [Tukey 95% CI]), which had an almost identical latency to non-kin eggs from the same population, and eggs from a different population were eaten most quickly (Fig. 1b). When contrasting responses to eggs from the same or the different population, neonates ate eggs from the different population marginally faster (ANOVA: $F_{1,\,16} = 3.26$, $P = 0.090$) than eggs from the same population.

In the second trial, there was not a significant difference between full and maternal half siblings in the number of eggs consumed (Fig. 1c; *t* test: $t_{15} = 2.13$, $P = 0.33$). There was also no difference in the latency to cannibalize eggs based on relatedness (Fig. 1d; *t* test: $t_{10} = 2.23$, $P = 0.88$), with a very similar latency to eat a full sibling or a maternal half sibling.

Experiment 2: Neonate Preference for Viable or Inviable Eggs

Sterile eggs vs. fertile eggs

In the no-choice test, neonates ate fewer total fertile eggs than sterile eggs (Fig. 2a; *t* test: $t_{108} = 3.80$, $P = 0.0002$), and waited marginally longer to eat fertile eggs (Fig. 2b; *t* test: $t_{80} = 1.69$, $P = 0.094$), with an average of 47±55 min (95% CI) longer.

Dead (frozen) eggs vs. live eggs

Neonates from the field-derived population were more cannibalistic on dead eggs than live eggs in the choice test. They consumed a dead egg first more often than a live egg (Fig. 3a; Chi-square: $\chi^2_1 = 8.23$, $P = 0.004$). They also ate more dead eggs than live eggs (Fig. 3b; paired t test: $t_{53} = 4.03$, $P = 0.0002$). However, neonates from the lab-reared population did not show a preference in the first egg eaten (Fig. 3a; Chi-square: $\chi^2_1 = 0.02$, $P = 0.88$), or in the total number of each type of egg eaten (Fig. 3c; paired t test: $t_{41} = 1.22$, $P = 0.23$).

Eggs of different ages

There was not an effect of egg age on the number of eggs consumed (ANOVA: $F_{5,\,87} = 0.97$, $P = 0.44$). There was also no effect of egg age on latency to cannibalize (ANOVA: $F_{5,\,60} = 0.97$, $P = 0.44$).

Delayed vs. non-delayed eggs

There was no effect of developmental delay on the number of eggs consumed by neonates (paired t test: $t_{29} = 0.64$, $P = 0.53$).

Discussion

Neonates did not show a significant preference for less-related eggs within their own population, although this result may have been due to small sample size. Even with a true mean difference of 1.5 eggs eaten or a true average latency difference among groups of 3.5 hours, power would have been less than 0.5. Neonates did, however, discriminate between eggs from their own and a different population, as the latter were eaten slightly earlier and a greater number were eaten than any other type. Although neonates would not normally encounter eggs from another population in the field, such discrimination indicates that they are able to distinguish between eggs. Discrimination is expected if neonates are under selection to avoid eating very similar (i.e. kin) eggs, although discrimination at a finer scale (close vs. distant relatives or full vs. half siblings) might have been reduced if colony inbreeding increased relatedness for all members within the same population.

I also predicted that neonates should be able to distinguish maternal half siblings from full siblings and, if developing embryos release cues that indicate paternity, paternal half siblings from nonkin. The lack of discrimination between these groups suggests that neonates may not recognize paternal cues. However, as paternal cues in embryos may increase as the embryos develop and their own gene expression increases (Mehlis et al. 2010), the use of immature eggs may have limited the strength of these cues, further diminishing discriminatory ability.

In all trials, neonates were unfamiliar with the test eggs, so the ability to recognize foreign eggs had to be direct rather than indirect. Preferential cannibalism of nonsiblings over unfamiliar siblings is observed in *Heliconius erato phyllis* caterpillars (De Nardin and de Araújo 2011) and in *Adalia bipunctata* (Agarwala and Dixon 1993) and *Harmonia axyridis* (Joseph et al. 1999) ladybird beetles. De Nardin and de Araújo (2011) hypothesize that the ability to discriminate unfamiliar kin may come by matching their sibling's phenotype to their own, learned by consuming their own chorion. In the present study, hatchlings cannibalized sibling eggs prior to eating their own chorion, but the potential influence of the chorion on kin recognition ability merits further examination.

Neonates from the field population were able to distinguish between eggs that were capable of hatching and those that were not, and they preferentially consumed inviable eggs. However, they showed no preference for eggs based on degree of development. These two results taken together demonstrate that neonates are able to detect something inherent in the egg that indicates its viability apart from its degree of development, as they did not consume freshly-laid or delayed eggs more readily than those on the verge of hatching. However, neonates from the standard laboratory population showed no discrimination between live and dead eggs. This colony has been reared under laboratory conditions for 20 years, and so over many generations, high density may have selected for neonates that reduce competition by increasing their propensity to consume viable eggs or by relaxing selection to maintain their discriminatory ability.

Just as kin discrimination abilities vary between species, the ability of species to distinguish between eggs according to their developmental status is varied. For example, Northern dusky salamander, *Desmognathus fuscus*, mothers are able to differentiate between their own cold-delayed and normally developing eggs, preferring to brood the non-delayed eggs (Forester et al. 2005). Female predatory mites also discriminate between their own young and old eggs (Schausberger and Hoffmann 2008). However, *Heliconius erato phyllis* caterpillars do not discriminate between normal and cold-delayed eggs (De Nardin and de Araújo 2011), and land snail hatchlings do not distinguish between unfertilized eggs and developing embryos when cannibalising (Baur 1992).

Selection for neonates to recognize inviable eggs should be strong. If neonates are able to preferentially consume sterile or dead eggs within their clutch, they gain all the nutritional benefits of consuming an egg (Collie et al. 2013) without incurring the cost of killing a sibling. Of all eggs consumed in the no-choice trial, 63% were sterile eggs; in the choice trial 70% of the first eggs eaten by neonates from the field population were dead. While the discriminatory abilities observed in our experiments were not perfect, they could still somewhat reduce kin selection against cannibalism. Up to 8% of eggs in field clutches may be sterile (Harcourt 1971); if neonates in field clutches similarly reject fertile eggs in 70% of encounters, less than 85% of eggs eaten would be viable siblings (rather than 92%, if eating is random).

According to kin selection theory, the costs of sibling cannibalism decrease as the likelihood that the victim egg would otherwise hatch decreases, since no inclusive fitness is lost by eating an inviable egg. The necessary balancing benefits also decrease as larval mortality rates increase, as even small absolute benefits become larger relative benefits (Eickwort 1973). Preferential consumption of inviable eggs means that the probability that a victim egg would have hatched is lower than the population average hatch rate (e.g. 85% vs. 92%). Thus, cannibalism with a preference for inviable eggs can compensate for inclusive fitness losses when larval mortality rates are lower than if there is no preference for inviable eggs. For example, if the probability of an average egg hatching, taking into account both egg infertility and other sources of mortality, is 57% (the lowest hatch rate observed in the field by Harcourt [1971]), at these levels of discrimination, the probability that the average cannibalized egg would have hatched is less than 52%. Based on the decrease in mortality resulting from the developmental benefit of cannibalism (Collie et al. 2013), this reduction in average hatch potential of cannibalized eggs allows selection for cannibalism when the probability of larval survival is up to 3% higher than in the absence of a sterile egg preference (e.g. 64% vs. 61%).

The actual survival rate at which cannibalism is favoured depends on the exact proportion of inviable, full-sibling, and half-sibling eggs in the average clutch. *L. decemlineata* is polyandrous (Szentesi 1985), but the average number of mates in field populations is unknown, making it difficult to estimate the proportion of full- and half-sibling eggs in a clutch. As the proportion of inviable eggs increases within a clutch, hatchlings are predicted to become more cannibalistic, risking eating viable siblings to gain access to these eggs before their siblings do, which increases parent-offspring conflict (Perry and Roitberg 2005). Females are only selected to increase the opportunity for sibling cannibalism when it increases the probability of survival of her offspring by at least 100% (i.e. when survival rates are less than 50%), so inviable eggs could be unavoidable rather than part of a female strategy (Perry and Roitberg 2005). However, if neonates discriminate sterile eggs from viable siblings, this parent-offspring conflict would be reduced, allowing the use of sterile eggs as part of a female strategy at higher survival rates, especially if sterile eggs are cheaper to produce than viable ones (Crespi 1992; Perry and Roitberg 2006). There is evidence that females do increase the proportion of inviable eggs in some clutches when laying on poor-quality plants (Appendix C), so that environmental conditions influence not only larval survival rates, but also the opportunity to cannibalize inviable eggs, further increasing the benefit of cannibalism as natal plant quality declines.

While *L. decemlineata* neonates do seem able to discriminate between sterile and viable eggs, their preference for sterile eggs is not perfect. The partial selectivity displayed by neonates may be limited by physiological capabilities. Alternatively, neonates may be adapted to eat some viable eggs to reduce competition on limited resources. In agro-ecosystems, uncontrolled populations (or uncontrollable pesticide-resistant populations) can defoliate an entire potato field within a growing season (Alyokhin et al. 2008), and starvation is a major cause of mortality (Harcourt 1971). Collie et al. (2013) found that neonates consume more eggs than can be explained by obtained nutritional benefits, implying that competition reduction may be an important selective factor in cannibalism evolution (see also Chpt. 4). Otherwise, if cannibalism of additional eggs provides no benefit, either nutritionally or by decreasing competition, excess sibling cannibalism that decreases inclusive fitness without increasing direct fitness would be under negative selection and should not still be observed at such a high frequency. Indeed, in cane toad tadpoles, competition reduction may be more important in driving cannibalism than nutritional benefits (Crossland et al. 2011). In the imported willow leaf beetle, a species in which young larvae remain in their natal groups, the nutritional benefits gained by egg cannibalism favour the behaviour, while the costs of group-size reduction result in opposing selection (Breden and Wade 1989). A similar phenomenon may be occurring in the potato beetle, with kin selection driving preference for inviable eggs but the costs of competition opposing the behaviour.

Figure 1. Effect of cannibal-egg relatedness on (a, c) the number of eggs eaten and (b, d) the latency to cannibalize in the Colorado potato beetle, *Leptinotarsa decemlineata*. Data are shown with least square (LS) mean values and their 95% confidence limits.

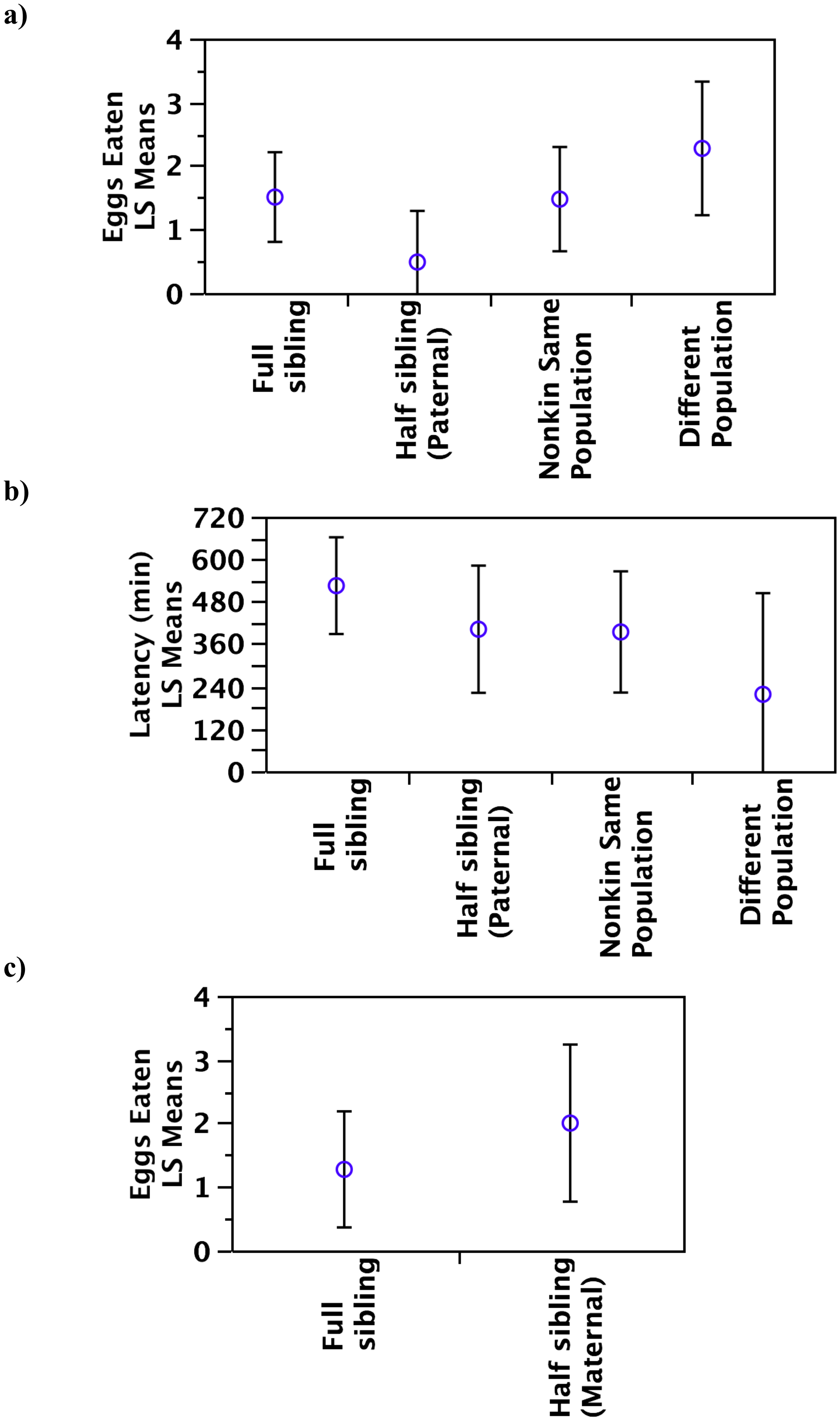


Figure 1 cont.

d)

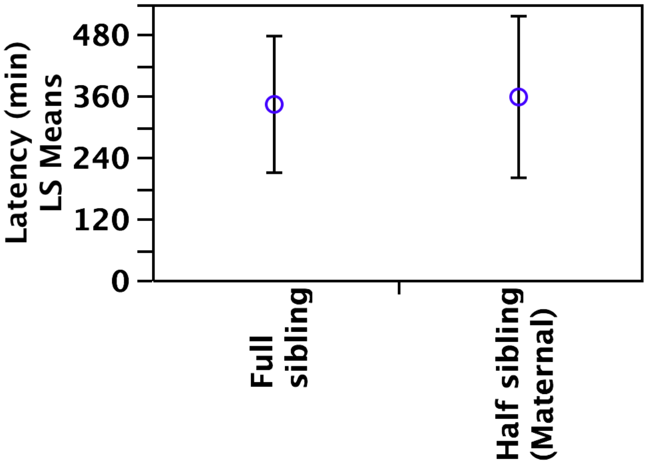


Figure 2. Effect of egg viability on (a) the number of eggs eaten, and (b) the latency to cannibalize in a no-choice test in the Colorado potato beetle, *Leptinotarsa decemlineata*. Data are shown with mean values and their 95% confidence limits.

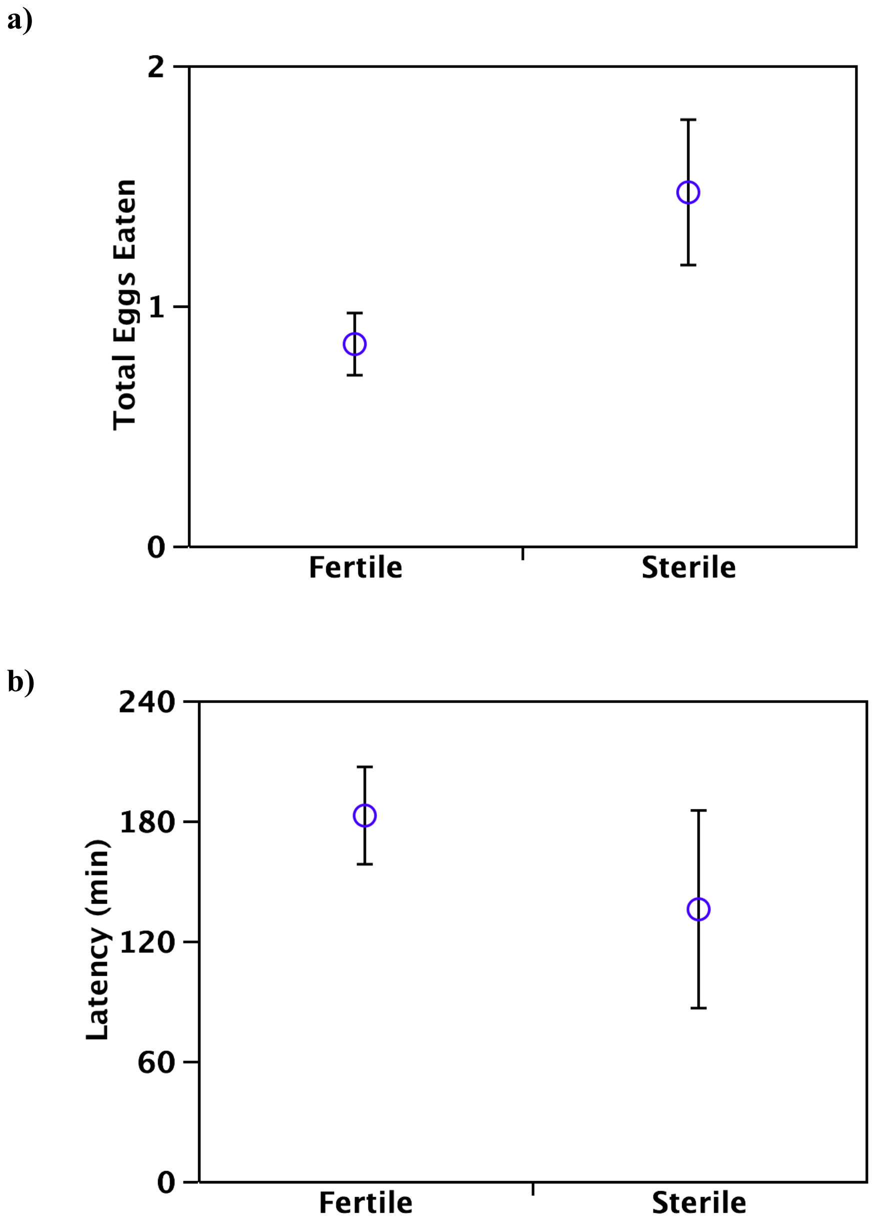
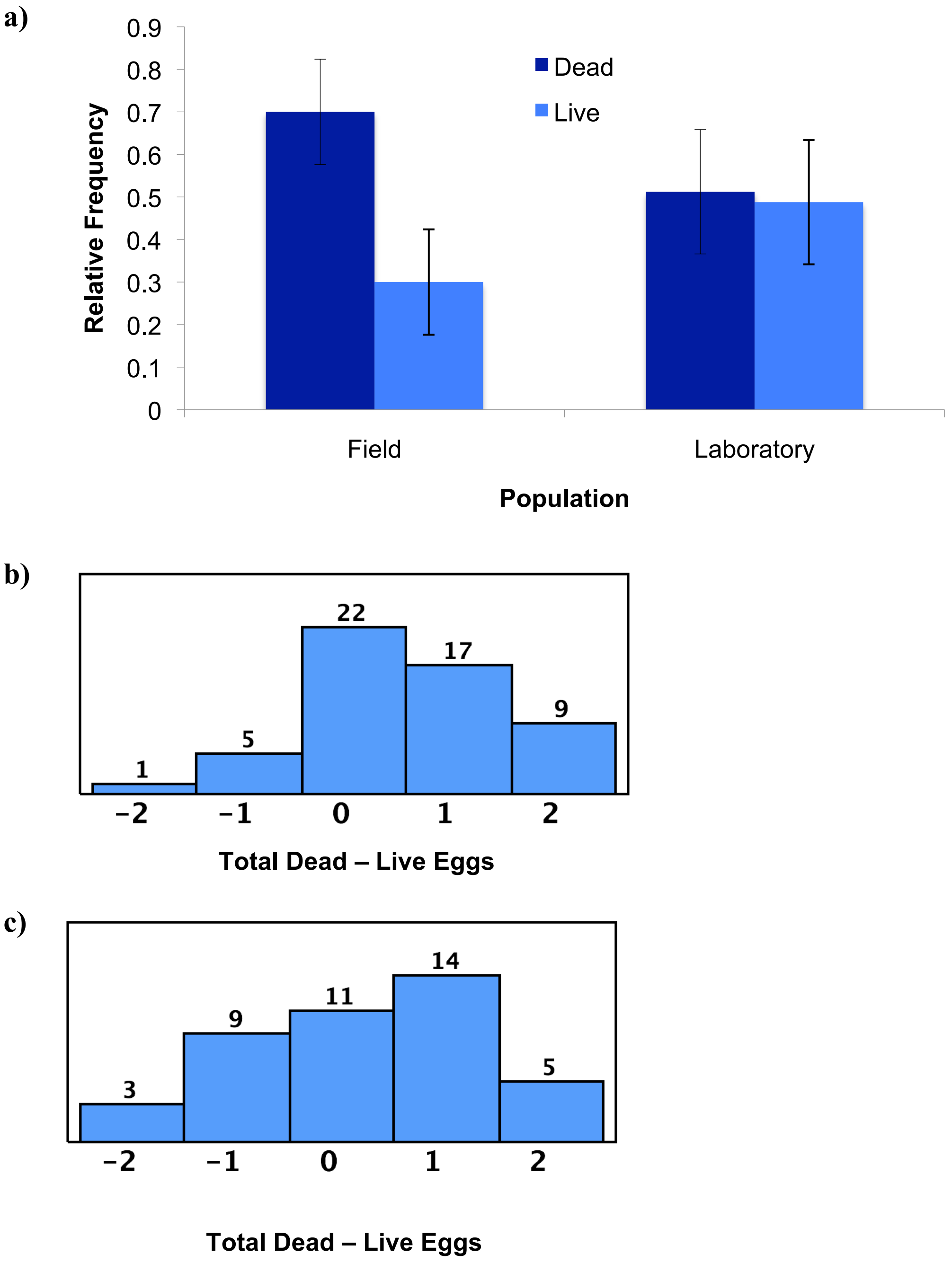


Figure 3. The effect of egg viability (alive or dead) on (a) the choice of the first egg eaten by neonates and the difference between the total number of live and dead eggs eaten by neonates from (b) a field population or (c) a laboratory population of the Colorado potato beetle, *Leptinotarsa decemlineata*. (a) Proportions are shown with their 95% Agresti-Coull confidence intervals. (b, c) The difference between the number of dead and live eggs eaten by each individual is shown, with negative numbers indicating more live than dead eggs eaten. Numbers above columns indicate frequency.



CHAPTER 4: Population differences in cannibalism between indigenous field populations of Colorado potato beetle, *Leptinotarsa decemlineata*

Geographic variation is expected in behavioral traits in response to differing environmental conditions and selective pressures. Cannibalism brings nutritional benefits and reduces competition and so may vary between populations facing different levels of predictable resource limitation. In the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, neonates cannibalize unhatched eggs within the natal clutch before assessing resource availability, suggesting an evolutionary response to potential resource limitation that should vary with competition between populations. Three CPB populations and a population of closely-related *L. undecimlineata* (LU) were studied on their native host plants in their natural range in Mexico over three years to assess differences in population density, intraclutch cannibalism rates, and cannibalism propensity. Plant size and population density were measured, and clutch size and the number of hatchlings were observed in unmanipulated clutches from each population. Neonate latency to cannibalize the first egg and the number of eggs eaten within 14 hours were also measured. In two years, intraclutch cannibalism rates and cannibalism propensity mirrored apparent competition in CPB populations, with faster cannibalism in the populations with the highest egg densities, although competition and cannibalism rates also changed over time in some populations. Although LU had much larger clutches, they also exhibited much lower cannibalism, in contrast to expectations. The effect of competition on fitness and plastic response to immediate environmental conditions need to be directly assessed. A common-garden experiment comparing cannibalism between native and invasive populations on the same host under the same conditions would further elucidate genetic differences between populations.

Geographic variation in genetic, morphological, physiological, and life-history traits is widely observed between populations within species. In the same way, though less often studied, there exist population differences at the behavioral level (Huettel 1986, Foster 1999). Some differences may be due almost entirely to climatic or environmental differences, as may be the case with temperature-dependent life history variation in European wolf spiders (Edgar 1971). Morphological and behavioral adaptations are also often driven by selection to eat and avoid being eaten, with population differences arising when food availability and predation risk differ between locations.

Adaptations are often correlated, with selection resulting in an entire suite of morphological and behavioral differences. Fish populations facing differing predation risks may exhibit color differences that are also correlated with female mate-choice preferences and anti-predator behavior, which may also be correlated with parental care behavior (Foster 1999). Desert spiders, *Agelenopsis aperta*, in arid and riparian regions show population differences in territory size and aggression that are correlated with normal prey abundance in the different regions; these differences are expressed between the populations even under identical conditions, independent of food provisioning, indicating genetic differences rather than temporal responses to the immediate environment (Riechert 1986). A social spider, *Metepeira spinipes*, also shows fixed population differences in minimum spacing and communal web-building behavior, although it also demonstrates plasticity in territory size dependent on food availability (Uetz et al. 1986). Checkerspot butterflies, *Euphydryas editha*, often exclusively use a single host species, even when other host species are available; host preference is population-specific, and individuals from populations feeding on different host plants also differ in oviposition preference, clutch size, height of oviposition, larval foraging height, and larval performance

(Singer and McBride 2012). Different populations of milkweed bugs, *Oncopeltus fasciatus*, vary in timing of reproduction, flight distance, wing and body length, fecundity, and clutch size; most, but not all, of these traits are correlated (Dingle et al. 1986). Populations also differ in how temperature and day length influence the expression of these traits (Dingle et al. 1986).

Cannibalism is also expected to vary between populations because it is sensitive to selection under local conditions, such as food availability and population density. Cannibalism is predicted to be higher in populations that have nutritional deficiencies in their diet, those with higher mortality or lower relatedness, and those with higher populations densities or more readily available conspecific prey (Whitman et al. 1994). Benthic insects in nutrient-poor sections of a stream are more cannibalistic than the same species at nutrient-rich sites downstream, and perch may be more cannibalistic in nutrient-poor than nutrient-rich lakes (Fox 1975). Three-spine stickleback populations in shallow lakes forage on benthic invertebrates and also frequently raid nests to cannibalize eggs, while those in deep lakes are planktonic feeders and do not raid nests; loss of egg cannibalism has also resulted in loss of parental males' diversionary displays and an increase in courtship conspicuity (Foster 1999). In some cannibalistic populations of threespine sticklebacks, males are more cannibalistic than females, while in others, females are more cannibalistic than males; population differences may be due to differences in food or egg availability (FitzGerald & Whoriskey 1992). In land snails, egg size, hatching synchrony, and cannibalism propensity vary between populations (Baur 1992).

When resource limitation is predictable, cannibalism may be a pre-emptive means to reduce competition for these resources, which may vary between populations in scarcity or importance. In the United States, larvae of the southwestern corn borer, *Diatraea grandiosella*, overwinter at the base of the corn stalk, in the portion below the ground. Space is limited, and larval cannibalism typically reduces the number of larvae to one per stem. Cannibalism is lower in a native Mexico population, where winters are milder and below-ground overwintering sites are less critical (Tarpley et al. 1993). Higher cannibalism in the U.S. population may also have selected for delayed pupation, as pupae are more vulnerable to cannibalism (Tarpley et al 1993). Predatory mites in California, with dry summers and predictable prey crashes, are more cannibalistic than those from the wetter Pacific Northwest (Fox 1975). Spadefoot tadpoles have more cannibal morphs in the dry parts of the species' range that has more temporary habitats, although their frequency still varies in time and between neighboring pools (Fox 1975). Conversely, cannibal morphs of the salamander, *Ambystoma tigrinum mavortium*, are more likely to be found in playas with longer hydroperiods, perhaps because these playas have higher population densities, which may encourage the formation of cannibal morphs by increasing intrapopulation size variation (Ghioca and Smith 2008). Growth rates, development time, and size at metamorphosis also vary between populations, likely because of variation in environmental factors, such as population and congeneric density, temperature, food availability, and hydroperiod. The presence of cannibal morphs itself also decreases the growth rates of other morphs and suppresses the presence of intermediate morphs, resulting in further population differences (Ghioca and Smith 2008).

Population differences in cannibalism rates may be due both to environmental factors and to genetic differences in proclivity. Environmental factors can be studied by measuring aspects of the population under natural conditions, such as encounter rates, size heterogeneity, and immediate food availability (reflecting hunger levels). Genetic differences are a response to evolutionary rather than ecological conditions and are expected to reflect persistent population differences in resource limitation and competition; they are better studied experimentally by measuring the cannibalism rates of individuals from each population when in the same state and presented with the same opportunity to cannibalize.

One species in which population differences in cannibalism are likely is the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*. CPB is an invasive species that extended its range from Mexico, where it is indigenous, into the U.S. in the early 1800s; it switched from its native host buffalo bur, *Solanum rostratum*, to cultivated potato, *S. tuberosum*, in the mid-1800s (Casagrande 1987). It then spread across the U.S. in 25 years (Casagrande 1987) and was introduced into France in the 1920s (Grapputo et al. 2005), from where it has spread across Europe and into Asia (Liu et al. 2012). Genetic variation has been observed across the species range (Grapputo et al. 2005, Piiroinen et al. 2013). Geographic variation has also been observed in the number of years adults overwinter, development rates and their response to different temperatures, and, where insecticide resistance is present, mechanisms of resistance, sometimes varying between populations in close proximity (Alyokhin et al. 2008).

CPB females mate with multiple males and lay mixed-paternity clutches on the underside of host leaves. Eggs hatch four to five days later, and neonates consume unhatched (though often viable) sibling eggs before dispersing from the clutch, sometimes cannibalizing within an hour of hatching and generally before eating available foliage (personal observation); egg cannibalism results in greater mortality than interspecific predation (Harcourt 1971). Neonate cannibalism of sibling eggs is likely to be more greatly influenced by historical than current conditions, as neonates consume eggs before they have an opportunity to assess food availability. Cannibalism in this species is thus expected to increase in populations with typically greater competition.

To test this hypothesis, three CPB populations were studied over three years on their native host plant in their original range in Mexico. Buffalo bur plants are generally smaller than cultivated potato, with a greater concentration of toxic steroidal alkaloids (Piiroinen et al. 2013), and are patchily distributed and covered with inedible thorns. Potato beetle populations can completely defoliate these patches within a growing season, making them limiting resources (Horton and Capinera 1988). Populations in the native range are more isolated than those on agricultural potato fields, increasing the likelihood of local selection. Population density at each site was estimated by measuring the size of each plant and recording the number of beetles at each life stage on each of these plants. Local competition was estimated as the average number of individuals at each stage per plant when controlling for plant size. Populations with higher egg density are predicted to be more cannibalistic. To estimate the occurrence of natural intraclutch cannibalism, clutch size was measured, as well as the number of intact and viable eggs and the number of resulting hatchlings in each clutch. Neonates from each population were also tested for their latency to cannibalize under common conditions to directly measure population differences in cannibalism propensity. A closely related species, *L. undecimlineata* (LU), was similarly studied on its host plant, *S. lanceolatum,* to measure interspecific differences in cannibalism.

Methods

Field sites in Mexico were established by locating areas with buffalo bur, typically on roadsides. Patches of plants were checked for CPB populations, and three locations were found with sufficient numbers to carry out population density surveys: El Rodeo, Morelos (18°46’48” N, 99°18’47” W, 1139 m), Villa de El Carmen Tequexquitla , Tlaxcala (“El Carmen”; 19°19’40” N, 97°38’45” W, 2353 m), and El Limón Totalco, Veracruz (“Totalco”; 19°30’04” N, 97°20’48” W, 2352 m). A recent study found that a CPB population in Puebla (~46.5 km southwest of El Carmen) and one in Morelos (~2.5 km northeast of El Rodeo) showed very strong genetic differentiation, though the authors did not propose that the populations be considered separate subspecies (Piiroinen et al 2013). As their populations were in between my study sites, the populations in the present study likely show similar genetic divergence.

All three sites were surveyed in the middle of the breeding season—in September 2008, September 2009, and August 2010—with the exception of El Rodeo, which had been turned into a construction site in 2010, precluding study that year. An additional site in the cloud forest near Coatapec, Veracruz (19°28'56" N, 96°59'48" W, 1585 m) was used in 2008 and 2010 to study a LU population feeding on *S. lanceolatum*, which is in the same subgenus as *S. rostratum* but a different subgenus than *S. tuberosum*.

The length, width, and height of *S. rostratum* plants were measured in 2009 and 2010, and plants were surveyed for the number of adults, egg clutches, new hatchlings (prior to dispersing from the clutch), small larvae (first and second instars), and large larvae (third and fourth instars) on each plant. Surveys were conducted in El Rodeo (29 plants) and El Carmen (24 plants) in 2009 and in El Carmen (18 plants) and Totalco (11 plants) in 2010, although the number of adults per plant was not counted in 2010. The number of eggs on each plant was estimated in 2009 by multiplying the number of clutches by the average number of eggs per clutch for each population. In 2010, clutches were matched to plant so that the exact number of eggs on each plant was known. A similar survey was conducted on LU on 22 plants in 2008. *Solanum lanceolatum* grows to much greater heights than *S. rostratum* and has larger, more sparsely distributed leaves. Rather than measuring the volume of entire plants, the number of leaves on each plant was counted, and total leaf area was estimated from the average leaf area of several representative leaves (measured by tracing leaves on graph paper). Total leaf area was similarly estimated for a *S. rostratum* plant at El Carmen for comparison.

Clutches were collected from all sites in all years by removing the portion of the leaf to which the eggs were attached; each leaf clipping with a clutch was placed in an individual petri dish and labeled to location. Eggs were kept at ambient temperature under natural light. The total number of eggs in each clutch was counted under a dissecting microscope. Some eggs were damaged prior to collection, presumably by predation, so the number of intact (undamaged) eggs were also counted. In 2009, clutches were examined daily prior to hatching for maturation, evidenced by the appearance of pigmentation spots that begin to develop on embryos approximately 24 hours prior to hatching (personal observation). The absence of such spots on individual eggs in otherwise mature clutches indicated egg inviability. The number of viable (visibly developing) eggs were thus counted. In 2008 and 2009, the number of hatchlings emerging from unmanipulated clutches was also counted.

In 2009, within 30 minutes post-hatching, individual CPB neonates were placed on 5 immature eggs and observed hourly for 14 hours to determine latency to cannibalize their first egg, as well as the total number of eggs eaten in that time. In 2010, CPB and LU neonates were placed on two eggs each, and only latency to cannibalize was measured.

Statistical Analyses

All statistical transformations and tests were performed in JMP® 8.0.2 (SAS Institute, Inc., Cary, NC).

Clutch size

The total number of eggs and numbers of intact and viable eggs and hatchlings were all square-root transformed to best improve their fit to a normal distribution. Clutch sizes, the number of intact and viable eggs, and the number of hatchlings were compared between populations and between years by ANOVA or *t* tests. Separate ANCOVAs for each year also compared the number of intact eggs between population, accounting for the total number of eggs and their interaction as fixed effects; models for each population included year, the total number of eggs, and their interaction. All nonsignificant interactions were removed from models. Similar models compared the number of viable eggs between populations while accounting for the number of intact eggs. Models comparing hatchlings per clutch accounted for intact eggs in 2008 and when comparing years and accounted for viable eggs in 2009.

Plant size

S. rostratum plant volumes were log transformed to make the distributions more normal. Because not every location was surveyed every year, separate *t* tests compared populations within year and compared years for El Carmen; due to unequal variance between populations, Welch's *t* tests were used for comparisons in 2009. The sizes of plants used for oviposition (containing eggs or larvae) were compared between populations and years for each life stage utilizing the plants. *S. lanceolatum* leaf area was also log transformed and compared to the similarly-measured *S. rostratum* from El Carmen.

Population density

For most life stages, the number of beetles on each plant was transformed to best fit a normal distribution. The number of adults and eggs on each plant were square-root transformed; the number of clutches, small larvae, and large larvae were log transformed; the number of hatchlings was not transformed.

The number of adults per plant was compared between El Carmen and El Rodeo, with population, log-transformed plant volume, and their interaction as predictor variables; all nonsignificant interactions were removed from models. Plants with no adults were included in the analysis, as adults are able to walk and fly between plants and so distribute themselves freely.

For all other measures, all populations and years were analyzed together, with each combination of population and year as a separate level [e.g. El Rodeo (2009), El Carmen (2010)] to standardize the effect of plant size. The likelihood of plants of a given size having eggs present was compared between the CPB populations with a nominal regression that included plant size, population-year, and their interaction. The number of clutches, eggs, small larvae, and large larvae were fit to multiple regression models with the same predictor variables and their interactions, including only plants with the relevant life stage, as these life stages were not able to freely disperse to empty plants. Planned comparisons between populations within year and between years within population were conducted.

CPB and LU feed on different host plants, which were measured in different ways, so direct comparisons of density cannot be made. However, the number of individuals per plant was compared by correcting the number of beetles at each life stage for plant size, with corrections made for each host plant separately. This correction effectively allowed for a comparison of the least-squares means of individuals at each life stage per plant between all populations.

Cannibalism

Latency to cannibalize (including only neonates that ate at least one egg) was square-root transformed; latency and the number of eggs eaten in 2009 were analyzed with mixed models including population as a fixed effect and the natal clutch of each neonate as a random effect. In 2010, latency to cannibalize was analyzed with a mixed model including population as a fixed effect and natal clutch and date as random effects. (There was no overlap in assay dates between populations in 2009, precluding adding date as a random effect). Latency to cannibalize was also analyzed within population by mixed models, with year as a fixed effect and natal clutch as a random effect. Due to unequal replications with random effects, all tests were by restricted maximum likelihood (REML).

Results

Clutch Size

Clutch sizes differed between populations and, in some populations, between years. Population differences in the total number of eggs, the number of intact and viable eggs, and the number of hatchlings in a clutch, as well as the hatch rate of intact and viable eggs for each year are shown in Figure 1. The statistical results of population comparisons for each year are given in Table 1; the results of within-population comparisons between years are given in Table 2. For all significant results, pair-wise comparisons were made by Tukey Honestly Significant Difference (HSD) tests ($\alpha = 0.05$).

In 2008, El Carmen and Totalco had clutch sizes that were not different from each other, but both had fewer eggs than clutches from El Rodeo ($P < 0.0001$). In 2009, clutches from El Rodeo were again larger, and clutches from Totalco were also significantly smaller than those from El Carmen ($P < 0.0001$). In 2010, there was no difference in clutch size between Totalco and El Carmen ($P = 0.11$). Clutch size did not change between years in El Rodeo ($P = 0.18$) or in El Carmen ($P > 0.99$). However, clutch size decreased over time in Totalco, with clutches in 2010 significantly smaller than those in 2008; clutches in 2009 were intermediate ($P = 0.005$).

The number of intact eggs when taking clutch size into account, a measure of the proportion of intact eggs, varied between populations and years. When taking clutch size into account, there was not a population difference in the number of intact eggs in a clutch in 2008 ($P = 0.41$), but there was an interaction between population and clutch size ($P = 0.008$), with Totalco having fewer than expected intact eggs in larger clutches. Overall, El Rodeo had more intact eggs in each clutch ($P < 0.0001$). El Rodeo had fewer intact eggs than expected based on clutch size in 2009 ($P = 0.022$), but still more intact eggs overall ($P < 0.0001$). In 2010, there was no population difference in the number of intact eggs, when corrected for clutch size ($P = 0.21$) or overall ($P = 0.86$). In El Rodeo, there were marginally fewer intact eggs than expected in 2009 compared to 2008 based on clutch size ($P = 0.055$) and overall ($P = 0.056$). In El Carmen, there were fewer intact eggs than expected in 2010 compared to other years ($P = 0.0006$), as well as an interaction between clutch size and year ($P = 0.010$), with fewer intact eggs than expected in small clutches in 2010; there were also marginally fewer intact eggs overall in 2010 compared to other years ($P = 0.068$). In Totalco there were also fewer intact eggs in 2010 than expected by clutch size ($P < 0.0001$) and overall ($P < 0.0001$).

The number of hatchlings when accounting for the number of intact or viable eggs was used as an indication of natural intraclutch cannibalism. While seemingly viable eggs (those that develop pigmentation spots as they mature) sometimes fail to hatch even without cannibalism, such hatching failure is rare (< 3% of eggs in video recordings of hatching clutches from these populations) and did not differ between populations ($\chi^2_2 = 1.60$, $P = 0.45$). Differences in the hatch rate of eggs are thus expected to be an accurate reflection of differences in intraclutch cannibalism rates.

Hatch rate did vary between populations in some years. When correcting for the number of intact eggs, there was no significant population difference in the number of hatchlings per clutch in 2008 ($P = 0.12$), but there was an interaction between population and the number of intact eggs ($P = 0.030$). El Carmen and Totalco had regression slopes of 0.95 and 0.96, respectively, indicating a hatch rate independent of clutch size, while El Rodeo had a regression slope of only 0.74, with fewer hatchlings than expected in large clutches. However, there were still more hatchlings from El Rodeo clutches than those from Totalco ($P = 0.0002$). There was no population difference in the number of viable eggs when correcting for the number of intact eggs in 2009 ($P = 0.54$), but El Rodeo had no more viable eggs overall than the other populations ($P = 0.20$). Additionally, there were fewer hatchlings than expected in El Rodeo clutches, based on the number of viable eggs ($P = 0.0002$), although there were still more hatchlings from El Rodeo clutches than those from Totalco ($P = 0.025$). There was no difference between 2008 and 2009 in the number of hatchlings per clutch, when correcting for the number of intact eggs, in El Rodeo ($P = 0.29$), El Carmen ($P = 0.16$), or Totalco ($P = 0.11$), and regression slopes remained nearly the same between years (0.73, 1.00, and 0.94, respectively, in 2009). However, there were marginally fewer hatchlings overall in 2009 in El Rodeo ($P = 0.078$) and Totalco ($P = 0.095$), but not in El Carmen ($P = 0.16$).

Plant Size

In 2009, plants in El Rodeo were larger than those in El Carmen (Welch's *t* test: $t_{51} = 2.88$, $P = 0.007$). In 2010, plants in El Carmen were the same size as those in Totalco (*t* test: $t_{27} = 0.84$, $P = 0.41$). As expected due to plant growth, plants in El Carmen were marginally larger in 2010 than in 2009 (*t* test: $t_{40} = 1.75$, $P = 0.087$). However, the size of plants utilized for eggs or larvae were not different between populations or year for any life stage ($P > 0.20$).

Population Density

The statistical results of the effect of population-year and plant size on the number of beetles at each life stage are given in Table 3. Least-squares means for the standardized number of each life stage for each population-year are shown in Figure 2.

Adults

When taking into account the size of the plants on which the adults were found, there was no main effect of population ($P = 0.17$), although there was a positive effect of plant size ($P < 0.0001$), and an interaction between plant size and population ($P = 0.003$), with a higher density of adults on larger plants in El Rodeo.

Eggs

Larger plants were more likely to have clutches on them (G test: $G_1 = 29.47$, $P < 0.0001$). There was a population effect on the likelihood of plants to be used for oviposition (G test: $G_3 = 15.38$, $P = 0.002$), with plants in Totalco (2010) more likely to have eggs. There were no other differences between populations or years. For plants with clutches present, plant size had no effect on clutch number ($P = 0.21$), but egg number increased with plant size ($P = 0.024$). There was also a marginal interaction between population-year and plant size on egg number ($P = 0.070$), with egg number increasing more rapidly with plant size in El Rodeo (2009) than elsewhere. Taking plant size into account, there was no difference in clutch number per plant between populations in 2009 ($P = 0.95$), but El Rodeo's larger clutches resulted in more eggs per plant than in El Carmen ($P = 0.027$). In 2010, plants in Totalco had more clutches than expected by plant size compared to El Carmen ($P = 0.020$), with marginally more eggs per plant ($P = 0.074$). There was no difference between years in El Carmen in clutch number ($P = 0.27$) or egg number ($P = 0.27$).

Larvae

There was no effect of plant size on the number of hatchlings per plant ($P = 0.66$), nor was there a difference between populations or years in hatchling number ($P > 0.23$). Plant size did not affect the number of small larvae per plant ($P = 0.31$), and there was no difference in the number of small larvae per plant between El Rodeo and El Carmen in 2009 ($P = 0.57$), but there were more small larvae than expected in Totalco than in El Carmen in 2010 ($P = 0.006$). There was no difference between years in El Carmen ($P = 0.29$). The number of large larvae per plant increased with plant size ($P = 0.004$), but there were no population or year differences ($P > 0.28$).

L. undecimlineata

S. lanceolatum, on average, had less leaf area per plant than the *S. rostratum* estimated in the same way (*t* test: $t_{21} = 3.46$, $P = 0.0023$), although some plants (3 of 22) were much (more than 3 times) larger than the comparison *S. rostratum*. Analyses on population density pooled across years, taking plant size into account separately for CPB and LU; statistical results are shown in Table 4, and species differences are demonstrated in Figure 2.

The number of LU adults per plant increased with increasing plant size (ANOVA: $F_{1, 20} = 39.75$, $P < 0.0001$). There were more LU adults per plant, compared to CPB ($P = 0.001$), with up to eight LU adults on a single plant, compared to three adult CPB per plant in El Rodeo and no more than one per plant in El Carmen.

The likelihood of a plant having LU eggs on it increased marginally with plant size (*G* test: $G_1 = 3.70$, $P = 0.054$); the number of clutches (ANOVA: $F_{1, 13} = 7.38$, $P = 0.018$) and eggs (ANOVA: $F_{1, 13} = 6.72$, $P = 0.022$) per plant also increased with increasing plant size. There was an effect of population on the proportion of plants with clutches ($\chi^2_3 = 39.01$, $P < 0.0001$), with *S. lanceolatum* more likely to have eggs than *S. rostratum* plants at any CPB site. There was a population effect on the number of clutches per utilized plant ($P = 0.022$), but this was due to differences between CPB populations, as LU was not different from any population. However, LU females are larger and lay larger clutches than CPB (Fig. 1; Welch's ANOVA: $F_{3, 54.9} = 105.91$, $P < 0.0001$), so that there were more LU eggs per plant than for any CPB population (*P*

< 0.0001). When correcting for clutch size, LU clutches observed in the lab produced as many hatchlings as expected compared to El Carmen and Totalco, but more than compared to El Rodeo (ANOVA: $F_{3,\,107} = 8.40$, $P < 0.0001$). Overall, there were more LU hatchlings per clutch than for any CPB population (Fig. 1; Welch's ANOVA: $F_{3,\,41.0} = 36.03$, $P < 0.0001$).

The number of LU hatchlings increased with increasing plant size (ANOVA: $F_{1,\,3} = 44.90$, $P = 0.007$), but plant size did not have an effect on the number of small larvae (ANOVA: $F_{1,\,4} = 2.06$, $P = 0.22$) or large larvae (ANOVA: $F_{1,\,3} = 0.15$, $P = 0.72$). There were more LU hatchlings per plant than for any CPB population ($P = 0.009$), and also more LU small larvae per plant ($P = 0.0005$). However, there was no population difference in the number of large larvae per plant ($P = 0.26$).

Cannibalism

Latency to cannibalize for each population for both years is shown in Figure 3. The statistical results for these comparisons are given in Table 5.

In 2009, neonates from El Carmen waited longer to eat their first egg than did neonates from Totalco or El Rodeo ($P = 0.001$). However, there was no difference in the total number of eggs eaten (LS mean ± SE: 1.8 ± 0.1 eggs; $P = 0.14$). In 2010, the trend reversed, with neonates from Totalco waiting longer to eat their first egg than did neonates from El Carmen ($P = 0.040$). There was no latency difference between years in El Carmen ($P = 0.13$), but Totalco waited longer to eat in 2010 than in 2009 ($P = 0.007$).

Pooling across years, LU neonates were much more likely to refuse to eat an egg than CPB neonates (Chi-square test: $\chi^2_1 = 118.39$, $P < 0.0001$). Only 13 of 29 LU neonates ate at least 1 egg in 14 hours, compared to 259 of 263 CPB neonates. However, the LU neonates that did eat an egg did not wait longer to eat than CPB neonates (*t* test: $t_{270} = 0.70$, $P = 0.48$).

Discussion

Of the surveyed CPB populations, El Rodeo laid the largest clutches and had the most intact eggs and hatchlings, generating the highest within-clutch competition. This higher competition was reflected in El Rodeo having a higher density of eggs on plants than El Carmen. Females in Totalco laid the smallest clutches but were more likely than those in El Carmen to lay eggs on plants that already had clutches present, generating a slightly higher density of eggs than in El Carmen and more between-clutch competition. Interestingly, while egg number increased with plant size, the numbers of hatchlings and small larvae on plants were not correlated with plant size, perhaps indicating density-independent mortality. However, the number of large larvae per plant was positively correlated with plant size, which may reflect resource limitation by the time larvae complete development. While El Rodeo and Totalco had the highest potential competition (i.e. greatest egg density), and Totalco had the highest actual competition during early development (i.e. greatest density of small larvae), there were no population differences in the density of large larvae, suggesting similar equilibria reached by all populations by this life stage.

In unmanipulated clutches, there is evidence for higher intraclutch cannibalism rates in El Rodeo, the population with the highest intraclutch competition. Overall, all populations had the same hatch rate in 2008, although El Rodeo had lower hatch rates in large clutches, indicating greater cannibalism in those clutches, even though there were still more hatchlings produced per clutch than in the other populations. When taking into account the number of viable eggs in clutches in 2009, El Rodeo had lower hatch rates than the other populations, again indicating higher cannibalism rates, with only slightly more hatchlings resulting from each clutch than in other populations.

Observed egg cannibalism rates may depend directly on clutch size and hatch time variation, which affect egg availability when neonates are old enough to consume eggs. Cannibalism rates were found to increase generally with clutch size in U.S. CPB populations (M. B. Baker, K. Hossain, K. Collie, unpublished data), but this seems to only be the case in El Rodeo in Mexico, as the regression of hatchlings on intact eggs had a slope nearly equal to 1 in the other populations.

While clutch traits influence the opportunity to cannibalism, the individual assays, which presented all tested neonates with the same opportunity to consume eggs, thus measuring their actual propensities, demonstrated that there are population differences in cannibalism behavior independent of clutch differences influencing opportunity. In 2009, cannibalism propensity reflected apparent competition, as neonates from Totalco and El Rodeo ate eggs earlier than did neonates from El Carmen. However, neonates waited longer to eat an egg in Totalco in 2010 than they had in 2009, resulting in a longer latency than observed in neonates in El Carmen.

This reversal of cannibalism propensity may reflect temporal variation in within-population competition. Clutch size in Totalco decreased between 2008 and 2010; although plants in Totalco were not surveyed in 2009, this decrease in clutch size over time should have had the effect of reducing egg density on plants, and thus lessened pressure for cannibalism to reduce this competition. The higher density of small larvae in Totalco than El Carmen in 2010 could be an indication of higher competition (a counter-example to the above hypothesis), or it could be an effect of reduced cannibalism. As the population surveys only give a snapshot of density at one point in time, they do not provide the data necessary to determine the direction of causality.

The temporal difference in cannibalism propensity may alternatively have been a result of the different assay conditions between years, as neonates were given 5 eggs in 2009 but only 2 eggs in 2010. If neonates adjust cannibalism propensity to clutch size, the smaller "foster" clutches in 2010 may have reduced perceived competition and cannibalism rates, although the lack of effect on El Carmen neonates would still indicate population differences in plasticity. As average clutch sizes remained remarkably invariable across years in El Carmen, there may be reduced selection for behavioral plasticity in this population (Mock and Parker 1997).

There was not a population difference in the number of eggs eaten by neonates, with neonates from all populations eating an average of approximately two eggs, indicating that latency to cannibalize may be the more important factor in reducing competition. Hatchlings facing greater competition may need to act more quickly to remove potential future competitors before they hatch, since larvae do not cannibalize other larvae in this species. Neonate consumption of multiple eggs, even in populations with longer latency, indicate that reduction of competition is still important, if less urgent, as neonates do not gain an additional nutritional benefit from eating multiple eggs (Collie et al. 2013).

Egg cannibalism may be a form of parent-offspring conflict, with neonates increasing their own fitness at the expense of the fitness of their mother (Perry and Roitberg 2005). However, excess egg-laying may be a maternal strategy to defend against egg predation, which varies between populations and years. If predation risk is unpredictable from season to season, females may do well to lay extra eggs; in years when predation is high, a few eggs may still survive in a large clutch, and in years when predation is low, sibling egg cannibalism can reduce brood size to what can be supported by available resources (Mock and Parker 1997). Females in different populations may thus be utilizing different strategies to maximize their fitness in the face of unpredictable predation and predictable resource limitation.

LU was found at a higher density than CPB at all life stages except large larvae and laid much larger clutches, resulting in much higher expected competition, especially since the average leaf area for its host *S. lanceolatum* was lower than that for the CPB host, *S. rostratum*. However, LU neonates were much less likely to cannibalize an egg than CPB neonates. This unexpected result may be due to higher predation reducing competition without necessitating cannibalism; in some clutches, we observed multiple unidentified maggots living in burrows they had made within LU clutches and feeding on the eggs, and the shift from a high density of small larvae to a low density of large larvae may also indicate high predation at this life stage. Competition is, however, still important in this species, as the study population is known to largely defoliate the available plants by the end of the season (Baena and Macías-Ordóñez 2012). Reduced cannibalism in this species may also be due to higher costs of cannibalism (if eggs are chemically defended) or reduced benefits (if eggs are less nutritious) than are present in CPB (Collie et al. 2013).

In addition to population differences in per capita plant quantity, populations (and species) may differ in plant quality, which could also influence selection for cannibalism. Other CPB populations in between El Rodeo and El Carmen showed genetic differentiation at the *AChE2* locus; the AChE enzyme is inhibited by steroidal alkaloids that are concentrated in the native host (Piiroinen et al. 2013). Population genetic differences may thus indicate differences in plant alkaloids between the sites. Plant differences may also play a selective role in the differences between CPB and LU in willingness to cannibalize.

Genetic structure within populations is largely unknown. Goff and Stevens (1995) hypothesized that kin selection, even in the absence of kin discrimination, should result in lower intraclutch cannibalism rates in populations with higher average within-clutch relatedness. CPB clutches are a mixture of full- and half-sibling eggs. In the laboratory, females may mate up to 20 times in under 2 months (Szentesi 1985), but mate number in the field is unknown. If average mate number, and thus intraclutch relatedness, differs between populations, this may also contribute to population differences in cannibalism.

While the present study clearly identifies population differences in cannibalism behavior, many questions still remain. Population comparisons are insufficient to establish selective causality, but they provide insight into probable causes of adaptive divergence and so are valuable starting points for further experimental studies (Foster 1999). The extent to which cannibalism as a means of brood reduction is adapted to local competition can be investigated by manipulating the number of hatchlings on experimental plants to determine the influence of density on growth, mortality, and adult fecundity.

Measuring behavior under common conditions, as was the case in the present study, can identify population differences, but measuring phenotypic plasticity can be more informative, especially when studying conditional strategies. Female response to the presence of egg predators can be directly measured to ascertain whether females strategically adjust clutch size. Neonate response to perceived clutch size also needs to be further investigated.

Finally, this study does not compare population differences of indigenous CPB populations on buffalo bur to U.S. populations on potato. Cannibalism may have played a role in the host-plant shift and subsequent spread of CPB; egg cannibalism, as an alternative food source for neonates, may have enhanced the ability of larvae to develop on the novel host, potato.

Increased development rates resulting from egg cannibalism (Collie et al. 2013) may also have enhanced migration into northern climates with shorter growing seasons. Cannibalism rates may thus be expected to be higher in U.S. populations than in the native populations; cannibalism increased with northern expansion in southwestern cornborers, reducing competition for limited below-ground overwintering sites in climates with harsher winters (Tarpley et al. 1993). However, population comparisons are confounded by these very differences in climate and host plant. A common-garden experiment, in which individuals from multiple pure and hybrid populations are reared in the same environment on the same host-plant species, should reduce developmental differences and maternal effects, allowing genetic differences in the cannibalism rates of their offspring to be better elucidated.

Table 1. Effect of population on the number of total, intact, and viable eggs and hatchlings within a clutch in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	2008		**2009** **Population**		**2010**	
	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	***t* (*df*)/*F* (*df*, *df*)**	***P***
Unadjusted						
Total Eggs	66.48 (2, 146)	< 0.0001	89.24 (2, 112)	< 0.0001	1.65 (45)	0.11
Intact Eggs	36.44 (2, 31.6)	< 0.0001	38.49 (2, 61.4)	< 0.0001	0.18 (18)	0.86
Viable Eggs			1.75 (2, 20.1)	0.20		
Hatchlings	13.48 (2, 18.8)	0.0002	4.19 (2, 29.7)	0.025		
Adjusted						
Intact Eggs	0.90 (2, 143)	0.41[1]	3.95 (2, 111)	0.022	1.67 (1, 17)	0.21
Viable Eggs			0.63 (2, 38)	0.54		
Hatchlings	2.13 (2, 91)	0.12[2]	10.86 (2, 35)	0.0002		

Unadjusted tests are direct comparisons between populations; adjusted tests take into account the total number of eggs (for the number of intact eggs), the number of intact eggs (for the number of viable eggs and the number of 2008 hatchlings), or the number of viable eggs (for the number of 2009 hatchlings). These correlations were all significant ($P = 0.001$ for viable eggs by intact eggs; $P < 0.0001$ for all others). Unadjusted tests were by *t* test (2010), ANOVA (total eggs), or Welch's ANOVA for unequal variance (all others). Adjusted tests were by ANCOVA.

[1] There was a significant interaction between the total number of eggs and population (ANOVA: $F_{2,143} = 5.05$, $P = 0.008$).

[2] There was a significant interaction between the number of intact eggs and population (ANOVA: $F_{2,91} = 3.63$, $P = 0.030$).

Table 2. Effect of year on the number of total and intact eggs and hatchlings within a clutch in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	El Rodeo		**El Carmen**		**Totalco**	
			Year			
	***t* (*df*)/*F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***
Unadjusted						
Total Eggs	1.34 (86)	0.18	0.001 (2, 41)	> 0.99	5.37 (2, 176)	0.005
Intact Eggs	1.94 (87)	0.056	2.90 (2, 37)	0.068	14.92 (2, 153)	< 0.0001
Hatchlings	1.79 (57)	0.078	2.15 (19)	0.16	1.72 (86)	0.095
Adjusted						
Intact Eggs	3.79 (1, 85)	0.055	9.27 (2, 34)	0.0006[1]	14.94 (2, 152)	< 0.0001
Hatchlings	1.15 (1, 51)	0.29	2.17 (1, 15)	0.16	2.56 (1, 80)	0.11

Unadjusted tests are direct comparisons between years; adjusted tests take into account the total number of eggs (for the number of intact eggs) or the number of intact eggs (for the number of hatchlings). These correlations were all significant ($P < 0.0001$). Unadjusted tests were by Welch's *t* test for unequal variances (El Rodeo intact eggs and Totalco hatchlings), *t* test (all other tests for El Rodeo or hatchlings), or ANOVA (all others). Adjusted tests were by ANCOVA.

[1] There was a significant interaction between the total number of eggs and year (ANOVA: $F_{2, 34} = 5.36$, $P = 0.010$).

Table 3. Effect of population and plant size on the number of adults, clutches and eggs, hatchlings, and small and large larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, per *Solanum rostratum* plant.

	Population-year		**Plant size**		**Population-year*plant size**	
	***F*/*t* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***	***F* (*df*, *df*)**	***P***
Adults	1.96 (1, 48)	0.17	17.94 (1, 48)	< 0.0001	9.75 (1, 48)	0.003
Clutches			1.66 (1, 24)	0.21		
ER/EC (2009)	0.06 (12)	0.95				
EC/T (2010)	2.49 (14)	0.020				
2009/2010 (EC)	1.13 (9)	0.27				
Eggs			5.94 (1, 21)	0.024	2.73 (3, 21)	0.070
ER/EC (2009)	2.38 (12)	0.027				
EC/T (2010)	1.88 (14)	0.074				
2009/2010 (EC)	1.12 (9)	0.27				
Hatchlings			0.22 (1, 6)	0.66		
ER/EC (2009)	0.98 (3)	0.37				
EC/T (2010)	1.34 (4)	0.23				
2009/2010 (EC)	0.64 (2)	0.54				
Small Larvae			1.08 (1, 15)	0.31		
ER/EC (2009)	0.58 (7)	0.57				
EC/T (2010)	3.18 (9)	0.006				
2009/2010 (EC)	1.10 (10)	0.29				
Large Larvae			10.28 (1, 23)	0.004		
ER/EC (2009)	0.27 (17)	0.79				
EC/T (2010)	0.94 (7)	0.36				
2009/2010 (EC)	1.10 (9)	0.28				

Adults per plant were averaged over all plants, including those that had no adults, which were free to disperse to empty plants. All other life stages were averaged only over plants that had that life stage present, as eggs and larvae cannot disperse to empty plants. Analyses included a separate level for each population each year. The effect of plant size and its interaction with population-year were taken over all population-years. The effect of population-year was assessed through planned comparisons between El Rodeo (ER) and El Carmen (EC) in 2009, between El Carmen and Totalco (T) in 2010, and between 2009 and 2010 in El Carmen.

Table 4. Effect of *Leptinotarsa* species and *Leptinotarsa decemlineata* population on the number of adults, clutches and eggs, hatchlings, and small and large larvae per *Solanum* plant.

	Population/Species	
	***F*/*t* (*df*, *df*)**	***P***
Adults	8.43 (2, 34.6)	0.001
Clutches	3.59 (3, 40)	0.022
Eggs	22.06 (3, 17.3)	< 0.0001
Hatchlings	13.63 (3, 4.7)	0.009
Small Larvae	43.13 (3, 5.0)	0.0005
Large Larvae	1.40 (3, 29)	0.26

Adults per plant were averaged over all plants, including those that had no adults, which were free to disperse to empty plants. All other life stages were averaged only over plants that had that life stage present, as eggs and larvae cannot disperse to empty plants. Comparisons involved *Leptinotarsa undecimlineata* and the Colorado potato beetle, *L. decemlineata*, populations, pooled across years, with beetle counts corrected separately by species for plant size. Rows with decimal *df* were tested by Welch's ANOVA to correct for unequal variances.

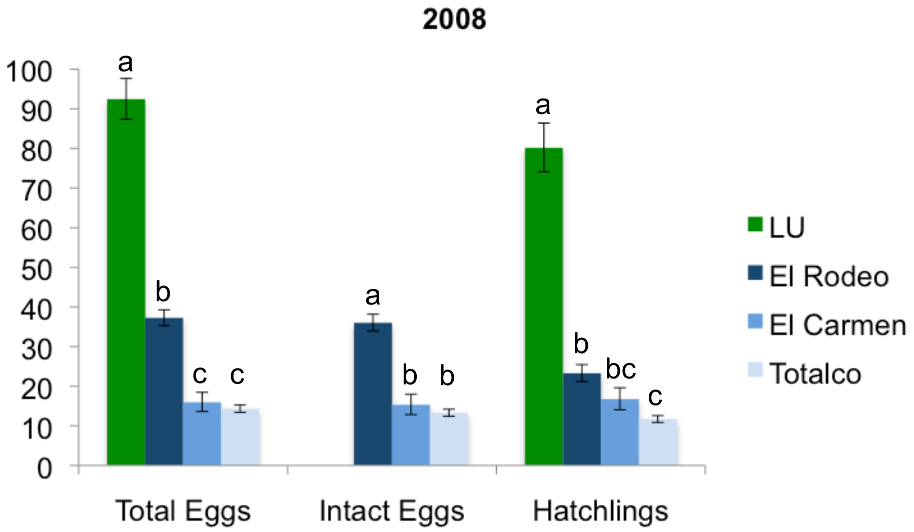
Table 5. Effect of year or population on latency to cannibalize, in minutes, and the total number of eggs eaten in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	***F* (*df*, *df*)**	***P***
Latency to Cannibalize		
2009	12.86 (2, 11.4)	0.001
2010	4.57 (1, 34.9)	0.040
El Carmen	2.88 (1, 7.3)	0.13
Totalco	10.51 (1, 12.2)	0.007
Number Eaten (2009)	2.47 (2, 9.1)	0.14

In 2009, latency to cannibalize and the number of eggs eaten were tested for population differences; in 2010, population differences for only latency were tested. Differences in latency between 2009 and 2010 were tested for El Carmen and Totalco. All tests were by REML.

Figure 1. The number of total, intact, and viable eggs and hatchlings from unmanipulated clutches for three populations of Colorado potato beetle, *Leptinotarsa decemlineata*, and one population of *L. undecimlineata* (LU) in a) 2008, b) 2009, and c) 2010, and d) the hatch rate of intact (H/I) eggs in 2008 and 2009 and viable (H/V) eggs in 2009. Intact eggs were those that were undamaged at collection (damaged eggs had presumably been predated). Viable eggs were those with developing embryos. Reverse-transformed averages for each population and year are shown ± SE. Hatch rates were calculated as the reverse-transformed LS means of hatchlings (± SE) divided by the reverse-transformed standardized number of intact or viable eggs from which the LS means of hatchlings was calculated. H/I for 2009 is presented for comparison to 2008, but separate statistical analyses were not conducted. Statistical comparisons between populations are given in Table 1, and comparisons between years are given in Table 2. Different letters indicate significant differences within each category. See text for discussion of year-to-year differences.

a)



b)

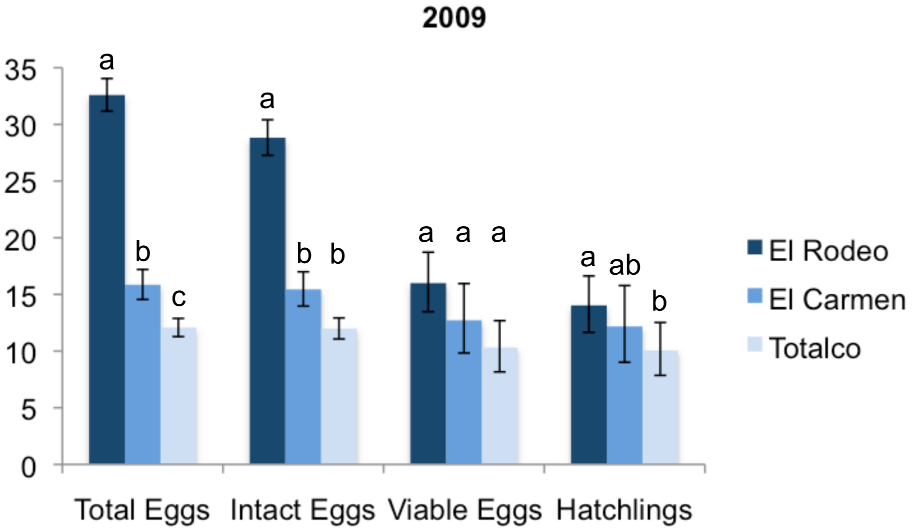


Figure 1 cont.

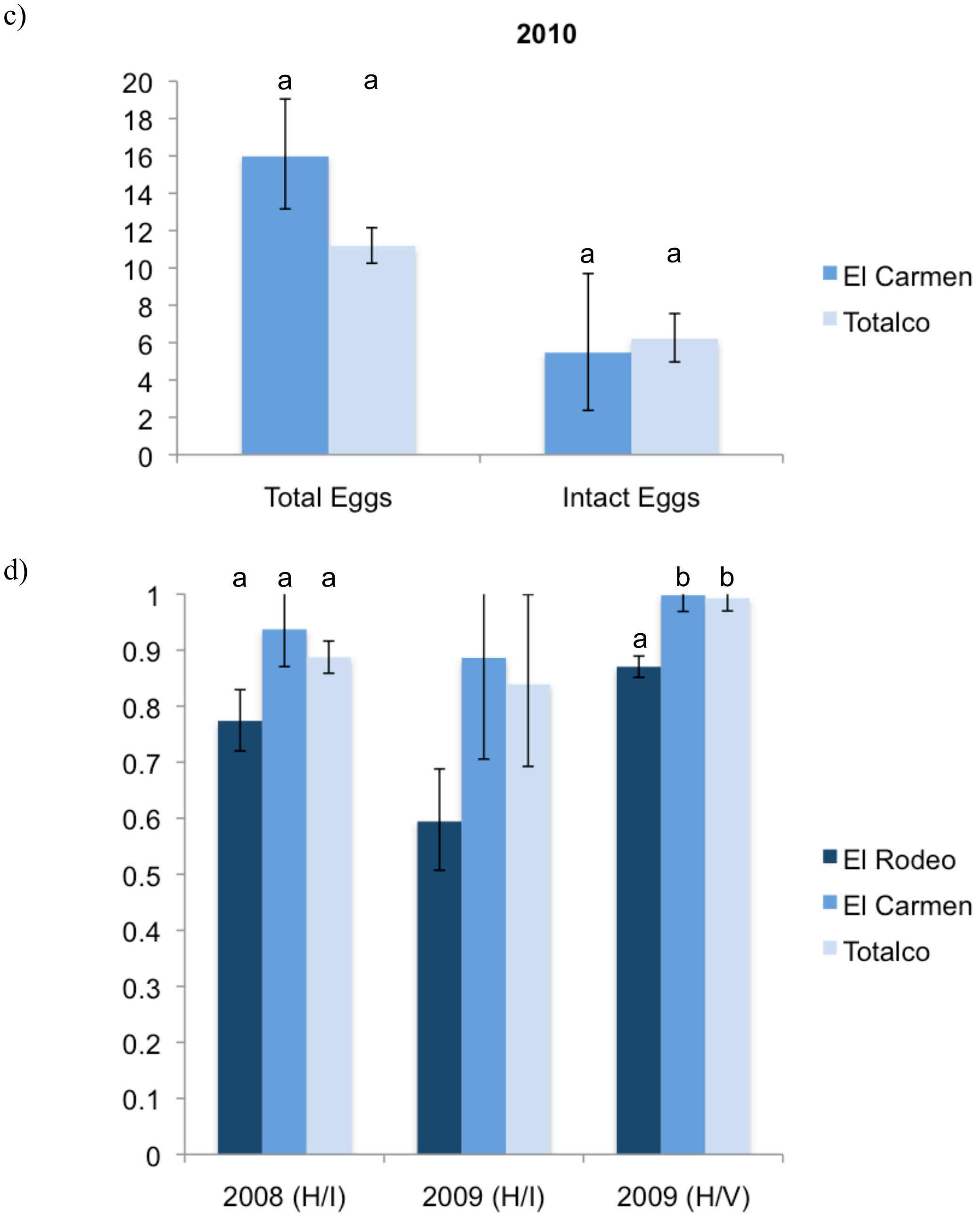


Figure 2. Population density of a) adults and b) other life stages of *Leptinotarsa undecimlineata* (LU) in 2008 and Colorado potato beetle, *L. decemlineata* at three different sites over two years. Reverse-transformed LS means for each population and year are shown ± SE, controlling for plant size within each species; note the log scale in b). Statistical comparisons between populations are given in Table 3, and comparisons between years for El Carmen are given in Table 4. * indicates $P < 0.05$; # indicates $P < 0.10$.

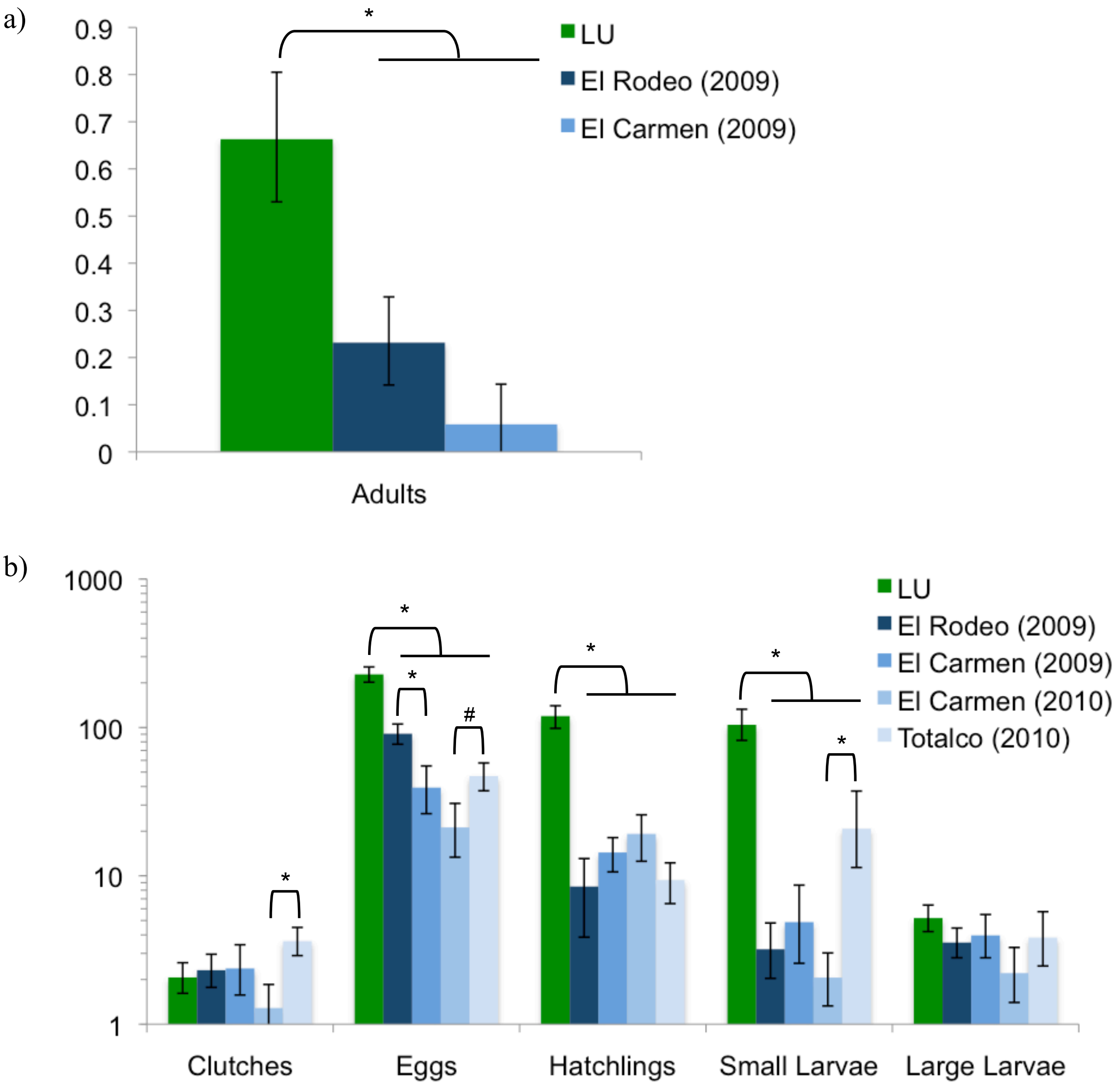
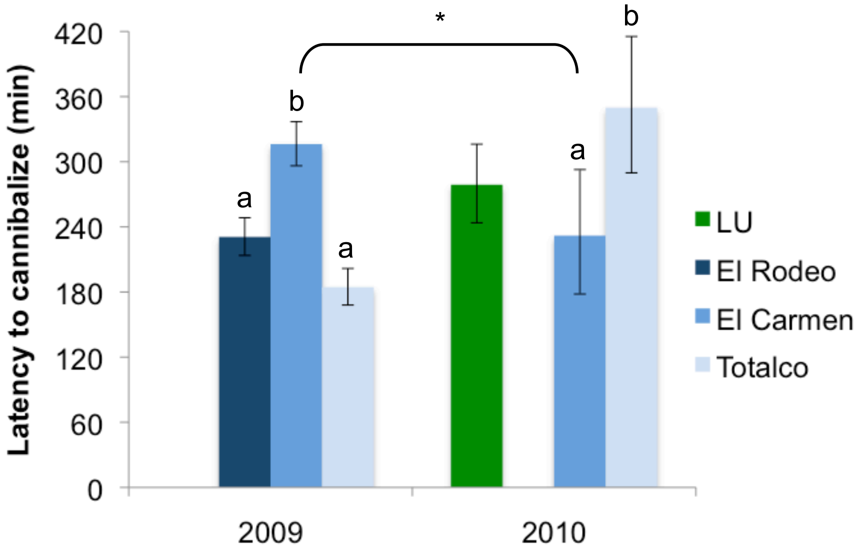


Figure 3. Latency in minutes for neonates of the Colorado potato beetle, *Leptinotarsa decemlineata*, and of *L. undecemlineata* (LU) to cannibalize their first egg. Reverse-transformed LS means for each population and year are shown ± SE, controlling for natal clutch in 2009 and controlling for natal clutch and assay date in 2010. Different letters indicate significant differences within each category. LU was not different from any population pooled across years. * indicates $P < 0.05$ between years.



APPENDIX A: Fitness benefits of cannibalism on a low-quality diet

METHODS

Animals

Leptinotarsa decemlineata used in this experiment were from a lab colony derived from beetles collected from potato fields during the summer 2008 in Riverhead, NY.

Insect Rearing

Beetles were maintained prior to the experiment under the same conditions as described in CHAPTER 1: *Insect Rearing*.

Experimental Procedure

The experiment began on 27 January 2009 and terminated on 6 March 2009. All clutches collected from the lab colony were examined daily under a dissecting microscope to determine the degree of development, as indicated by pigmentation spots that appear approximately 24 hours before hatching and continue to develop as hatching nears. Well-pigmented eggs were checked hourly for hatching. Within 30 minutes of completing hatching, determined either by direct observation of hatching or by incomplete post-hatching melanization, neonates were moved by paintbrush into individual wells of plastic artificial diet trays. Any individuals of unknown hatching time that completed melanization prior to set-up were excluded to ensure that experimental individuals did not consume anything before receiving their experimental treatment. In total, 183 individuals from 20 clutches were used.

Diet trays consisted of 4 rows of 8 wells each. Each diet well was 2.7 cm x 2.7 cm and was filled approximately 1 cm deep with Colorado potato beetle artificial diet (Forrester, unpublished data). Neonates were either placed directly on the artificial diet with no eggs or were placed directly on either 1 or 2 immature eggs on the diet. All larvae provisioned with eggs were checked after 24 hours to verify consumption of the egg(s). Each well, containing a single individual, was sealed with a porous plastic sheet to prevent larval escape while permitting sufficient ventilation. All diet trays were incubated at 25° C and 16:8 L:D for the entirety of the experiment; the artificial diet was changed as needed.

Larval growth and development rate

All hatchlings were weighed with clutchmates prior to placing them in their individual wells, and the average mass for the group was used as the estimated hatching mass for each individual. As nearly as possible, larvae were weighed daily at the same time as they had hatched to reduce individual differences due only to age. The instar stage of each individual was determined at the time of weighing. Wells were resealed after each weighing.

Statistical Analyses

All statistical tests were performed in JMP® 8.0.2 (SAS Institute, Inc., Cary, NC).

No larvae survived to pupation, so complete larval growth slopes could not be calculated. Instead, the effect of egg consumption on daily mass was tested for each day with sufficient surviving larvae using a mixed REML model with egg consumption as a fixed effect and larval family (clutch) as a random effect.

Only a single larva survived to the third instar, so only the time to the second instar could be examined. The time to reach this moult was estimated by logarithmically regressing life stage as an ordinal factor on the age at each observation, using maximum likelihood to estimate the slope and intercept of an instar probability curves for each individual. These values were used to calculate the midpoint of the curve as the best estimate of the time to the moult. When individuals were actually observed moulting, the observed time was used as the estimate. The age at the moult was fit to a one-way ANOVA with egg consumption as the fixed effect.

Mortality curves were calculated for each experimental group. Mortality differences were analyzed by log-rank tests, grouping by egg consumption.

RESULTS

Egg consumption significantly increased larval mass through day 7 (Table 2; Fig. 1; $P < 0.03$). Egg consumption did not have a significant effect on the time to reach the second instar, although the trend was in the predicted direction (Table 2; $P = 0.33$). Egg consumption did have a significant effect on mortality (Fig. 2; Wilcoxon test: $\chi^2_2 = 11.6$, $P = 0.003$)—median age at mortality was 5.0 days for individuals eating 0 eggs, 6.0 days for those eating 1 egg, and 7.0 days for those eating 2 eggs.

Table 1. Effect of egg consumption with artificial diet on daily larval mass (in mg) of the Colorado potato beetle, *Leptinotarsa decemlineata*.

	0 Eggs Eaten	**1 Egg Eaten**	**2 Eggs Eaten**	R^2	**Clutch %**	**Cannibalism *F* (*df*, *df*)**	***P***
Day 1	0.2±0.05^a	0.8±0.05^b	1.0±0.06^c	0.75	37.1	164.70 (2, 148.3)	<0.0001
Day 2	0.4±0.06^a	0.8±0.06^b	1.0±0.07^c	0.68	49.4	84.14 (2, 141.7)	<0.0001
Day 3	0.4±0.08^a	0.8±0.07^b	1.0±0.09^c	0.57	34.9	36.68 (2, 86.7)	<0.0001
Day 4	0.4±0.09^a	0.7±0.08^b	1.0±0.10^c	0.53	36.0	19.00 (2, 81.6)	<0.0001
Day 5	0.6±0.10^a	0.8±0.09^{ab}	1.1±0.11^b	0.28	9.3	5.58 (2, 46.6)	0.007
Day 6	0.8±0.13^a	1.1±0.12^{ab}	1.3±0.13^b	0.18	1.7	3.88 (2, 40.1)	0.029
Day 7	0.8±0.21^a	1.7±0.22^b	1.5±0.21^b	0.52	27.5	6.56 (2, 27.9)	0.005
Day 8	1.1±0.48^a	1.6±0.29^a	1.5±0.28^a	0.16	7.0	0.52 (2, 16.9)	0.60
Day 9	1.2±0.74^a	1.8±0.45^a	1.7±0.52^a	0.08	2.9	0.26 (2, 13.0)	0.78

Statistical comparisons were made by restricted maximum likelihood (REML). Least square means ±SE are indicated for each egg consumption group, as well as the percentage of random variation explained by clutch effects. Different superscripted letters indicate significantly different means by Tukey's HSD test ($\alpha = 0.05$).

Table 2. Effect of egg consumption on the time to reach the second instar on artificial diet in the Colorado potato beetle, *Leptinotarsa decemlineata*.

	Mean ± SE	R^2	$F_{2,34}$	P
0 Eggs Eaten	5.3±0.8	0.06	1.13	0.33
1 Egg Eaten	4.6±0.5			
2 Eggs Eaten	4.0±0.4			

Figure 1. The effect of egg consumption on larval growth on artificial diet in the Colorado potato beetle, *Leptinotarsa decemlineata*. Lines connect the least square means of daily mass for each number of eggs consumed; error bars are ± SE. Restricted maximum likelihood (REML) tests on the daily masses included larval family (clutch) as a random effect and egg consumption as a fixed effect.

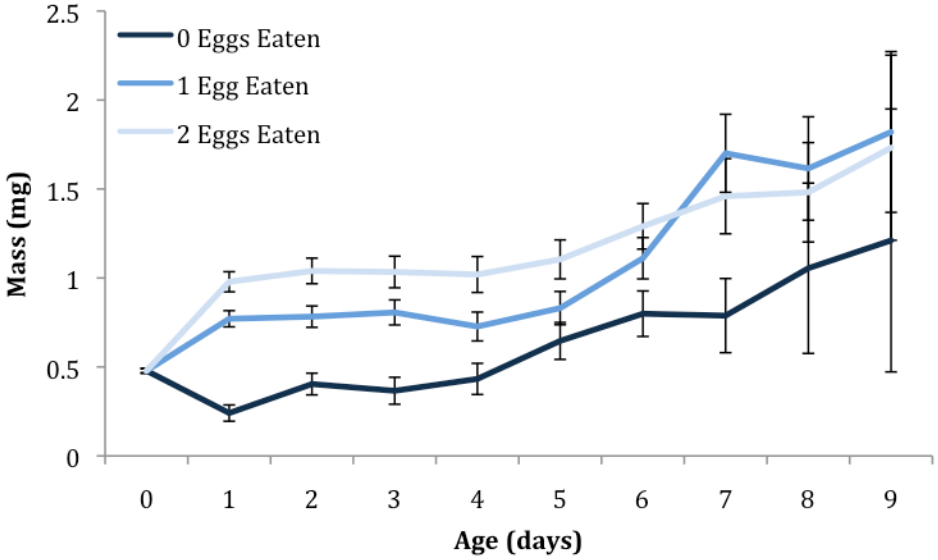
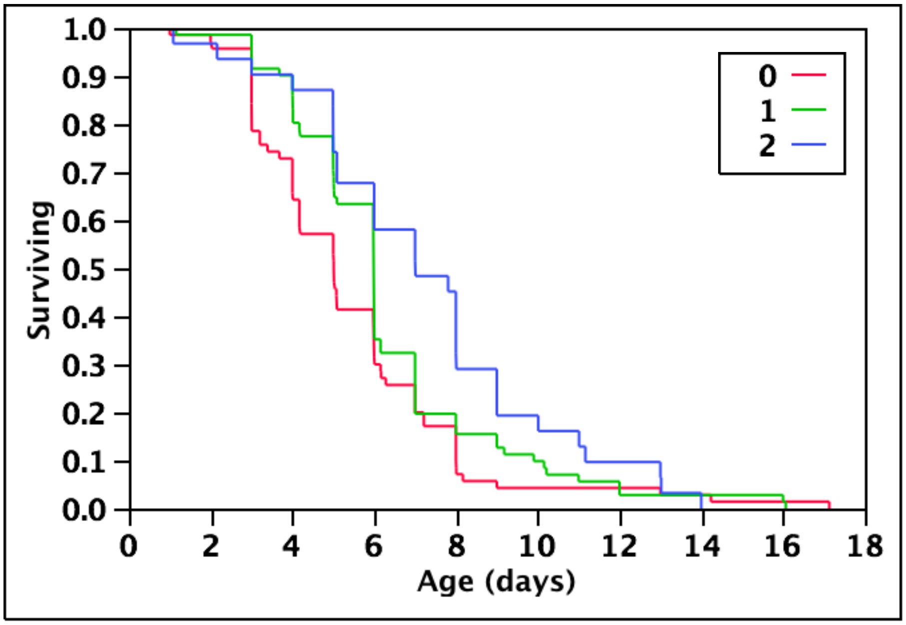


Figure 2. Mortality curves for larvae of the Colorado potato beetle, *Leptinotarsa decemlineata,* on artificial diet. Each line represents the mortality distribution for individuals provided no, one, or two eggs at hatching, with the proportion of each group still alive at each age interval.



APPENDIX B: Hatch time as a function of pesticide resistance

Baker et al. (2007) found that the eggs of imidacloprid-resistant beetles took 9.7-15 hours longer to hatch than the eggs of imidacloprid-susceptible beetles, with hybrid eggs intermediate in hatch time. I wanted to determine whether the more resistant larvae would hatch later within clutches with eggs of variable resistance.

METHODS

Animals

L. decemlineata used in these experiments were from two source populations, one imidacloprid-susceptible and one imidacloprid-resistant. The susceptible population was derived from clutches from a United States Department of Agriculture greenhouse-reared population in Maryland; this population was originally collected from fields in Beltsville, MD. The resistant population was founded with adults collected from potato fields in Riverhead, NY.

Trial 1

Experimental procedure

Susceptible and resistant adults were paired to created hybrid offspring. These F_1 hybrids were bred to form an F_2 hybrid generation, which was expected to show maximal diversity in resistance. F_2 eggs were collected from 9 F_1 pairs. Mature clutches were checked every 3 hours for hatchlings, up to 24 hours after the first egg hatched. Hatchlings were removed at each observation and reared under standard conditions by clutch and observation time to the second instar, for a total of 86 neonates (2 to 17 per clutch).

Second-day second instars were assayed with imidacloprid at a dose of 6E-6 mg/ml. Each larva received a 1µl drop applied to the dorsal abdomen. Assayed neonates were placed on a potato leaf in a petri dish and maintained in the standard incubator for another 24 hours before scoring. Larvae were scored for mortality by gently prodding larvae with a probe and looking for movement within 10 seconds. Larvae that did not move were scored as dead.

Statistical analysis

Mortality was calculated for each age group within each clutch as the number of dead larvae divided by the total number of larvae within that hatch time range. Mortality was Freeman-Tukey arcsin transformed (Zar 1999) to improve the fit to a normal distribution. The hatch time range was condensed to the median time within the range (e.g. 0-3 hours was recorded as 1.5 hours). The effect of hatch time on mortality was tested by REML, with hatch time as a fixed continuous effect and clutch as a random effect; mortality was weighted by the total number of larvae in each hatch time in each clutch.

Trial 2

Experimental procedure

F_2 hybrid eggs were generated as in *Trial 1*. F_2 eggs were collected from 4 F_1 pairs, with 13 to 20 clutches collected for each female. Mature clutches were checked every hour for hatchlings, up to 10 hours after the first egg hatched. Hatchlings were removed at each observation and reared under standard conditions by clutch and observation time to the second instar, for a total of 1239 neonates.

Assays were conducted as in *Trial 1*. Larvae were scored for mortality by gently prodding larvae with a probe and looking for movement within 10 seconds. Larvae that did not move were scored as dead. Larvae that moved but could not right themselves were scored as intoxicated.

Statistical analysis

Mortality was calculated for each age group within each clutch as the number of pooled dead and intoxicated larvae divided by the total number of larvae within that hatch time range. Mortality was Freeman-Tukey arcsin transformed to improve the fit to a normal distribution. The hatch time range was condensed to the median time within the range. The effect of hatch time on mortality was tested by REML, with hatch time as a fixed continuous effect. Family, clutch nested within family, and scorer (to account for scoring variation in undergraduate assistants) were included as random effects; mortality was weighted by the total number of larvae in each hatch time in each clutch.

RESULTS/DISCUSSION

Trial 1

While all clutches with larvae in multiple hatch times showed a general decrease in mortality in late-hatching larvae, the overall effect of hatch time was not significant (REML: $F_{1, 24.9} = 2.02$, $P = 0.17$).

Trial 2

Mortality significantly decreased with hatch time (REML: $F_{1, 311.5} = 4.67$, $P = 0.032$), with predicted reverse-transformed mortality (Miller 1978) 60% for larvae hatching in the first hour and only 50% for larvae hatching in the tenth hour.

Later hatching larvae within a clutch of variable resistance do appear to be more resistant than their earlier hatching siblings. In the field, especially in fields neighboring untreated refuges that may harbor more susceptible beetles, eggs within clutches are expected to show some variation in resistance. The delayed hatching of resistant larvae may make them more vulnerable to being cannibalized by their susceptible siblings, which would have the affect of reducing resistance alleles and slowing resistance evolution.

APPENDIX C: Female mediation of cannibalism in response to oviposition plant quality

Cannibalism may be a form of parent-offspring conflict (Mock and Parker 1997), or it may be a means that females use to provision offspring in the absence of other maternal care (Perry and Roitberg 2006). The conditions for females to facilitate cannibalism are more stringent than those for neonates to consume siblings (Perry and Roitberg 2005), so facilitation is only expected when larval survival is very low, as when eggs are deposited with little or low-quality food. Under these conditions, females may lay infertile eggs to guarantee an initial food source for neonates, increasing their chances of survival. To determine whether females respond plastically to plant condition when ovipositing, well-fed females were given access to either a high-quality plant (young and well-fertilized) or a low-quality plant (either beginning to senesce or given only half as much fertilizer) for oviposition.

METHODS

Experimental Procedure

High-quality plants were grown under standard conditions, planted with 2 tsp of fertilizer. They were used when they were in peak condition, four weeks post-sprouting. Low-quality plants were either planted with only 1 tsp of fertilizer and used at four weeks post-sprouting or planted with 2 tsp of fertilizer and used at six weeks post-sprouting, when they were just beginning to senesce (but would be senescent before larvae completed development).

Virgin females were collected from the lab colony upon emergence and maintained individually on high-quality foliage until they reached sexual maturity (five days post-eclosion), at which time they were mated with a single male each. Each pair was maintained on high-quality foliage at least until the female laid her first clutch. Reproductive females were placed singly in cages containing either a high-quality or one of the low-quality plants to use for oviposition. Females were left with the plants for 24 hours, insufficient time to affect female condition but enough time to lay eggs. Clutches were collected at the end of 24 hours. The total number of eggs and the number of clutches were counted upon collection. When clutches reached maturity three to four days later, evidenced by the appearance of pigmentation spots in mature eggs, the number of inviable eggs, those lacking such pigmentation spots, was counted.

Statistical Analysis

The probability of a female laying eggs on the plant was compared across treatments by a Chi-square test. For only those females laying eggs, the total number of eggs laid and the number of clutches were compared by separate ANOVAs. The number of eggs in each clutch was compared across treatments by REML, with treatment as a fixed effect and female as a random effect. The likelihood of a female laying infertile eggs was compared across treatments by a *G* test, with treatment, female, and clutch size as fixed effects (JMP does not support random factors in *G* tests). For only those females laying infertile eggs, the number of infertile eggs laid was compared across treatments by REML, with treatment and clutch size as fixed effects and female as a random effect. The ratio of infertile to fertile eggs within each clutch was also analyzed by REML, with treatment and clutch size as fixed effects and female as a random effect. The ratio of infertile to fertile eggs was also contrasted between high- and low-quality plants.

RESULTS/DISCUSSION

Females were less likely to oviposit on low-quality plants (Chi-square: $\chi^2_2 = 7.94$, $P = 0.019$). For females that did lay eggs, there was no effect of plant quality on the total number of eggs laid (ANOVA: $F_{2,45} = 0.02$, $P = 0.98$). However, females on under-fertilized plants distributed their eggs in slightly more clutches than those on old or high-quality plants (ANOVA:

$F_{2, 45} = 3.02$, $P = 0.059$), resulting in fewer eggs in each clutch (REML: $F_{2, 72.7} = 6.22$, $P = 0.003$). The likelihood of laying infertile eggs increased with clutch size (G test: $G_1 = 3.90$, $P = 0.048$) and varied among females (G test: $G_{37} = 54.08$, $P = 0.035$) but not plant quality (G test: $G_2 = 0.47$, $P = 0.79$). For only those females that laid infertile eggs, there was no effect of clutch size (REML: $F_{1, 2.7} = 0.22$, $P = 0.68$) or plant quality (REML: $F_{2, 12.1} = 0.32$, $P = 0.73$) on the number of infertile eggs laid, although there was a strong female effect (REML: 93.3% of random variation). The proportion of infertile eggs thus decreased with clutch size. The ratio between infertile and fertile eggs similarly decreased with clutch size (REML: $F_{1, 7.7} = 9.10$, $P = 0.017$), and a contrast between high- and low-quality plants showed a marginally higher ratio on low-quality plants ($F_{1, 11.9} = 3.55$, $P = 0.084$).

Females thus do not increase the number of inviable eggs when ovipositing on low-quality plants, but they divide eggs into a greater number of smaller clutches, so that at least some clutches have more inviable eggs available for each viable hatchling. Females may also mediate the opportunity for cannibalism by altering the hatching synchrony of their eggs (Perry and Roitberg 2005), a prospect for future research.

APPENDIX D: Cannibalism rates for U.S. populations

Table 1. Latency to cannibalize and the number of eggs eaten in 14 hours for U.S. populations of the Colorado potato beetle, *Leptinotarsa decemlineata*.

Population	Year	N	Eggs eaten	Eggs eaten SD	Latency to cannibalize (minutes)	Latency SD	Notes
MD	2007	10 (9)	1.1	0.6	479.3	186.0	Kin selection experiment (excluding neonates tested with eggs from a different population)
MD	2008	17 (12)	1.5	1.4	349.8	151.8	Kin selection experiment (excluding neonates tested with eggs from a different population)
MD	2008	11 (11)	1.9	1.3	366.4	245.0	Artificial selection experiment
Field MD	2008	6 (5)	1.8	1.3	476.2	318.1	Artificial selection experiment
MD x NY hybrid	2008	7 (7)	1.4	1.0	528.9	274.6	Artificial selection experiment
NY	2007	10 (9)	1.2	0.8	429.0	184.1	Kin selection experiment (excluding neonates tested with eggs from a different population)
NY	2008	34 (35)	1.4	0.6	403.3	205.3	Artificial selection experiment
NY	2009	8 (8)	1.4	0.5	424.8	234.5	Cannibalism videos
NY	2010	16 (15)	1.6	0.6	247.1	171.2	Cannibalism videos
Field NY	2008	8 (9)	2.5	0.9	226.7	88.5	Artificial selection experiment
ME	2008	(505)			357		Median of maximum latencies in an artificial selection experiment
ME	2010	4 (5)	1.25	0.5	283.4	163.1	Cannibalism videos

Samples sizes (N) in parentheses indicate the sample size for which latency was calculated (excluding individuals that did not eat an egg). For all populations except ME, larvae were recorded with a video camera or a digital microscope, and latencies and the number of eggs eaten represent the mean. For ME, larvae were checked periodically under a stereomicroscope, and latency is expressed as the median.

APPENDIX E: Clutch size and hatch time variability

METHODS

Clutches were collected from field populations in Mexico as described in Chpt. 4. The data for these populations on buffalo bur is from clutch sizes in the field and are presented here again for comparison. Adults from these populations were brought back to the U.S. and reared on potato in the laboratory. No clutch data could be collected from El Rodeo on potato, as adults from this population refused to eat potato and so laid no eggs in the lab. Adults from U.S. field populations and one laboratory standard colony (NJ) were similarly maintained on potato. Clutches were collected from these adults and counted. The number of viable eggs in clutches was also counted on potato in 2010.

Clutches from all three Mexico populations and NY were recorded under a Sony® model HDR-HC9 MiniDV HD Handycam® camcorder (Sony Corporation, Tokyo, Japan) using BTV Pro Carbon® 5.4.1 time-lapse software (Ben Bird, www.bensoftware.com) to determine the time that each egg hatched.

RESULTS/DISSCUSION

Clutch sizes for each population are given in Table 1. The means, standard deviations, medians, and interquartile ranges of hatch times (from the time the first egg in each clutch hatched) are given in Table 2.

In 2008, clutches laid by adults from NY were larger than those laid by adults from El Carmen and Totalco on potato (ANOVA: $F_{2, 149} = 18.80$, $P < 0.0001$). In 2010, clutches laid by adults from NY were larger than those laid by adults from ME, MI, and El Carmen on potato (ANOVA: $F_{4, 117} = 7.39$, $P < 0.0001$), while those laid by females from the NJ laboratory standard population were not different from any other (Tukey HSD, $\alpha = 0.05$). Just as clutch size did not change between years on buffalo bur in El Carmen (Chpt. 4), clutch size did not change between years on potato for this population (t test: $t_{80} = 0.66$, $P = 0.51$). Nor did clutch size change between years in NY (t test: $t_{101} = 0.28$, $P = 0.78$). Clutch size did not change between buffalo bur and potato for El Carmen in 2008 (t test: $t_{48} = 1.02$, $P = 0.31$), in 2010 (t test: $t_{50} = 0.39$, $P = 0.70$), or when pooling across years (ANOVA: $F_{1,\,99} = 1.03$, $P = 0.31$); there was no interaction between year and host plant ($P > 0.82$). In contrast, clutch sizes were larger on potato than on buffalo bur for Totalco in 2008 (t test: $t_{138} = 3.86$, $P = 0.0002$).

There was not a population difference in the number of viable eggs when controlling for clutch size (ANOVA: $F_{4,\,64} = 0.47$, $P = 0.90$). Neither was there an interaction between clutch size and population on the number of viable eggs in each clutch ($P > 0.99$). There was not a population difference in the number of hatchlings when controlling for the number of viable eggs (ANOVA: $F_{4,\,24} = 0.31$, $P = 0.87$), but there was a marginal interaction between population and the number of viable eggs, with slightly fewer hatchlings than expected from clutches with many viable eggs in NY (ANOVA: $F_{4,\,24} = 2.28$, $P = 0.090$), a result similar to another study comparing hatch rates of U.S. populations (M. Baker, K. Hossain, and K. Collie, unpublished data). There was no difference between El Carmen and Totalco in the number of hatchlings when controlling for clutch size on potato in 2008 (ANOVA: $F_{1,\,80} = 0.72$, $P = 0.40$), nor was there an interaction between population and clutch size ($P > 0.23$). There were slightly fewer hatchlings than expected in 2010 compared to 2008 when controlling for clutch size on potato for El Carmen (ANOVA: $F_{1,\,42} = 3.64$, $P = 0.063$).

There were no population differences in any measure of hatch time variation ($P > 0.13$). However, hatch time variation may be underestimated in some clutches, as it only includes eggs that successfully hatched. Those that were eaten before they hatched were not included; had cannibalism been prevented, their greater latencies to hatch would have increased overall variability.

Table 1. Average clutch size for populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, by year and host plant.

Population	**Year**	**Host plant**	**Clutch size**	**SD**	**N**
El Carmen	2008	Buffalo bur	16.9	8.9	14
El Carmen	2008	Potato	19.6	8.0	36
El Carmen	2009	Buffalo bur	16.9	4.2	21
El Carmen	2010	Buffalo bur	16.7	7.0	6
El Carmen	2010	Potato	18.3	9.7	46
Near El Carmen	2008	Buffalo bur	19.0	8.3	10
El Rodeo	2008	Buffalo bur	39.9	19.0	47
El Rodeo	2009	Buffalo bur	33.6	11.2	41
Near El Rodeo	2008	Buffalo bur	30.0	15.4	6
Totalco	2008	Buffalo bur	14.8	5.8	88
Totalco	2008	Potato	19.2	7.2	52
Totalco	2009	Buffalo bur	12.4	5.1	47
Totalco	2010	Buffalo bur	12.0	6.2	41
ME	2010	Potato	22.0	19.2	17
MI	2010	Potato	19.7	19.7	14
NJ Lab	2010	Potato	30.0	10.5	6
NY	2008	Potato	32.9	18.7	64
NY	2010	Potato	33.9	17.2	39

Table 2. Hatch time variation (in hours) by population of the Colorado potato beetle, *Leptinotarsa decemlineata*.

Population	1st Quartile	Median	3rd Quartile	Last hatch	Mean	SD	Clutch size
NY	1.20	1.89	2.61	5.00	2.08	1.12	58
NY	2.68	3.87	5.83	10.27	4.21	2.29	40
NY	0.87	1.50	2.20	5.90	1.69	1.24	47
NY	2.79	3.58	5.24	8.88	4.06	1.95	22
NY	1.77	4.40	8.58	11.33	5.27	3.72	15
NY*		1.38		31.27	10.88	17.67	3
NY*		0.49		1.23	0.55	0.59	4
NY*	0.83	1.28	2.23	3.45	1.54	1.09	14
NY*	0.42	0.61	1.28	2.1	0.81	0.67	8
NY*	1.68	3.29	4.98	13.00	4.11	3.94	8
NY*	0.08	0.72	2.35	3.47	1.14	1.30	7
NY*	1.10	2.59	3.98	6.92	2.66	1.83	22
NY*	0.46	1.26	2.28	4.60	1.54	1.60	6
NY*	0.80	1.84	3.78	5.84	2.21	1.65	20
NY*	0.97	1.92	2.40	2.68	1.63	0.87	11
NY*	0.40	1.41	2.20	3.83	1.45	1.15	12
El Rodeo	0.23	1.16	1.91	2.05	1.10	0.83	8
El Rodeo	3.55	5.52	9.05	15.68	6.69	4.42	27
Near El Rodeo	0.70	1.25	2.13	3.68	1.44	0.95	31
Near El Rodeo	1.03	1.5	2.16	4.35	1.64	0.92	53
Totalco	0.50	2.95	3.32	4.50	2.20	1.53	17
Totalco	3.97	4.48	5.57	6.90	4.56	1.57	15
Totalco	1.33	1.95	2.69	4.25	1.99	1.17	16
El Carmen	1.68	2.53	3.22	4.55	2.49	1.20	18
El Carmen	0.70	1.13	1.79	3.58	1.35	0.90	16
Near El Carmen	0.31	1.77	3.19	5.97	1.95	1.96	9

*Clutches laid in the laboratory. All other clutches were collected in the field.

APPENDIX F: Effect of cold incubation on hatching times

METHODS

Individual clutches were divided, with some eggs maintained at 26° C until hatching and others maintained at 12° C for a fixed amount of time and then at 26° C until hatching. Hatching clutches were recorded under a Sony® model HDR-HC9 MiniDV HD Handycam® camcorder (Sony Corporation, Tokyo, Japan) using BTV Pro Carbon® 5.4.1 time-lapse software (Ben Bird, www.bensoftware.com) to determine the exact time of hatching for each egg.

The delay in hatching for each cold incubation period was calculated as the average hatching time for the delayed portion of the clutch minus the average hatch time for the portion of the clutch maintained in the warm incubator. The effect of cold incubation was estimated by regressing hatch delay on the duration of cold incubation, weighted by the number of delayed eggs hatching in each clutch. The intercept was set at 0.

RESULTS

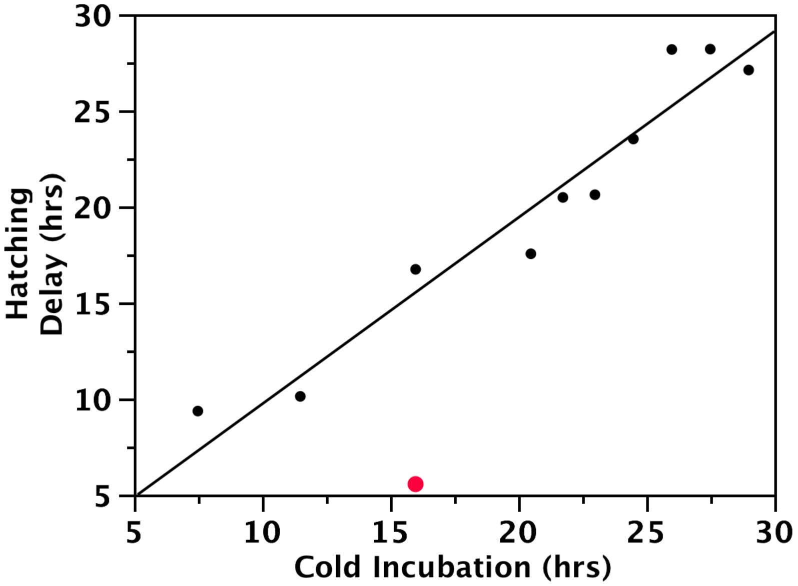
Including all delayed clutches, hatching delay increased significantly with the duration of cold incubation (ANOVA: $F_{1, 10} = 130.28$, $P < 0.0001$), with each hour in cold incubation resulting in a delay of 0.87 hours. However, one clutch appeared to be an outlier, with only a 5.54 hour delay resulting after a 16 hour cold incubation. Re-examination of the video of this clutch revealed that the warm-incubated portion of the clutch took longer than normal to hatch (over 5 days, compared to the 4.53 to 4.86 days for the other clutches for which clutch age was known within 6 hours). As this warm-incubated clutch took four to eight hours longer to begin hatching than any other clutch, it was excluded an the analysis repeated with the remaining clutches. With this clutch excluded, hatching delay still increased significantly with the duration of cold incubation (ANOVA: $F_{1,9} = 1299.11$, $P < 0.0001$), with each hour in cold incubation resulting in a delay of 0.97 hours (Fig. 1).

When cold incubation time was longer than 20 hours, some eggs failed to hatch, though not in every case (Table 1). Thus, cold incubation up to at least 16 hours serves as an effective and fairly predictable technique for delaying egg hatching.

Table 1. Hatch rates of cold-incubated (12° C) eggs of the Colorado potato beetle, *Leptinotarsa decemlineata*.

Time (hrs) incubated at 12° C	**No. eggs held**	**No. eggs hatched**	**Notes**
7.5	5	5	
11.5	13	13	
16	13	10	Final three eggs eaten by earlier hatchers
16	4	4	
20.5	3	2	Third egg observed over 13 hrs after second egg hatched
21.75	3	2	Third egg observed over 9 hrs after second egg hatched
23	3	2	Egg fell off-screen
24.5	3	2	Third egg observed over 7 hrs after second egg hatched
26	3	2	Third egg observed only 1.5 hrs after second egg hatched
27.5	3	3	
29	3	3	

Figure 1. Hatching delay of eggs of the Colorado potato beetle, *Leptinotarsa decemlineata*, resulting from incubation at 12° C. Hatch time increased with the duration of cold incubation. The bold (red) point was excluded from analysis, as the control (incubated at 25° C) portion of the clutch also exhibited delayed hatching.



APPENDIX G: Heritability of cannibalism

METHODS

Neonates were taken from lab-reared colonies originating from MD or NY source populations, a MD x NY hybrid population, or MD or NY field populations. Neonates were kept separately by source population throughout cannibalism assays and rearing.

Neonates within 30 minutes post-hatching were placed on five immature eggs from the same source population and recorded under a Sony® model HDR-HC9 MiniDV HD Handycam® camcorder (Sony Corporation, Tokyo, Japan) using BTV Pro Carbon® 5.4.1 time-lapse software (Ben Bird, www.bensoftware.com) to determine their latency to cannibalize and the number of eggs eaten within 14 hours. Hatchlings from the NY field population that ate an egg within four hours were moved into a rearing cage with other larvae from the same source population to form a “Fast” line. Hatchlings from other populations that ate an egg within five hours were moved into similar “Fast” rearing cages for their source populations. Remaining neonates were recorded until they ate their first egg. Those from the lab-reared and field NY that ate their first egg after at least eight hours were moved into rearing cages with other larvae from the same source population to form “Slow” lines. Similarly, neonates from the lab-reared MD and hybrid populations that first cannibalized after 9.5 hours and those from the MD field population that cannibalized after 13 hours were moved to “Slow” rearing cages for their populations.

In addition to these assays, for which exact latency was known, additional neonates from each source population were placed on two immature eggs from their source population and were checked for cannibalism under a dissecting microscope at the above-described threshold times. Those neonates eating within the appropriate time frames were added to those rearing cages to increase the selection population sizes. All neonates eating in between the two selection thresholds were discarded, while those larvae meeting the selection thresholds were reared to adulthood and were allowed to mate within their selection line. Their offspring were recorded to determine exact latency, and thus the response to selection.

Only the lab-reared NY "Fast" line and the MD field "Fast" and "Slow" lines yielded enough offspring for further analyses. Heritability (h^2) was estimated for each line according to the equation $h^2 = R/S$, where R, the response to selection, is the difference between the means of the original source population and the offspring of the selected line, and S, the selection differential, is the difference between the means of the entire source population and those individuals selected to be the parents of the next generation.

RESULTS/DISCUSSION

All three selection lines shifted in the predicted direction, but not equally (Table 1). The NY lab-reared population barely moved, while the MD field population responded more strongly to selection in both directions. The difference may be due to greater genetic variability in field populations compared to potentially inbred laboratory populations. The NY lab-reared population was less variable (s = 205 min) than the MD field population (s = 319 min), supporting the hypothesis of reduced genetic variability in the lab population.

Table 1. Heritability estimates for cannibalism rates in three selection lines of the Colorado potato beetle, *Leptinotarsa decemlineata*.

Population	**Selection line**	**Overall mean latency (min)**	**Parental mean latency (min)**	***S***	**Offspring mean latency (min)**	***R***	h^2
NY lab	Fast	403	203	200	386	17	0.08
MD field	Fast	477	216	261	360	117	0.45
MD field	Slow	477	821	344	606	129	0.38

S is the difference between the overall and parental mean latencies. *R* is the difference between the overall and offspring mean latencies. h^2 is calculated as R/S.

APPENDIX H: Pesticide resistance of larvae from Mexico populations

METHODS

Hatchlings from eggs collected from El Carmen and Totalco and from eggs laid in the lab by adults collected from these populations, were reared to the second instar on a diet of potato in 2009. In addition, hybrids resulting from crosses between adults from Long Island, NY and Totalco were similarly reared to the second instar. At this stage, larvae were assayed with a single drop of imidacloprid diluted in acetone to a concentration ranging from 2.5 x 10^{-7} to 1.0 x 10^{-4} applied to the dorsal abdomen. Assayed larvae were maintained by population and dose in a petri dish with potato foliage for 24 hours and were then scored as alive or dead. Assays were repeated with larvae from El Carmen in 2010.

To estimate the LD_{50}, the lethal dose for 50 percent of a population, a nominal logistic regression was carried out for each population, with dose as a continuous predictor variable and alive versus dead as a categorical response variable. The resulting curve was used to generate an inverse prediction of the dose for which 50 percent of the population would be dead.

RESULTS/DISCUSSION

Interestingly, the population from El Carmen was more resistant than that at Totalco, with no overlap of their LD_{50} confidence intervals (Table 1). Resistance did not change between years in El Carmen. For comparison to Table 1, the LD_{50} for a resistant Long Island, NY population was 3.95 x 10^{-5} (95% CI: 3.41 x $10^{=5}$ to 4.67 x $10^{=5}$) in 2010, while that of a standard susceptible laboratory strain was 5.57 x 10^{-7} (3.20 x 10^{-8} to 1.64 x $10^{=6}$) (Baker, Hossain, and Collie, unpublished data). Thus, even the more susceptible population at Totalco, which has presumably never been exposed to imidacloprid, is still more resistant than the U.S. susceptible strain.

Table 1. Second-instar mortality for Mexico populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, at a range of concentrations of imidacloprid.

Population	**Year**	**Dose**	**Alive**	**Dead**	**LD_{50}**	**95% UCL**	**95% LCL**
El Carmen	2009	0	3	0	1.48E-5	2.85E-5	1.09E-5
El Carmen	2009	7.81E-6	7	2			
El Carmen	2009	1.25E-5	7	4			
El Carmen	2009	1.56E-5	16	19			
Totalco	2009	0	2	0	3.54E-6	4.62E-6	2.46E-6
Totalco	2009	3.91E-6	3	5			
Totalco	2009	7.81E-6	0	5			
Totalco	2009	1.56E-5	0	5			
NY x Totalco	2009	0	26	0	1.17E-5	1.36E-5	1.01E-5
NY x Totalco	2009	1.95E-6	27	3			
NY x Totalco	2009	3.91E-6	26	4			
NY x Totalco	2009	7.81E-6	20	10			
NY x Totalco	2009	1.25E-5	13	17			
NY x Totalco	2009	1.56E-5	10	20			
NY x Totalco	2009	3.13E-5	0	30			
El Carmen	2010	0	46	3	1.48E-5	2.20E-5	8.89E-6
El Carmen	2010	2.5E-7	20	10			
El Carmen	2010	5.0E-7	19	11			
El Carmen	2010	1.56E-6	16	13			
El Carmen	2010	3.13E-6	15	15			
El Carmen	2010	6.25E-6	14	16			
El Carmen	2010	1.25E-5	11	19			
El Carmen	2010	2.5E-5	12	19			
El Carmen	2010	5.0E-5	3	25			
El Carmen	2010	1.0E-4	3	25			

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