

# The Life History of *Chelymorpha alternans* (Coleoptera: Chrysomelidae: Cassidinae) in Panamá

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## Abstract

Here we describe an investigation of the life history characteristics, behavior, immature and adult morphologies of the Neotropical tortoise beetle *Chelymorpha alternans* (Boheman; Coleoptera: Chrysomelidae: Cassidinae) from the Republic of Panamá. Developmental times and behavioral characteristics for individuals maintained under laboratory and field conditions are evaluated. Survival rates of individuals maintained under field and laboratory conditions are compared. A list of convolvulaceous host plants utilized by *C. alternans* is provided. Empirical studies investigating the biology of *C. alternans* are reviewed. This study contributes to our knowledge of quantitative and qualitative aspects of this species' biology. These findings demonstrate that *C. alternans* is an organism that develops, behaves, and interacts with host plants, much as other tortoise beetles do. Rigorous, multi-year investigation of this ecologically representative species has produced a comparative life history description that expands our knowledge of general Cassidinae and Coleoptera biology. The natural history background, life history descriptions, diet breadth information, and literature review provided in this contribution make a compelling case for continued utilization of *C. alternans* as a representative tortoise beetle species for a variety of field and laboratory based studies in plant–insect interactions, chemical ecology, natural enemy ecology, and phenotypic trait and diet breadth evolution.

**Key words:** Coleoptera, Cassidinae, Panamá, behavior, morphology

The genus *Chelymorpha* Chevrolat 1836 is a member of the Cassidinae tribe Mesomphalini Chapuis 1875. Mesomphalini is the second largest tribe of the Cassidinae, representing 553 species in 25 genera (Borowiec and Świętojańska 2014). Monophyly of the tribe is supported by a mtDNA based molecular phylogeny of the Cassidinae assembled by Hsiao and Windsor (1999), and presence of a basal tooth on the pretarsal claw in all members (Borowiec 1995). This genus *Chelymorpha* is currently considered to contain more than 70 nominal species (Borowiec and Świętojańska 2014), although historical authorities recognized more than 100 nominal species (Blackwelder 1944). *Chelymorpha* is the second most diverse genus in the Mesomphalini, following Stolas (187 species). *Chelymorpha* species are distributed from southern Canada, throughout Central and South America, to Northern Chile and Argentina. The genus reaches its greatest species diversity in lowland wet forests of South America (Borowiec and Świętojańska 2014). Member of this genus can be some of the most numerically abundant tortoise beetles in Neotropical areas, especially those characterized by anthropogenic disturbance. Species with host plant records are monophagous, or oligophagous, exclusively utilizing plants in the morning glory

family, Convolvulaceae. The entire *Chelymorpha* life cycle occurs on the host plant, often with immatures and adults on the same individual plant. Cohabitation of the host plant is generally the case for tortoise beetles (Chaboo 2007). *Chelymorpha* contains high levels of adult color pattern polymorphism between species and within populations. Prevalence of adult polymorphism in the genus suggests that current species estimates may decrease as further empirical studies are published (Zolessi 1968, Vasconcellos-Neto 1988). Alternatively, current species estimates may increase if convergent color polymorphisms are concealing cryptic species.

Champion (1893; also Blackwelder 1944) list *Chelymorpha alternans* (Boheman; Coleoptera: Chrysomelidae: Cassidinae) (Boheman 1854) as occurring in Costa Rica, Panamá, Colombia and Brazil and a red color morph, *Chelymorpha rufipennis* Boheman 1854, occurring in Panamá, Colombia, Venezuela, Brazil, and Argentina. Borowiec (1999) restricts the name *Chelymorpha testaceomarginata* Boheman 1854 to only the metallic-striped morph, *C. alternans*, described in Champion (1893) and lists its occurrence in Costa Rica, Panamá, Dominican Republic, Columbia, Venezuela, and northern Brazil. This determination was based on a morphological comparison

of similar color pattern between nominal species. Windsor et al. (1992) recognize the red morph, *C. rufipennis*, and a black-striped morph, *Chelymorpha militaris* Boheman 1862, as synonymous with the metallic-striped *C. alternans*, and occurring widely across the Republic of Panamá. This determination is supported by mating experiments demonstrating that viable offspring result from mating pairs of all possible adult color morphs (L. Strickland, unpublished data). The present study follows Windsor et al. (1992) in recognizing these three color morphs as phenotypic variants of *C. alternans*, and identifies *C. alternans* Central American distribution as western Costa Rica to Darien Province of Panamá. The actual distribution of *C. alternans* in South America remains unresolved due to taxonomic confusion and misidentification of its color morphs with those of a widely co-occurring species, *C. cribraria* Fabricius 1775 (Vasconcellos-Neto 1988, Borowiec 1999).

*C. alternans* is found from sea level to elevations of 1,000 m in Panamá inhabiting light-rich forest gaps, and edge habitats such as roadsides, pasture land, river edges, and transitional zones from disturbed areas into secondary forest where light is abundant. Individuals are often abundant where host plants have persisted for several years. Conspicuous elytral coloration and diurnal feeding habits make the species ideal for many field studies. Several studies have contributed perspectives about the life history of *C. alternans* that were relevant to the questions that those authors were interested in addressing (Pullin and Knight 1992, Rodriguez 1993, Rodriguez 1994, Rodriguez 1995, Keller et al. 2004, Vencl et al. 2005, Cuignet et al. 2008, Van Bael et al. 2009). However, none of these investigations intended to detail the complete life history of *C. alternans*, and thus did not comprehensively describe this species' basic biology. These investigations encompass all empirical research conducted on *C. alternans* to date. They highlight the utility of employing *C. alternans* as a research organism and will receive detailed review in the discussion section.

Empirical research on various aspects of *C. alternans* biology are currently being conducted in field and laboratory settings. This research momentum motivated us to conduct a thorough investigation of this species' life history. A detailed life history of this species will establish a foundational ecological context for upcoming research articles to reference. This article is the first to present a complete life history description of *C. alternans*. There is a need for researchers to comprehensively review the biology of morphologically, behaviorally, and ecologically representative organisms so that the scientific community is able to develop a more complete understanding of the basic biology of species. Detailed life history descriptions of biologically representative organisms also provide researchers with considerable comparative power that can be wielded to address questions about functionally and phylogenetically similar organisms. We satisfy this need by describing the life history of *C. alternans* and review its morphology, development, behavior, and diet breadth using field and laboratory observations, and published empirical studies. A foundational life history description is requisite for researchers to begin creating synthesis with previous literature and asking broader questions aimed at elucidating mechanisms and contexts governing *C. alternans* trophic interactions and genetics. We conclude this contribution by making a brief case that the comprehensive natural history observations, life history descriptions, and diet breadth information that we have provided about *C. alternans* creates an advantageous context for employing this species as a focal study organism. Our results suggest that this species is appropriate for a wide variety of field and laboratory based investigations on Cassidinae and Coleoptera biology, plant-insect

interactions, chemical ecology, natural enemy ecology, and phenotypic trait and diet breadth evolution.

## Materials and Methods

The principal goal of this study was to raise a large number of *C. alternans* individuals on multiple host plants, under field and laboratory conditions, to contribute an accurate review this species' life history characteristics, behavior, immature, and adult morphologies. Adult *C. alternans* were collected from populations in Coclé, Colón, Panamá, and Darien provinces of the Republic of Panamá between April 2015 and July 2016 to accomplish this goal. Intensive sampling was conducted in multiple provinces to establish a captive breeding population capable of producing the large number of individuals necessary to conduct a life history review that truly exemplifies this species' biology. Additionally, we collected from widely distributed populations on both sides of the Panamá Canal to account for possible variation in localized life history characteristics. In total, 373 egg masses were oviposited by 83 females representing 9,246 individual eggs over 15 continuous months. 5,997 larvae hatched from these eggs (64.86% gross larval eclosion rate); 1,752 of which completed larval development and pupated (33.02% gross pupation rate) producing 1,298 adults (74.09% gross adult eclosion rate). Subsets of these individuals were randomly selected and compared for morphological descriptions.

Native host plants were harvested in Parque Nacional Soberania, and Parque Nacional San Lorenzo, Panamá. *Ipomoea batatas*, commonly known as sweet potato, plants were grown from organic tubers purchased at a local produce market located in Corozal, Panamá. Cuttings were made of harvested host plants and maintained in an ambient shade house at the Smithsonian Tropical Research Institute (STRI) Greenhouse Complex in Gamboa, Panamá.

Adults were maintained in 17 × 17 cm plastic 'Solo' containers with moistened filter paper and ventilation holes punched into the lids. Several males and a single female were placed in each container. Eggs were excised from the host plant following oviposition and maintained individually in plastic petri dishes with moistened filter paper, and sealed with plastic paraffin. Split-brood sibling groups were randomly assigned one of four previously reported convolvulaceous host plants (Jansegers 2004) following larval eclosion: *Merremia umbellata* (L.) Hallier f., *Ipomoea batatas* (L.) Lamand, *Ipomoea phillomega* (Vell.) House, or *Ipomoea tiliacea* Willd. Sibling groups were maintained in 5.5 × 6 cm plastic containers with moistened filter paper and ventilation holes punched into the lid. Leaves were provided to clutches ad libitum. Laboratory reared clutches were maintained in the ambient space of the STRI Gamboa Schoolhouse (09° 6'59.01"N, 79°42'1.45"W), located at an elevation of ~35 m in Gamboa, Panamá. Light cycle was approximately 15:9 (L:D) h, average temperature ca. 28°C, and average relative humidity approximately 85%.

A subset of clutches ( $N = 12$  clutches) were taken from the laboratory immediately following larval eclosion and placed inside fine mesh enclosures surrounding potted host plants in the field. This allowed for comparison of survival rates of clutches reared under field and laboratory conditions. Disparate survival rates between individuals reared in the laboratory and the field may indicate that rearing conditions bias an accurate description of *C. alternans* life history characteristics. Field sites were located at Kent's Marsh and Cerro Escapaldo (09° 7'12.70"N, 79°41'51.44"W), Gamboa, Panamá at an elevation of 50 m.

Developmental and survival rate calculations apply weighted averages recorded from individual clutches. Statistical analysis of survival rates was conducted using Welch's *t*-test, as sample size between treatments and variance within replicates were not homogeneous. Analyses were conducted in the R statistical package 3.3.2 (R Core Team 2016).

Voucher specimens of beetles were deposited in the STRI Insect Collection and the Fairchild Entomological Collection at the University of Panamá.

A comprehensive literature review of *C. alternans* empirical studies was conducted on the Web of Science citation indexing service and Google Scholar search engine. Every hit of the keyword '*Chelymorphism alternans*' was investigated to ascertain whether the authors conducted experiments involving *C. alternans*, or simply mentioned this species in the text. Only publications known to have employed *C. alternans* as a study organism were reviewed in this article. These studies were grouped into three categories: mating behavior and reproductive phenology, microbial interactions, and natural enemy ecology.

## Results

Eggs (Fig. 1A; 1.75–2 mm Tall, 0.5–0.75 mm Wide; N = 257 Eggs)

Ovular, light green, slightly transparent, and soft following oviposition. Eggs harden and become dull, green-beige 6 h following

oviposition. There is a dark brown tip at the terminal apex of each egg. Eggs are oviposited by gravid females in tight groups on host plant leaves. Eggs are normally oviposited on ventral leaf surfaces in loose rows. Eggs are adhered to the leaf, and to one another, by a thin, transparent excretion known as extrachorion, that is expelled by females during oviposition. Inner rows contain erect eggs while outer rows contain eggs oriented towards the periphery of the egg mass. Average number of eggs laid in single oviposition event by a gravid female is  $24.69 \text{ eggs} \pm 9.53$  ( $N = 379$  egg masses). A circular break forms around apex of egg prior to larval eclosion. Larvae emerge from this break in the egg. Average hatching success of larvae is  $64.15\% \pm 32.81$  ( $N = 367$  egg masses). Average number of days from egg mass oviposition to larval eclosion is  $11.15 \text{ d} \pm 2.81$  ( $N = 329$  egg masses). Larvae eclose from the egg mass in near synchrony. Late eclosing larvae have never been observed hatching from an egg mass more than 4 h following eclosion of their siblings.

## Larvae

*C. alternans* possesses five larval instars. Feeding occurs at all hours of the day, primarily on ventral leaf surfaces. Larvae will feed on dorsal leaf surfaces more often as they develop into later instars. Larvae always remain on the host plant. Average number of days from larval eclosion to pupation is  $19.08 \text{ d} \pm 3.83$  ( $N = 294$  clutches). Average laboratory survival of larvae, from eclosion to pupation, is  $44.84\% \pm \text{SD } 32.67$  ( $N = 242$  clutches). Average survival of larvae in the field is  $43.76\% \pm \text{SD } 19.55$  ( $N = 12$  clutches). These weighted average

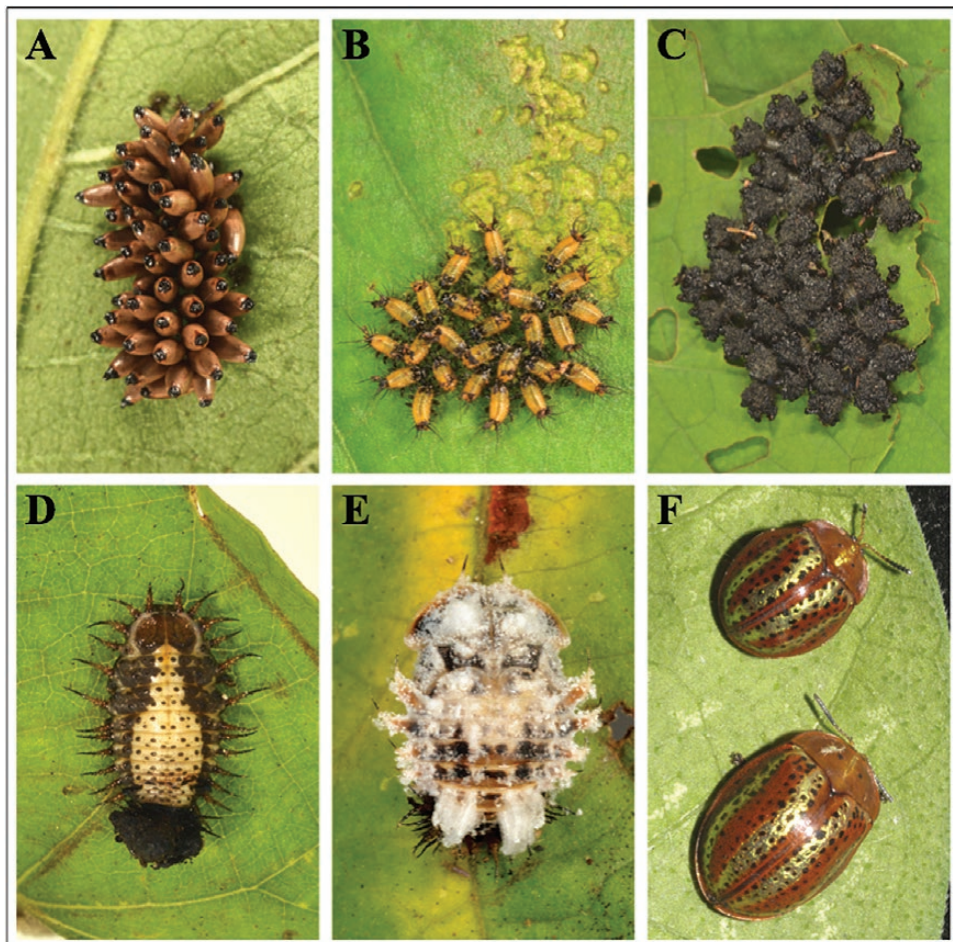


Fig. 1. Egg mass (A); 1<sup>st</sup> instar larvae (B); 4<sup>th</sup> instar larvae (C); pre-pupal larva (D); pupa (E); mature adults, male on top, female on bottom (F). Images by D.M.W.



survival rates do not exhibit a statistically significant difference with one another (Welch's *t*-test;  $t = -0.173$ ;  $df = 13.991$ ;  $P = 0.865$ ; Fig. 5A). Survival rates of clutches reared under field and laboratory conditions are 9–10% greater in this study than previous tortoise beetle studies that permitted predation in the field (Cox 1996, Olmstead 1996). There was no qualitative seasonal effect on survival of individuals maintained under laboratory conditions.

#### Early Instar: 1<sup>st</sup>–3<sup>rd</sup> (Fig. 1B; N = 278 Individuals From 11 Clutches)

1<sup>st</sup> instar larvae average 2 mm body length, average 0.5 mm width. Body length increases by avg. 1 mm per molt until the 3<sup>rd</sup> instar. Width increases by average 0.25 mm per molt until the 3<sup>rd</sup> instar. Larvae are light yellow–green with brown head capsule following larval eclosion. Larvae become light green 3 h after eclosion. Third instar larvae are dark brown, with areas of green along the dorsum. Early instar larvae possess black heads, legs and prothoracic shields. Larvae possess long, dark gray scoli on each thoracic and abdominal segment. Longest scoli are located on thoracic segments, and are approximately one-half width of the body length. Scoli length decreases posteriorly. Principal scoli are branched with many smaller spines. Larvae possess an ambulatory caudal fork called the furca, on the 8<sup>th</sup> abdominal segment. The furca has two prong-like processes, dark brown with light brown tips. Larvae hold the furca laterally over dorsum. A muscular, telescopically protrusible anus attaches fecal material to the furca. Fecal material and exuviae are combined by the furca to form an exuvio-fecal shield. The furca is oriented anteriorly so that larvae can maneuver fecal shields over their bodies.

The moment of larval eclosion is not synchronous for all individuals. However, eclosion occurs for approximately 70% of individuals within the first hour, with most individuals eclosing by the fourth hour following the emergence of the first individual larva. Feeding begins immediately upon eclosion from the egg mass, normally adjacent to mid-vein of the leaf near base of the petiole. Feeding proceeds outward towards leaf margins, and occurs at all hours, primarily on ventral leaf surfaces. Early instar larvae are gregarious, systematically consuming leaves (Fig. 1B). Early instar larvae normally begin consuming leaves from the ventral cuticle. Inner layers of mesophyll are consumed until larvae reach the opposing layer of waxy cuticle, where feeding ceases. This feeding process leaves a thin, transparent layer of cuticular cells on the side of the leaf opposite where feeding commenced (Fig. 4A). This characteristic form of herbivorous leaf damage can be used to identify *C. alternans* larvae in the field. Early instar larvae do not consume leaf veins. Third instar larvae consume all leaf layers.

Larvae move together along the plant stem to an adjacent leaf once a leaf is completely consumed. Feeding recommences immediately upon arrival at a fresh leaf. Larvae present on periphery of a group will orient their fecal shields away from the group (Fig. 4A). This behavior forms a barrier of fecal shields around the group. Larvae move their fecal shields together in the direction of a threat when disturbed.

#### Late Instar: 4<sup>th</sup>–5<sup>th</sup> (Fig. 1C; N = 244 Individuals From Nine Clutches)

Body length of 4<sup>th</sup> and 5<sup>th</sup> instar larvae approximately 5–8 mm, approximately 2–3 mm width. Fourth instar larvae are reddish-brown with dark brown dorsum. Fifth instar larvae are dull green, becoming cream colored as they become prepupal with dark brown spots, lateral markings, and prothoracic shields.

Posterior abdominal segments are held upwards, causing the caudal fork and fecal shield to be elevated. This allows larvae to completely cover their bodies with the fecal shield (Fig. 1C). Late instar larvae are loosely gregarious (Fig. 2A), occasionally forming tight groups (Figs. 1C and 2B). Late instar larvae develop sufficient mandibular strength that individuals can completely chew through leaves. They will consume host plant leaves and veins, with veins being the final parts to be consumed (Fig. 4C and D). Consumption of leaves generally proceeds from one leaf to the next. However, larvae have been observed passing three to four leaves before consuming a particular leaf.

Larvae become prepupal approximately 2 d into the 5<sup>th</sup> instar (Fig. 1D). Host plant consumption diminishes in preparation for metamorphosis into the pupal stadium. Feeding ceases 1 d prior to pupation. Larvae anchor themselves into position for pupation on a host plant leaf with their tarsal claws.

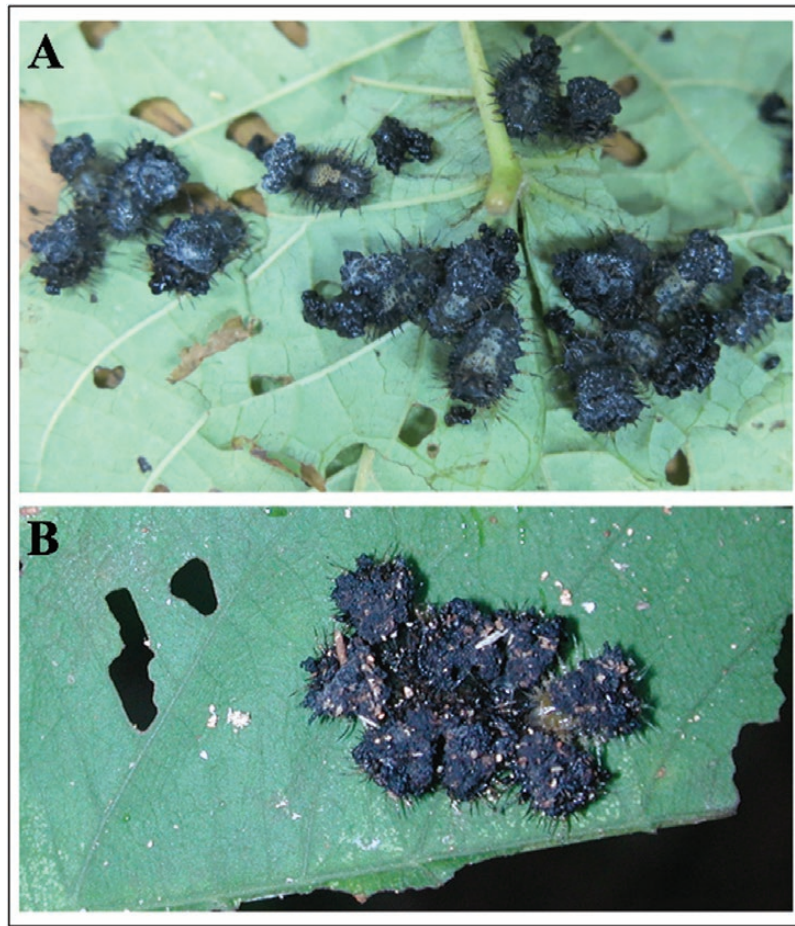
#### Fecal Shields

Construction of fecal shields by immature cassidines has been suggested as a physical (Eisner et al. 1967, Olmstead and Denno 1993, Eisner and Eisner 2000, Bacher and Luder 2005) and/or chemical defense against predation (Gomez et al. 1999, Müller and Hilker 1999, Vencl et al. 1999, Nogueira-de-Sá and Trigo 2005, Vencl et al. 2005), or to reduce effects of abiotic stressors such as wind and ultra violet radiation (Olmstead and Denno 1992). Vencl et al. (2005) demonstrated that *C. alternans* uses its fecal shield as a physical and chemical defense against natural predators. Individual larvae with shields removed suffered a significantly lower survival rate against generalist *Azteca* ants than those with shields. Larvae wielded shields to block attacking ants. When ants were unable to circumvent the ambulatory fecal shields they stopped attacking. *C. alternans* fecal shields also deterred *Azteca* ants via chemistry. Solvent leaching of fecal shields, with water or methanol, significantly reduced *C. alternans* fecal shield efficacy when compared to larvae with fecal shields that had not been treated with solvents. Vencl et al. (2009) demonstrated that *C. alternans* larvae co-opt plant chemistry into their fecal shields. They demonstrated that chlorophyll precursors present in *M. umbellata* host plant tissues are modified into chlorophyll catabolites as they pass through the larval gut and subsequently accumulate in fecal shields.

*C. alternans* larvae completely reassemble their fecal shields with new fecal material in less than 24 h following removal by natural abrasion, or with forceps ( $N = 204$  individuals). Incorporation of exuviae into the fecal shield only occurs following metamorphosis into a new larval instar. Loss of a fecal shield does not appear to harm larvae, nor accelerate feeding.

#### Pupae (Fig. 1E; N = 78 Individuals From Nine Clutches)

Body length of pupae approximately 12–13 mm, width approximately 5–6 mm. Average number of days from date of pupation to adult eclosion is  $5.33 \text{ d} \pm 3.04$  ( $N = 259$  clutches). Average laboratory survival of pupae from date of pupation to adult eclosion is  $77.54\% \pm \text{SD } 31.39$  ( $N = 212$  clutches). Average survival of pupae in the field is  $83.12\% \pm \text{SD } 16.13$  ( $N = 12$  clutches). These weighted average survival rates are consistent between pupae reared under field and laboratory conditions (Welch's *t*-test;  $t = 1.269$ ;  $df = 17.299$ ;  $P = 0.221$ ; Fig. 5B). As with larvae, there was no qualitative seasonal effect on survival of individuals maintained under laboratory conditions.



**Fig. 2.** Loose aggregation of 4<sup>th</sup> instar larvae (A); tight aggregation of 4<sup>th</sup> instar larvae (B). Body length of 4<sup>th</sup> instar larvae ca. 5–8 mm, 2–3 mm width. Image 2A by C.R.M. Image 2B by D.M.W.

Pupae are light yellow/cream, conspicuous, ovular, robust, with dark spots and blotches. They are bordered with a white flocculence, especially high concentrations on head and 1<sup>st</sup> thoracic segment (Fig. 1E). Flocculence attached to posterior abdominal segments is fibrous. Thoracic segment 1 is a semi-circle with 2 long, anteriorly facing setae. Thoracic segments 2 and 3 are semi-covered by segment 1. Lateral setae are present on abdominal segments 2 to 6. Fin-shaped segments form around base of each seta. Intersegmental space is light brown. Pupae often retain the fecal shield. However, it remains loosely attached to the caudal furca at the pupa's posterior and often becomes dislodged in the laboratory and the field from leaf movement or contact.

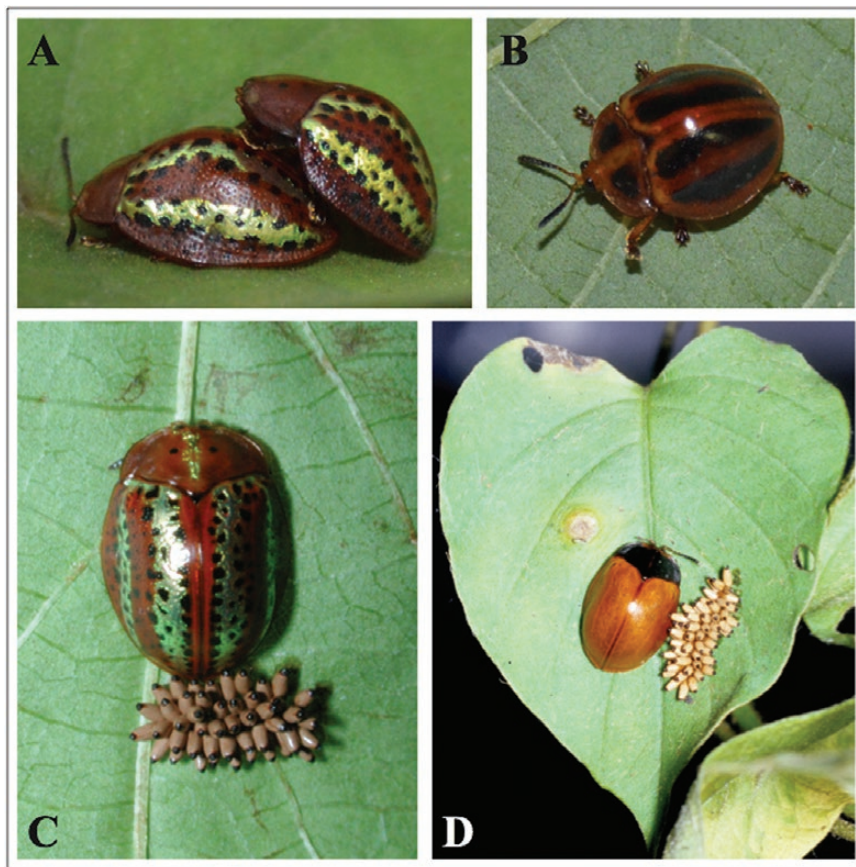
Larvae normally pupate on the ventral side of host plant leaves; 83% of pupae, that had pupation location recorded, pupated on the ventral side of a host plant leaf ( $N = 135$  pupae). Pupae will make slow, rhythmic jerks laterally when disturbed. Moment of adult eclosion from the pupa is often synchronous within clutches. However, members of individual clutches have been observed pupating over a period of 1 to 4 d.

#### Adults (Fig. 1F; $N = 69$ Individuals From Seven Clutches)

Female body length approximately 11–12 mm, width approximately 4–5 mm. Male body length is more variable, approximately 5–8 mm, width approximately 3–5 mm. Females are ovular with slight indentation at border of the pronotum and elytron.

Males are more circular than females with pronounced dentition between the pronotum and elytron. Elytra extend approximately 0.5 mm outwards from the body in both sexes. We recognize three phenotypic variants of *C. alternans* that were previously described (Champion 1893, Blackwelder 1944, Borowiec 1999) as separate nominal species: metallic-striped morph (Fig. 3A and C), black-striped morph (Fig. 3B), and red morph (Fig. 3D). All color aberrations exhibit some red color with varying degrees of iridescence. Average weight of a live, adult females is  $64.68 \text{ mg} \pm 15.69$  ( $N = 239$  individuals), while the average live, adult male weighs  $43.32 \text{ mg} \pm 8.97$  ( $N = 143$  individuals), over 30% lighter than females.

Sexual maturation is reached in 2.5–3 wk for females, 2 wk for males. Copulation time is variable, lasting between 30 min and 3 h (Fig. 3A). This observation corroborates previously reported copulation observations (Rodriguez 1994). It appears that females only need to mate once in their lifetime for achievement of continual egg fertilization. Six females, that mated only once, continually produced egg masses for over 4 mo, until they deceased. Females begin ovipositing egg masses 3–5 d after copulation (Fig. 3C and D). This species is multi-voltine, producing at least three broods per year in the area around the Panamá Canal. Gravid females will occasionally oviposit egg masses on non-host plants growing near larval food plants (see female with egg mass on *Jacquemontia* sp. [Convolvulaceae] in Fig. 3D). Adult *C. alternans* are long-lived. Individuals ( $N = 5$  males;  $N = 7$  females) have survived in plastic containers maintained in the



**Fig. 3.** Metallic-striped morph adults mating (A); black-striped morph male (B); metallic-striped morph female with egg mass (C); red morph female with egg mass (E). Images 3A and 3B by C.R.M. Female body length ca. 11–12 mm, width 4–5 mm; Male body length ca. 5–8 mm, width 3–5 mm. Images 3C and 3D by D.M.W.

ambient space of the STRI Gamboa Schoolhouse for greater than 11 mo. Adults feed at all hours, often conspicuously on dorsal leaf surfaces (Fig. 1F). Adults have never been observed feeding on the same leaf as larvae. However, adults and larvae can be found consuming separate leaves of the same host plant. Conspicuous feeding habit (Fig. 4B) and putative aposematic coloration suggests that adult *C. alternans* utilizes host plant metabolites as a chemical defense and advertises unpalatability to predators. Alternatively, these traits may be utilized for intraspecific signaling involved in mate recognition, sexual selection and reproduction. Adults utilize several strategies to avoid predation by crawling predators (e.g., ants). They firmly adhere themselves to substrates via utilization of their microscopic tarsal bristles, an anatomical characteristic shared by all cassidines (Eisner and Eisner 2000). They run away from predators at great speed. Or they withdraw all appendages beneath the elytra and release their grip on a substrate, causing them to fall into underlying foliage.

Adults are seldom observed flying distances greater than 10–20 cm. The primary function of *C. alternans* elytra and hindwings appears to serve a locomotory function. Adults extend their elytra and hindwings to turn themselves over and correct their position if they are rolled over onto the dorsum.

#### Host Plants

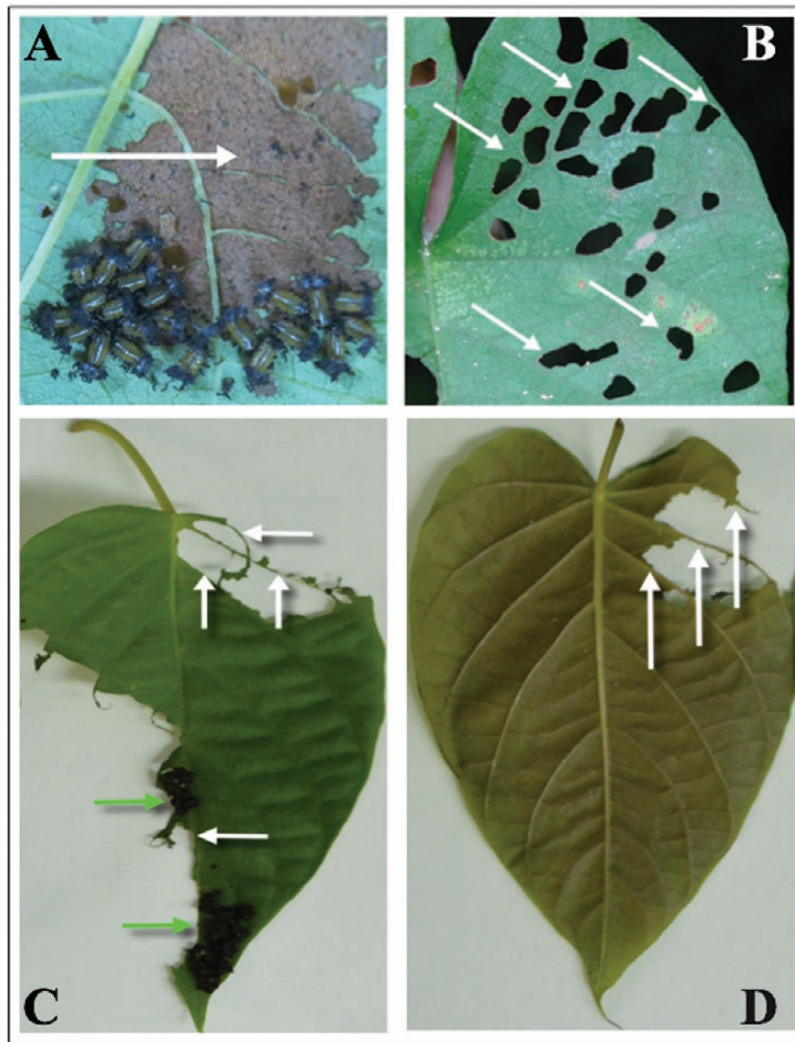
*C. alternans* utilizes at least eleven plant species, in three genera, of the morning glory family, Convolvulaceae (Table 1). Known host plants include: *M. umbellata* (L.) Hallier f., *I. lindenii* Martens and

Galeotti, *Ipomoea phillomega* (Vell.) House, *I. tiliacea* (Willd.) Choisy, *Ipomoea trifida* (Kunth) G. Don (C.R.M. personal observation, Jansegers 2004), *Ipomoea hederifolia* L., and *Ipomoea squamosa* Choisy (D.M.W. personal observation). *C. alternans* will consume several nonnative agricultural and horticultural plants including: *Ipomoea batatas* (L.) Lamand, *Ipomoea nil* (L.) Roth, *Ipomoea tricolor* Cav., and *Convolvulus arvensis* L.

#### Discussion

Member of the genus *Chelymorpha* can be some of the most numerically abundant tortoise beetles in the Neotropics. Despite their expansive distribution and abundance, only six species of *Chelymorpha* have had their life histories formally described: *C. cassidea* Fabricius 1775 (Chittenden 1924, Lawson 1991), *C. constellata* Klug 1829 (Marques 1932), *C. cribraria* Fabricius 1775 (Chittenden 1924), *C. indigesta* Boheman 1854 (Frers 1922), *C. reimoseri* Spaeth 1928 (Świętojańska et al. 2015), and *C. varians* Blanchard 1851 (Frers 1922, Zolessi 1968). These contributions present high quality figures and morphological descriptions that are useful to taxonomic description of the organism of interest. Some of these contributions contribute notes on the biology (Frers 1922, Chittenden 1924, Marques 1932) or to the systematics of the genus (Zolessi 1968, Świętojańska et al. 2015). However, they do not comprehensively review a complete life history (egg to immature to pupa to adult), developmental rates, survival, or behavioral characteristics. To our knowledge, this article is the first comprehensive life history





**Fig. 4.** *M. umbellata* leaf consumption by 1<sup>st</sup> instar larvae, notice skeletonization of leaf cuticle (A); *M. umbellata* leaf damage by adults (B); *I. phillomega* leaf damage by 4<sup>th</sup> instar larvae (green arrows) (C and D), notice that secondary veins are the last to be consumed and the thicker veins may be avoided entirely. White arrows indicate site of leaf damage. Images by C.R.M.

**Table 1.** Known *Chelymorpha alternans* convolvulaceous host plants

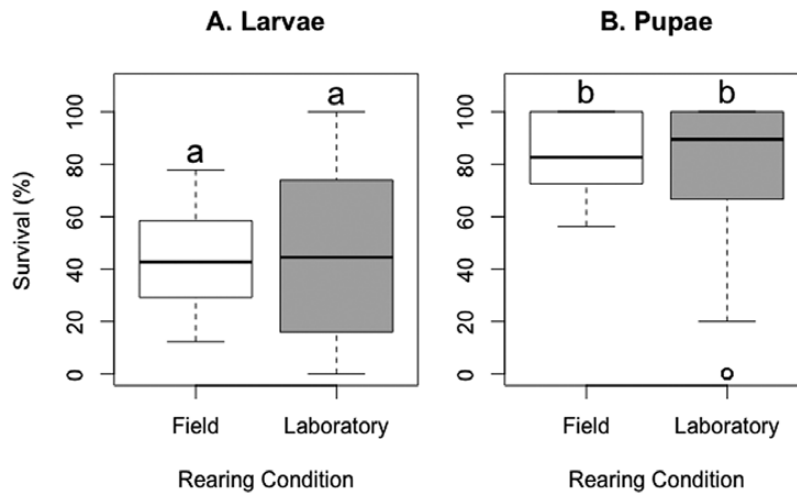
Host plant species	Plant geography
<i>Merremia umbellata</i> (L.) Hallier f.	Native
<i>Ipomoea hederifolia</i> L.	Native
<i>Ipomoea lindenii</i> Martens and Galeotti	Native
<i>Ipomoea phillomega</i> (Vell.) House	Native
<i>Ipomoea squamosa</i> Choisy	Native
<i>Ipomoea tiliacea</i> (Willd.) Choisy	Native
<i>Ipomoea trifida</i> (Kunth) G. Don	Native
<i>Ipomoea batatas</i> (L.) Lamand	Nonnative
<i>Ipomoea nil</i> (L.) Roth	Nonnative
<i>Ipomoea tricolor</i> Cav.	Nonnative
<i>Convolvulus arvensis</i> (L.)	Nonnative

Native signifies that wild *C. alternans* have been observed feeding on this host plant in nature; nonnative signifies that this host plant does not occur naturally in Panamá, but *C. alternans* will utilize it in captivity.

description of a *Chelymorpha* species from egg to the adult. The fact that this description is presented with data on developmental rates, survival, behavioral observations, and host affiliation data makes this

investigation the most complete *Chelymorpha* life history review to date. Developmental rates, survival, and observed behaviors are qualitatively consistent with that exhibited by other tortoise beetle species of the Mesomphalini whose life histories have not been detailed comprehensively (i.e., with comparable sample sizes, behavioral observations, study duration) as they have been in this study (Paterson 1931, Windsor 1987, Williams 2002, Świętojańska 2008, Świętojańska et al. 2015). For example, developmental rates between all developmental stadia of *C. cassidea*, a species with a more northerly distribution, are consistent with those observed in *C. alternans* (Paterson 1931).

Our results on patterns of host specificity and behavioral characteristics are generally consistent with those reported elsewhere (Buzzi 1988, Windsor et al. 1992, Jolivet and Verma 2002, Chaboo 2007). *C. alternans* appears to possess a moderately more expansive diet than many closely related members of its tribe, the Mesomphalini, and Cassidinae in general. One observation that supports this assumption is that *C. alternans* readily consumed four nonnative plant species in feeding trials, one of which is included in a genus that it never encounters in nature (*Convolvulus*). This observation suggests that the potential diet breadth of this species is considerably underestimated by our observations. However, this inference needs to be verified through



**Fig. 5.** Comparison of percent survival rates of *C. alternans* larvae (A) and pupae (B) reared under field and laboratory conditions. And  $N = 12$  clutches were reared in the field,  $N = 242$  clutches of larvae were reared in the laboratory.  $N = 12$  clutches were reared in field,  $N = 212$  clutches of pupae were reared in the laboratory. Outputs represent results from Welch's *t*-tests, as sample size between treatments and variance within replicates were not homogenous. No statistically significant differences were revealed between rearing conditions for either life history stadium.

systematic feeding experiments conducted with other representatives of the Mesomphalini, and related Cassidinae tribes.

Early instar (1<sup>st</sup> to 3<sup>rd</sup>) *C. alternans* larvae do not consume leaf veins. This observation is true for early instar larvae of several other tortoise beetle species found in the Republic of Panamá, such as *Acromis sparsa*, *Agroiconota propinqua*, *Charidotella* species, *Charidotis* species, and *Stolas plagiata* (C. Morrison, *unpublished observations*). This may be due to an inability to chew that material, or a nutritional deficiency inherent to leaf veins. Alternatively, this may be an adaptive behavior that circumvents milky latex flowing through veins of convolvulaceous plants. Milky latex can provide a primary plant defense against herbivory that many insects have evolved behaviors to avoid (for examples see Agrawal and Konno 2009).

Gregarious feeding is another early instar life history characteristic that warrants discussion. Immediately following eclosion from the egg mass, larvae begin consuming leaf mesophyll in close proximity to the egg mass and the main vein of the leaf. Gregariousness does not appear to be motivated by an external factor, rather the behavior occurs because of the close proximity to each other that larvae find themselves in upon eclosion from the egg mass. The gregarious feeding habit appears to facilitate collective breakage of the leaf cuticle, resulting in more efficient consumption of host plant material. This hypothesis is supported by the observation that larvae reared individually, or in small groups (3–5 individuals), develop at slower rates, and survive to a lesser extent, than those reared in large groups (>10 individuals). Larvae will remain in a tight group as they continue systematic consumption of the mesophyll layer of leaf cuticle until the final days of the 3<sup>rd</sup> instar. This hypothesis is also supported by the observation that late instar larvae (4<sup>th</sup>–5<sup>th</sup>) do not need to consume leaves in a group. In contrast to early instar larvae, the developmental rates of 4<sup>th</sup> and 5<sup>th</sup> instar larvae do not appear to be related to the number of individuals that comprise a given group.

This description of *C. alternans* life history characteristics, immature and adult morphology provides comprehensive background information that thoroughly describes the nuances intrinsic to the biology of this species. These results highlight this species' ability to serve as comparable representative of the Cassidinae for future research. A comprehensive natural history background and

life history description are essential to researchers that are intent on utilizing a representative phytophagous beetle species to advance biological research on the Cassidinae, Coleoptera, as well as theory driven research on insect ecology and evolution. *C. alternans* can be handled with ease, consistently located in the field by collectors lacking extensive training, maintained for long periods of time in captivity, and reared on host plants that are easy to locate, harvest and require minimal maintenance in a nursery. These logistical characteristics were essential for maintaining these beetles for sufficient time to generate the sample size that we desired for a thorough investigation of this species' biology. Ability to maintain these beetles over the course of 2 y and multiple changes of season also allowed us to evaluate potential temporal variation in their behavior or fitness parameters. Below we briefly discuss all known empirical studies involving *C. alternans*. This comprehensive review highlights the utility of this species for a wide variety of basic and applied research on the ecology and evolution of host plant specialized herbivorous insects.

### C. *alternans* Empirical Research Review

#### Mating Behavior and Reproductive Phenology

Rodriguez (1995) described *C. alternans* copulatory courtship and mating behavior. Males court females before, and during, copulation by mounting the female dorsally (Fig. 3A). Courtship is initiated when a male mounts a female and taps her elytra with his antennae. Antennations are interspersed with attempts at intromission. Females unwilling to copulate will raise their abdomens, bringing the abdomen into contact with their elytra, preventing intromission. Four behaviors were described that males exhibited during copulation: 'soft pumping of the aedeagus', 'swaying', 'body jerking', and 'vibration of the head'. Variation in individual performance of these behaviors was observed.

Females expelled a droplet, containing spermatozoa, from their genital opening during several attempts at intromission. This behavior was performed more often by nonvirgin females than virgins (83% vs 13%). Expulsion of the spermatozoa droplet extended copulation time, and increased intromission duration of mating pairs involving nonvirgins, relative to pairs containing virgins. Rodriguez



suggested that spermatozoa droplet expulsion, and variation in male copulatory behaviors, is indicative of sexual selection for cryptic choice by females. Further evidence for female sexual selection was provided by female preference for longer spermatozoa flagella length (Rodriguez 1993) and demonstration of the key role that female spermathecal muscles perform in transferring spermatozoa to the spermatheca (Rodriguez 1994).

Wild *C. alternans* enter reproductive diapause and forgo oviposition during the dry season in Panamá. The annual Panamanian dry season lasts from approximately December to May when photoperiod shortens from 13:11 (L:D) h to 12:12 (L:D) h. Pullin and Knight (1992) conducted experiments on environmental conditions hypothesized to be responsible for induction of this case of reproductive diapause. The authors experimentally simulated natural variation in photoperiod with wild collected *C. alternans* populations under controlled laboratory conditions. Reproductive diapause was induced in groups of individuals exposed to shorter photoperiods. Courtship attempts by males also declined precipitously in the shortened photoperiod treatment (12:12 h). The authors unsuccessfully attempted to compel females to oviposit by exposing them to longer photoperiods (13:11 h) and higher levels of relative humidity characteristic of wet season conditions. Females began mating and ovipositing egg masses 3–4 mo after induction of diapause, regardless of their photoperiod treatment. Pullin and Knight concluded that this behavior was to be expected because oviposition of egg masses 3–4 mo following onset of the dry season conditions resulted in propagation of offspring that reached sexual maturity by late April and May. This was the time of year that precipitation increased, host plants flushed new leaves, and wet season conditions materialized. Dry season induced reproductive diapause has also been reported with another Panamanian cassidine, *Acromis sparsa* Boheman 1854 (Windsor 1987).

#### Microbial Interactions

Microbial interactions with *C. alternans* have been investigated in two cases. Effects of bacteria on *C. alternans* fertilization ability was investigated by Keller et al. (2004), who demonstrated that Panamanian *C. alternans* populations were infected by two strains of the endocellular bacterial genus *Wolbachia*. Pacific coast populations, located west of the Panamá canal, were only infected by strain 1. Populations distributed along the Caribbean coast, and east of the Panamá Canal, displayed polymorphism in *Wolbachia* strain infection. These populations were infected by strain 1 only, or by both strains. No individuals were found infected with strain 2 only. Infected individuals (>700) suffered from varying degrees of cytoplasmic incompatibility (CI), a condition preventing spermatozoa and eggs from producing viable offspring. The authors concluded that this result may hold consequences for geographically separate *C. alternans* populations because eastern populations infected with both strains suffered high CI (~70–90%), relative to western Pacific populations infected with strain 1 only (~20% CI).

Van Bael et al. (2009) explored interactions between fungi and plant resistance to herbivory by studying whether *C. alternans* oviposition preference varied depending on the densities of foliar endophytic fungi in the leaves of its host plant. Density of a common foliar endophytic fungus, *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk, that colonize *M. umbellata*, a *C. alternans* host plant, was manipulated to produce plants with low and high fungal densities. Low endophyte density in *M. umbellata* individuals were obtained by growing seedlings in nurseries surrounded by plastic sheeting while high density *M. umbellata* individuals were obtained by growing seedlings in nurseries exposed to ambient precipitation. Adults that consumed low endophyte density plants as

larvae produced 80% more offspring than individuals fed high endophyte density plants. Van Bael et al. concluded that effects of high fungal endophyte density represent a significant fitness disadvantage for *C. alternans*. They also noted that this experiment does not elucidate whether variation in *C. alternans* fecundity is due to the herbivore's perception of food quality, or physiological constraints attributable to food quality.

#### Natural Enemy Ecology

Cox (1994) proposed that 'the Cassidinae are the most frequently parasitized subfamily', of chrysomelid beetles. While this conclusion may be driven by sampling bias, or spatial variation in data collection, it provides a compelling insight into the pressure exerted on cassidines by parasitoids, a ubiquitous natural enemy of holometabolous insects. Cuignet et al. (2008) conducted a long-term survey targeting parasitoid taxa utilizing 47 cassidine species as hosts, including *C. alternans*, from 2001 to 2005. A tachinid fly, *Eucelatoria* sp. (Diptera: Tachinidae) was found in the abdomens of 29.03% (72/248) of dissected *C. alternans* adults collected in five locations across the isthmus, and far western Panamá. Fifty percent (76/152) of *C. alternans* egg masses were found to be parasitized by one or more wasp species of the genus *Emersonella* (Hymenoptera: Eulophidae). Cuignet et al. suggested that lack of maternal care by *C. alternans* may explain the high levels of egg parasitism observed. This suggestion was supported by the fact that the total number of egg masses of a subsocial tortoise beetle, *Acromis sparsa*, that exhibits maternal brood care, were parasitized 43% less than those of *C. alternans*. Only 27 *C. alternans* larvae (2.7%) were parasitized out of more than 1,000 individuals sampled, suggesting that parasitoids do not commonly utilize *C. alternans* larvae as a host. The authors proposed that this observation may be because fecal shields provided larvae a defensive advantage against parasitoids, as suggested by Bacher and Luder (2005). In addition to arthropod parasitoids, eight unidentified Nematomorpha worms were obtained from over 800 *C. alternans* sampled over the course of this study.

*C. alternans* natural enemy interactions have also been investigated in a comparative framework. Vencl et al. (2005) assayed *C. alternans*, and two monophagous cassidines species, against three species of sympatric generalist predators to investigate whether host plant specialization confers immature cassidines enemy free space against generalist predators. The comparison was established by separately rearing *C. alternans* clutches on the same host plants utilized by the monophagous species employed in the study and offering individuals to predators. Individual *C. alternans* larvae were captured by the assassin bug, *Montina nigripes* Stål 1859 (Hemiptera: Reduviidae) in over 80% of laboratory based experiments, regardless of which host plant they consumed. *Montina nigripes* captured the monophagous cassidine species to a lesser degree than *C. alternans*. Individual *C. alternans* larvae were captured by the highly recruiting, generalist ant species, *Azteca lacrymosa* Forel 1899 and *A. chartiex* Forel 1896 (Hymenoptera: Formicidae) approximately 20% of the time in field predation experiments. Survival rate of all cassidines species was approximately equivalent against both *Azteca* species.

#### *C. alternans* as a Utilitarian Research Organism

Most fundamental to the utility of *C. alternans* as a study organism is the fact that many of its native host plants are robust and easily maintained in cultivation, it will readily consumes several agricultural and horticultural plants that are also easy to maintain in cultivation, husbandry is tractable year-round, and complete generation

time from egg to sexually mature adult is short (~5 wk). Moreover, this species' life history characteristics make it ideal for employment in investigation of numerous questions that are fundamental to basic and applied biology. Examples include: Cassidinae phenotypic evolution, immature defense, preference and performance, host plant choice, and herbivore-natural enemy interactions. In other words, *C. alternans* can be handled with ease while being employed in a variety of laboratory and field based research.

*C. alternans* possesses an oligophagous diet that allows researchers to design flexible experiments that address a variety of questions that contribute to investigation of the widespread evolution of restricted diet breadth characteristic of herbivorous insects. *C. alternans* could be employed in studies investigating the significance of 'enemy free space' (Price et al. 1980, Bernays and Graham 1988), or coevolution with plant defensive chemistry (Ehrlich and Raven 1964, Stamp 2001) by presenting individuals consuming different host plants to functionally distinct guilds of natural enemies (e.g., generalist predators or viruses, specialist parasitoids or entomopathogens). Questions about variation in ability of herbivores to utilize plant chemistry (Burghardt et al. 2001, Fordyce and Nice 2008, Jamieson and Bowers 2010, Dimarco et al. 2012, Glassmire et al. 2016) can be explored by sampling larval fecal shields (Vencl et al. 2009) or adult elytra.

Multiple, disparate, adult color phenotypes are present throughout the actual distribution of *C. alternans*. This mosaic of polymorphism, within and between populations, allows for investigation of the genetics of phenotypic variation. Classic model systems of host specialized insects characterized by spatial heterogeneity in phenotypic diversity, such as the *Heliconius* butterfly system (Joron et al. 2006, Hines et al. 2011), are not common. These systems provide researchers exceptional opportunities to study the genetics of polymorphic traits.

Synthesis of detailed natural history observations, ecological associations, and life history descriptions (Stearns 1992) provides researchers an informed context for asking meaningful questions about relative influences of ecology and evolution in governing organismal biology. The information presented in this article suggests that *C. alternans* has great potential to continue serving as a utilitarian organism for a wide variety of field and laboratory based research.

## Future Direction

*C. alternans* continues to be investigated by a number of researchers, including ourselves. Ongoing research includes, but is not limited to: preference and performance of individuals utilizing different host plants and dietary feeding regimes, efficacy of defense by individuals consuming different hosts against various natural enemy guilds, natural products chemistry of fecal shields recovered from individuals consuming different diets, role of plant compounds sequestered by adults against pathogens, and the metagenomics of multiple distinct color phenotypes. This species will continue to be a research target for many investigators interested in addressing questions about the biology of herbivorous insects. Research articles demonstrating the utility of using this species as a research organism will continue to be published over the coming years. We expect that the comprehensive investigation of *C. alternans* natural history, life history, morphology, and diet breadth presented here will contribute to ever-expanding general knowledge of Cassidinae, and Coleoptera biology. Furthermore, we conclude that the results, literature review, and case made for the utility of this species will compel investigators to consider employing *C. alternans* as a focal research organism in empirical research that addresses the topics mentioned in this article and much more.

## Data Availability Statement

Data from this study are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d71b0>.

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