

RESEARCH ARTICLE

Ontogenetic shift from aposematism and gregariousness to crypsis in a Romaleid grasshopper

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Abstract

Traits of chemically-defended animals can change as an individual grows and matures, and both theoretical and empirical evidence favour a direction of change from crypsis to aposematism. This study examines the suite of traits involved in an unusual opposite shift from aposematism to crypsis in a neotropical toxic-plant-feeding Romaleid grasshopper, *Chromacris psittacus* (Gerstaecker, 1873). Field surveys, behavioural observations and a rearing experiment compare host plant choice, aggregation, locomotion and thermoregulation between life history stages. Results showed that both nymphs and adults fed exclusively on a narrow range of Solanaceae plants, suggesting that the shift in defensive syndrome is not due to a change in chemical defense. Instead, nymphal aposematism appears linked to aggregation in response to plant-based selection pressures. Slow nymphal development suggests a cost to feeding on toxic plant compounds, and grouping could mitigate this cost. Grouping also increases conspicuousness, and hence can favour warning colouring in chemically-defended insects. The role of diet breadth in aposematism is poorly understood, and these results suggest how constraints imposed by feeding on toxic plants can generate bottom-up selection pressures shaping the adaptive suites of traits of chemically-defended animals.

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Introduction

Animals that feed on toxic plants are often themselves distasteful or toxic, and exhibit variable suites of associated traits, including some that enhance defense (e.g. defensive secretions, aposematism and group-living) and others made possible by lower predation pressure (e.g. large size, sluggish or conspicuous behaviour). These traits often change during ontogeny with size-dependent selection pressures [1]. Ontogenetic colour change in chemically-defended animals generally goes from crypsis early in development to aposematism at larger body size [2], and the increase in aposematism during herbivore ontogeny seems to be reinforced by both bottom-up and top-down forces [1]. Size-linked selection pressures are more likely to favour crypsis in small animals (which are both less conspicuous and less able to sequester a large enough dose of toxin to deter predators) than in larger ones [3]. An opposite shift from

Table 1. Life history traits of previously studied Romaleids. Table includes data from the literature on diet breadth, colouration and aggregation of nymphs and adults, nymphal development time, adult flight capacity and defensive secretions and final female adult size.

Species	NYMPHS				ADULTS						Source
	diet	color	group	develop. time	diet	color	group	flight	secrete	♀ size (mm)	
<i>Rhincoderma</i> spp. (<i>Bactrophorinae</i>)	P	C	s	?	p	c	s	no	?	40	[22]
<i>Xyleus discoideus</i> (<i>Procolpini</i>)	P	C	s	60 d. at 28°C	p	c, f	S	weak	No	50	[21]
<i>Tropidacris collaris</i> (<i>Tropidacrini</i>)	P	A	g	?	p	c, f	?	yes	No	100	[11,16,17]
<i>Brachystola magna</i> (<i>Romaleini</i>)	P	C	s	27 d. 20–25°C	p	C	S	no	No	65	[19]
<i>Zoniopoda tarsata</i> (<i>Romaleini</i>)	P	A	s	?	p	a	s	yes	?	60	[20]
<i>Taeniopoda eques</i> (<i>Romaleini</i>)	P	A	g	39 d. in field	p	a	S	no	yes	51	[11]
<i>Romalea microptera</i> (<i>Romaleini</i>)	P	A	g	60 d. in field	p	A	G	no	yes	76	[18]
<i>Chromacris colorata</i> (<i>Romaleini</i>)	O	A	g	8–10 days/ instar at 28°C	o	c,f	s	yes	No	?	[23]
<i>C. psittacus</i> (<i>Romaleini</i>)	O	A	g	2 instars in 50 days at 26:18°C	o	c, f	s	yes	No	40	present study

The subfamily (for the one species that is not in the Romaleinae) or tribe is given for each species. The genus *Taeniopoda* is thought to be paraphyletic with respect to *Romalea microptera* [24]. Recorded nymphal development times are given (d. = days), with temperature conditions under which these measurements were made. Question marks indicate fields for which no information was found. Codes: diet: p–polyphagous, o–oligophagous; colour: a–aposematic, c–cryptic, f–flash; grouping: g–gregarious, s–solitary (‘solitary’ indicates that no evidence exists for active aggregation, although the species may occasionally reach very high densities and be important crop pests). Secrete = secretion from specialized glands only, not regurgitation.

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aposematism to crypsis could be favoured under particular circumstances where the costs of crypsis decrease over ontogeny [4]; a few examples exist (e.g. in a tropical frog [5]), notably in the Pyrgomorphidae or gaudy grasshoppers [6,7], but overall empirical studies of aposematism-to-crypsis ontogenetic shifts are rare.

Such a shift from aposematic to cryptic colouration appears to occur in some Romaleid grasshoppers (Table 1; Fig 1), providing an opportunity to test how colouration interacts with other traits, and how these trait associations vary across ontogeny. Romaleid grasshoppers (ca 500 species) exhibit various degrees of chemical defense, from distastefulness to toxicity to secretion of noxious compounds, which can be integrated with other traits in diverse defensive suites [8]. Romaleids are called lubbers due to their sluggish behaviour. The two best studied lubbers, *Romalea microptera* and *Taeniopoda eques*, both sequester compounds from plants and use them in defensive secretions expelled from the spiracles in response to attack [9–11]—the quantity and quality of these secretions is highly variable and affects deterrence [9]. The Romaleidae and sister families are thought to have diverged from Old World ancestors in the Cretaceous and to have diversified in South America [12,13]; feeding on toxic plants appears to be ancestral and to have driven associated changes in physiology, morphology, life history and ecology [8]

Many lubber species exhibit warning colouration at some stage in the life cycle and some are also gregarious. Lubbers are generally sluggish, some are flightless, and exhibit exposed diurnal behaviour, combined with behavioural thermoregulation and basking. They attain very large sizes, presumably linked with an absence of vertebrate predation [8]. The few lubber species studied to-date combine these traits in a variety of suites of traits [8]—see Table 1. Some variation in traits appears easily explained by local adaptation: for instance, desert-dwelling *T. eques* exhibits behavioural thermoregulation and early morning basking [14], but subtropical wetland *R. microptera* does not [15].

Distasteful lubbers obtain their defensive compounds from host plants [25], and, in the one species studied (*T. eques*), nymphs and adults show the same pattern of feeding and diet breadth [26]. However, other selection pressures vary during ontogeny: as insects grow bigger,



Fig 1. *Chromacris psittacus* adult and early-instar nymphal herd.

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thermal mass increases and basking need decreases—as expected, *T. eques* adults show less thermoregulatory behaviour than nymphs [17]. Predation risk has also been shown to decrease with increasing size in warningly-colored distasteful lubbers, as larger chemically-defended prey are more avoided by predators [8,27]. Some predators attack small nymphs (that contain a smaller dose of toxin) but not adults [17]. What limited evidence exists on romaleid ontogeny suggests that, among species with aposematic nymphs, some retain warning colouration as adults (e.g. *T. eques*, *R. microptera*) whereas others (e.g. *Chromacris* species) exhibit an unusual ontogenetic shift, switching to crypsis with flash-colouration as adults: the body is cryptic, but in flight the colourful hindwings appear [23,28–33]—see Fig 1. Adults appears to combine low

contrast with background vegetation and disruptive patterns, such that they are difficult for humans to detect on vegetation [29].

Several possible scenarios could underlie this unusual ontogenetic colour change: the simplest involves an ontogenetic change in host plant use that impacts effectiveness of chemical defense (1)—indeed, several Orthopteran species are aposematic as nymphs feeding on toxic plants but become cryptic as adults when they change their diet [3]. Alternatively, chemical defense could remain throughout the life-cycle, and aposematism could be favoured early in ontogeny by aggregation driven by other selection pressures, e.g. thermoregulation, or overcoming plant defenses [34]. These pressures are generally stronger in smaller animals [1].

The following questions were investigated in order to compare suites of traits of *Chromacris psittacus* adults and nymphs and to assess the above scenarios regarding ontogenetic trajectories:

1. Host plant choice: do nymphs and adults feed on the same range of plants and/or plant parts? Do they exhibit diet mixing [35] to enhance their chemical defense?
2. Aggregation: Do nymphs actively stay together (truly gregarious sensu Costa[30]) or are groups simply resource-driven aggregations [36]? Specifically, we will test whether synchronization keeps nymphs together during movement [37,38].
3. Underlying benefits of nymphal aggregation: do nymphal groups exhibit behaviours that suggest either thermoregulatory or plant-based advantages to grouping? Specifically, do they bask collectively to increase temperature and maximize growth rate [14]? Do they synchronize feeding to overcome plant defenses [39–41]?
4. Sluggishness: Do adults/nymphs exhibit sheltering or exposed behaviour? How mobile are they? Is movement sluggish?
5. Nymphal growth rate: do nymphs grow fast to attain large size and escape invertebrate predators as per the slow-growth-high-mortality hypothesis [8,42]?

Methods

The hypotheses listed above were tested with a combination of a field survey, field observations and laboratory rearings. Data were combined from these three approaches to characterize the *C. psittacus* ontogenetic strategy.

Study species

The genus *Chromacris* (9 species, several subspecies) feed on Solanaceae host plants, and are common in disturbed areas in the neotropics [43]. The black-and-red colouration of *C. psittacus* nymphs is typical of aposematism as it increases conspicuousness against the green of foliage [44], whereas the green-and-yellow of adults blends in with the environment [29]—see Fig 1. Adults also display flash colouration: the bright orange hind wings appear when they fly away in response to disturbance [33]. Nymphs undergo 6 instars before the moult to the adult stage [23]. *C. psittacus* adults shows no evidence of specialized defensive secretions but do regurgitate when handled (personal observation). The species is thought to be distasteful to predators [23,28,29,45]; distastefulness is more likely derived from gut-contents than from sequestered compounds [46,47], although this has not been tested.

Field survey of nymphs and adults

A field survey of adult and nymphal *C. psittacus* was conducted in 2017 in semi-natural partially shaded areas (overgrown old-fields, forest edges) in the Mindo valley (00°03'44.1''S

78°45'41.7''W), located in cloud forest at 1250 m a.s.l. on the Western slope of the Andes in the province of Pichincha, Ecuador.

The following variables were recorded for each individual or group observed: host plant, leaf position (expanding, mature or senescent), group size and whether the insect was in the sun or shade. Nymphs were scored for developmental stage: early instar = no visible wing pads, mid-instar = two pairs of wing pads visible or late instar = only forewing pads visible and beginning background colour change to green instead of red. Each host plant on which an individual or group was observed was flagged, and monitored daily to record changes in group size or movement between host plants. Field work was conducted on private land, authorized by land-owner (Maria Elena Garzon Jaramillo).

These data were used to test hypotheses about: 1. Host plant choice (are nymphs and adults observed on the same host plants?), 2. Aggregation (what is the frequency distribution of nymphal group sizes?), and 3. Sluggishness (do individuals react to the presence of the observer?).

Behavioural observations

The behaviour of 12 replicates (nymphal groups or adult individuals) was recorded in the field using Noldus Pocket Observer. Three classes of behaviour were continuously recorded, each comprising several mutually exclusive states: activity (resting, moving or eating), location (on host plant, on another plant or off plants) and basking. The basking variable was scored as follows: in sun (the weather is sunny and the insect is in direct sunlight), in shade (the weather is sunny and the insect is in the shade), overcast or light rain (in these two cases the question of basking behaviour does not arise). Observations were discontinued under heavy rain. Several point behaviours (events with no duration) were also recorded: flight (adults only), changing leaf and changing plant.

Observations were intended for 2 h each, but some were terminated early due to interruptions (e.g. by rain or the insect flying away and getting lost). Others were continued for longer, for a total of 24h of observation.

The behaviour of nymphal groups was recorded in a similar fashion as that of adult individuals, except that records also included the number of individuals involved in the behaviour. These data were used to test hypotheses about:

1. Host plant choice: diet-mixing was tested by recording each plant on which the individual/group fed during the course of the assay [35].
2. Aggregation: Active aggregation of nymphs was evaluated as synchronization of movement by testing for overdispersion relative to a binomial process [48]. For each replicate observation, the frequency distribution of the number of nymphs engaged in moving was compared with a binomial distribution around the mean number of moving individuals over the course of that observation. The goodness of fit to the binomial was tested with an overdispersion index, calculated as the ratio of deviance to the degrees of freedom. If behaviour of nymphs in the herd is independent (i.e. no synchronization), this index is equal to 1 [49]. A chi-square test was used to assess significance of the departure from independent behaviour [47].
3. Thermoregulation: periods of each observation in which weather was overcast or light rain were excluded from the analysis. Proportion of observation time spent in the sun was compared with that in the shade to test whether insects bask to increase their body temperature. Quasibinomial distribution was used because the data did not fit the binomial.
4. Overcoming plant defenses: As in 2, chi-square was used to test if feeding is synchronized based on goodness of fit to a binomial.

5. Sluggishness: Proportions of time spent moving and feeding were compared between nymphs and adults with quasibinomial GLMs.

Nymphal rearing

Ten early-instar nymphal herds (mean group size 9.6 +/- 2.2 S.D.) were collected in the field during October and November 2017 and reared in sleeve cages on *Brugmansia* spp (mean rearing period 50 days +/- 10 S.D.). Each herd was weighed with a portable balance (Ohaus Scout SPX123). Due to the low resolution of the balance (10 mg) and the gregarious behaviour of the insects, the group was weighed as a whole, and mass was divided by the number of individuals to calculate an individual relative growth rate, RGR ($\text{mg}\cdot\text{day}^{-1}\cdot\text{g}^{-1}$). Rearing was done under field conditions with a 12:12 photoperiod, 18°C: 26°C night- and day-time temperatures.

Results

Host plant use and diet mixing

In the field survey, of 60 adults seen, the sex ratio was balanced (32 females, 28 males). Most were observed on *Solanum* section *geminata* spp (N = 21) or *Brugmansia* spp (N = 20), but a few were on other Solanaceae as well (*S. acerifolium* (N = 5), *Cestrum* spp (N = 2), *S. candidum* (N = 1), *Acnistus arborescens* (N = 4), *Solanum lycopersicum* (N = 7)). All were on mature leaves. Of 30 nymphal groups seen, most were seen on *Solanum* section *geminata* spp (N = 21) and *Brugmansia* spp (N = 6). A few late instar groups were seen on *A. arborescens* (N = 2) and *Cestrum* spp (N = 1).

The behavioural observations showed no evidence for dietary mixing. Adults occasionally moved between individual plants, but never between species: movement between individual host plants was observed 20 times in 24h of observation. Some individuals changed host plant (N = 9 individuals moved between plants between 1 and 6 times each) but others (N = 3) never did so in the 2h observation period. Nymphal herds were never observed to move between plants. The field survey showed that nymphal herds were often observed on the same individual plant for several subsequent days, see Table 2.

Nymphal herds also moved from one leaf to another within the plant less often than adults (total of 16 times in 24h compared to 56 times). Adults appeared to be selective of feeding sites, moving from one leaf to another before settling to feed, whereas nymphal herds always fed on the leaf on which they were situated. Adults were observed to spend most of their time on host plants (85.6% of observation duration), and nymphs were never observed off hosts.

Both nymphs and adults showed a clear alternation between meals and intermeal intervals (sensu [50]). Adults exhibited meals of a median duration of 7 min (first quartile 2 min; third quartile 15 min; n = 27 uncensored bouts) of feeding, interspersed with 30 min (first quartile 23 min; third quartile 61 min; n = 17 uncensored bouts) intermeal intervals, time spent generally immobile with short, slow movements. Nymphal herds exhibited synchronized meals of

Table 2. Field observation of nymphal herd group size (mean +/- S.E.) and persistence (number of days the group was observed on the same plant, mean +/- S.E.) per developmental stage.

instar	# groups	group size	persistence (days)
early	15	22.73 +/- 8.2	7.66 +/- 3.05
mid	7	19.14 +/- 1.80	3.43 +/- 0.64
late	8	5.13 +/- 1.38	2.00 +/- 1.10

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median 23 min duration (first quartile 10 min; third quartile 32 min; $n = 28$ uncensored bouts), and 33 min (first quartile 18 min; third quartile 63 min; $n = 18$ uncensored bouts) quiescent intermeal intervals.

Nymphal aggregation and synchronization

In the field survey, most adults were observed alone, but nymphs were always observed in groups. Group size decreased as development progressed (see Table 2).

Overdispersion analysis reveals that 10 of 12 groups exhibited significant (χ^2 $p < 0.05$) synchronization of movement; the two exceptions are short observations with only approximately 10 moving bouts recorded, suggesting that the number of data points was insufficient for an adequate test of behavioural synchrony (see Fig 2). Whenever a nymph became separated from the group during movement around the plant's architecture, it quickly rejoined the group, orienting to visual stimuli [23].

For eating behaviour, 8 out of 12 groups exhibited significant synchronization. The 4 replicates in which synchronized feeding was not observed had again been short observations in which less than 10 feeding bouts were observed (see Fig 2).

Basking

When it was sunny, both nymphs and adults spent more time in the shade than in the sun (for both adults and nymphs $P < 0.0001$; binomial GLM comparing proportion of assay time spent

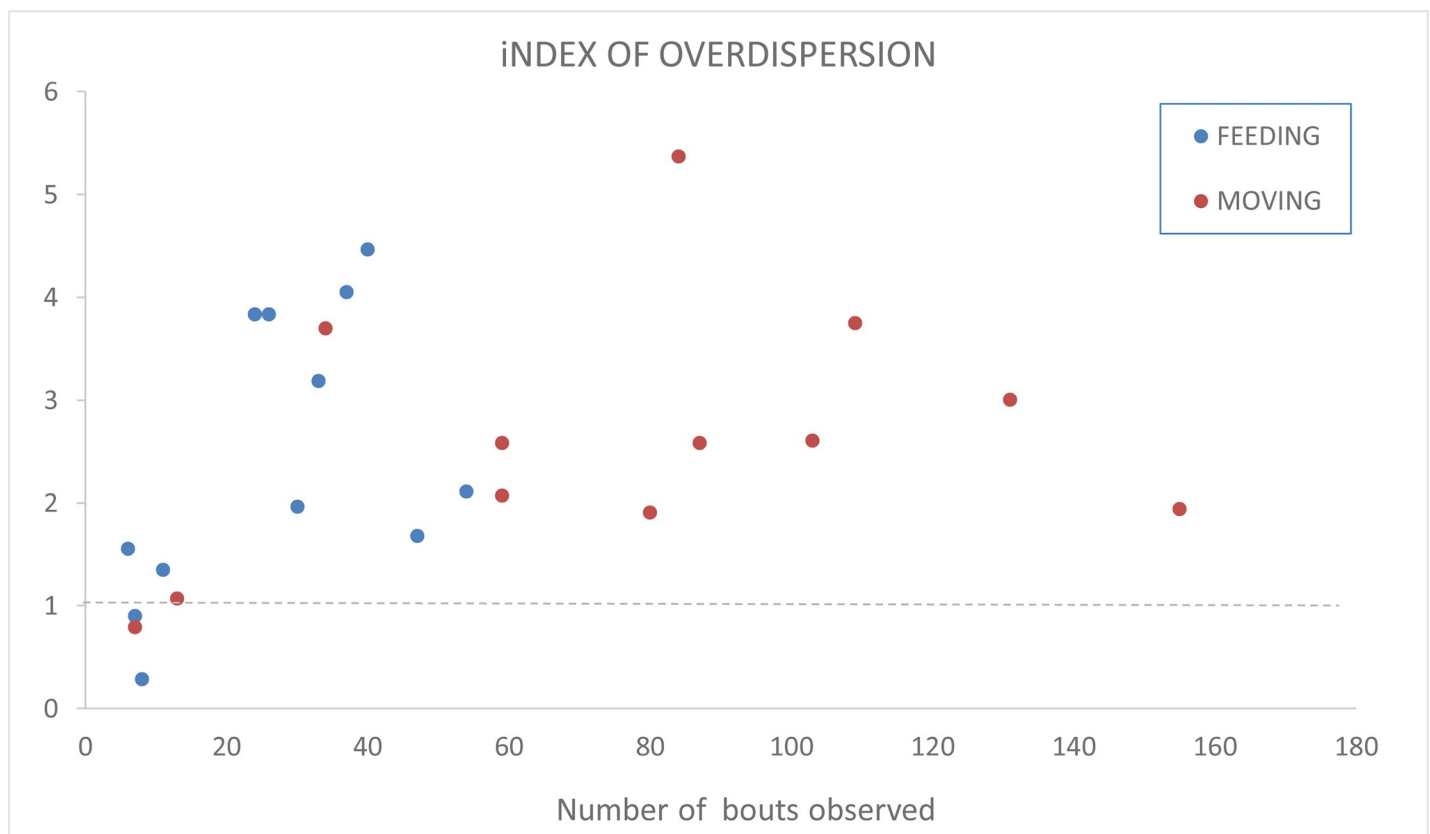


Fig 2. Synchronization of eating and moving bouts. The y-axis represents the index of overdispersion from the binomial distribution—values greater than one indicate synchronization of behaviour (indicated by the dashed line). The x-axis represents the number of behavioural bouts observed.

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in sun or in shade)—see Table 3. When clouds moved away and grasshoppers found themselves in full sun, they generally moved to the shade within minutes (the average duration of bouts in the sun was 4.9 min for adults and 4.8 min for nymphs, compared to 22 min in the shade for nymphs and 66 min for adults).

The persistence of nymphal colonies on the same host plant made it possible to observe their daily pattern of movement: they moved down to the ground in the evening and stayed at the base of plants overnight, where they remained until ca 10 AM the following morning, when they moved up the plant and onto a leaf. The early morning weather was generally overcast, and basking was never observed. By the time the sun emerged in mid-morning, the temperature was generally warm enough (ca 26°C) that the nymphs sought out the shade.

Sluggishness

The field survey showed that most adult individuals were no longer on the same plant the following day (N = 57). However, a few remained on the same plant for several days (N = 3): these individuals were seen on isolated individuals of infrequently used host-plant species (*Cestrum* and *S. acerifolium*), with no other Solanaceae nearby.

By contrast, some nymphal herds remained on the same plant for up to 3 weeks. Most groups were observed on the same plant for several days (mean: 5 +/- 1.96 S.D.), but this persistence decreased as nymphs grew (see Table 2).

The behavioural observations showed that nymphs were more active than adults, but not quite significantly so ($t = 2.07$; $p = 0.0504$; quasibinomial GLM on proportion of assay time spent moving). Nymphs spent significantly more time feeding than adults ($t = 4.38$; $p = 0.00026$; quasibinomial GLM on proportion of assay time spent feeding)—see Table 3. Both adults and nymphs spent most of the time immobile.

Nymphal development

Nymphs exhibited a mean RGR = 0.11 +/- 0.018 S.D. $\text{mg}\cdot\text{day}^{-1}\cdot\text{g}^{-1}$ (growth per day divided by initial mass) over an average period of 50 days. Moults were approximately synchronized within groups: most groups moulted twice during the observation period and averaged 24 days between moults.

Table 3. Behavioural observations, showing number of events for point behaviours (moving between leaves, between plants, and flight in response to a disturbance) and proportion of assay time spent in mutually exclusive timed behaviours (eating, moving, resting) or locations (on a host plant, on another plant, on the ground). Time spent in sun or shade is given as a proportion of the time in which the weather was sunny.

Behaviour	ADULTS		NYMPHS	
	# events	% time	# events	% time
Change leaf	56		16	
Change plant	20		0	
Fly	39			
Eating		7.33		20.8
Moving		7.41		10.8
Resting		85.3		62.2
Host plant		85.6		100
Other plant		9.70		0
Non plant		4.41		0
Shade		93.04		82.10
Sun		6.90		17.81

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Discussion

Ontogenetic shift in defensive suite of traits

Both nymphs and adults fed exclusively on a narrow range of Solanaceae plants, consuming only mature foliage, which is generally lower in defensive compounds than are developing leaves [51]. These results do not support the hypothesis that the ontogenetic switch in defensive traits is tied to a change in diet and resulting decrease in chemical defense in the adult stage, and suggest it is more likely linked to other selection pressures associated with aggregation.

The *C. psittacus* nymphs remain in very conspicuous behaviourally synchronized groups, suggesting that these insects are truly gregarious sensu [30], and are not merely exhibiting resource-driven aggregation [36]. Previous research also shows that congeneric *Chromacris colorata* rejoin groups if they are experimentally separated [23]. Active gregariousness suggests that individuals derive advantages from proximity to neighbours and hence exhibit behaviours that keep them together [37]. One benefit that these nymphs likely derive from aggregation is enhancement of the aposematic signal [52]; in the context of the unusual ontogenetic shift from crypsis to aposematism, we examine whether other selection pressures could also be involved in nymphal grouping. The observations provided no evidence of basking to increase body temperature, one commonly cited advantage to grouping; on the contrary, nymphs avoided direct sunlight, moving into the shade when the sun emerged. Advantages to gregariousness are thus not likely to include thermoregulation.

By contrast, nymphs did exhibit synchronized feeding, suggesting density-dependent manipulation of host quality. Synchronized feeding has been shown to increase consumption rates in several gregarious folivorous insect species [53–56]. For example, *Battus philenor* (Lepidoptera) larvae gain more weight in groups than singly, even when prevented from interacting directly, suggesting that simultaneous feeding with others improves host suitability [40], via a form of induced susceptibility involving overcoming of chemical defenses or creation of a nutrient sink [57]. Solanaceae, including *Solanum* and *Brugmansia*, contain complex mixes of alkaloids and it has been shown that, in *Brugmansia*, the tropane alkaloid scopolamine is induced following damage to leaves and inhibits subsequent herbivory [58]; synchronized feeding could limit exposure of nymphs to this noxious compound [41], providing a selective advantage to gregariousness in *C. psittacus* nymphs.

Nymphal development was surprisingly slow, refuting the prediction that nymphs should grow fast in order to achieve large sizes and escape invertebrate predators sooner [8,26]. Relative growth rate has not been investigated in other romaleids, but the values obtained in the present study are lower than those found for the sister family Acrididae [59–62]. Recorded development times for romaleids are shorter than those observed in the present study (see Table 1).

Both nymphs and adults *C. psittacus* thus exhibit adaptive suites of traits compatible with chemical defense, involving aposematism and gregariousness in the early instars, gradually shifting to crypsis and evasive flash colouration in the adult [29]. No evidence suggests that this shift is linked to lower chemical defense in adults since both feed exclusively on plants that contain compounds that can confer toxicity to vertebrate predators. A potential alternative explanation is based on bottom-up selection pressures associated with an increase in ability to metabolize host plant defenses with larger size [63]. For small nymphs, host plant defenses might favour grouping; indeed, previous work on gregarious chemically-defended insect larvae has suggested that the adaptive value of group-living in the early instars lies mainly in overcoming host plant defenses [40,64,65]. In this case, the cost of aposematism would be low since the grouped insects are already conspicuous [34,52]. In general, aposematism is favoured

when toxic compounds provide effective defense and costs of crypsis are too high [1]. In the present case, feeding on toxic plants appears to provide that defense and to impose constraints that make crypsis impossible for nymphs. In adults, size-related increased ability to feed alone would imply lower host-plant-related cost for solitary living. In addition, the acquisition of flight further lowers the cost of crypsis by enabling a second line of defense based on a startle display with rapid escape [52].

Diet breadth and defensive traits

Observations suggested no evidence of the dietary mixing seen in other romaleids: both adults and nymphs fed exclusively on a single plant species during an observation. By contrast, the gregarious aposematic *T. eques* and the solitary cryptic *Brachystola magna* (both Romaleidae) exhibited a high degree of individual polyphagy, with *T. eques* individuals feeding on up to 30 food items per day and single meals consisting of up to 11 different food items [35,66,67]. The present findings suggest that *C. psittacus* do not use the strategy suggested for *T. eques* of achieving defense against predators by mixing compounds from different host plants [67]. Conversely, being confined to a single host plant has been shown to increase efficacy of chemical defense in the polyphagous gregarious aposematic *R. microptera* [68]. The relative effectiveness of chemical defenses and aposematism in specialist vs generalist herbivorous insects is not clear: dietary specialization could enhance defense by increasing concentration of defensive compounds in the insect [54], or feeding from a diverse range of plants could allow synergies between plant compounds [35,69].

Solanaceae alkaloids are highly toxic to vertebrates, and are used by several aposematic Acridid grasshoppers as chemical defense [34,70,71]. Low growth rate and slow development, like those observed in the present experiment can be indicative of high chemical defenses in the host plant and associated cost of detoxification for the herbivore [62]. Indeed, even specialist feeders on toxic plants can suffer costs associated with their host plant's defenses: for instance, alkaloids in Solanaceae host plants negatively affect development, survival and fecundity of the specialist tobacco hornworm, but do not trigger avoidance behaviour and do protect the caterpillars from predators [72]. Aposematic insects that acquire defenses from their host plant face a trade-off between top-down (predation avoidance) and bottom-up (toxicity) effects of feeding on toxic plant compounds [52,63,73]. Specialist feeding could place *C. psittacus* at a different point along this trade-off continuum than other diet-mixing romaleids.

The behaviour of *C. psittacus* nymphs and adults resembles more that of the cryptic Acridid *Schistocerca shoshone* than that recorded for aposematic romaleids: insects remain perched on the host plant rather than on the ground, move rarely and feed exclusively on that plant [74]. Chambers et al (1996) proposed alternative foraging strategies for cryptic and aposematic generalist-feeding grasshoppers, and our results suggest broadening the scope to include diet as an important driver of behavioural traits and a key bottom-up selection pressure generating correlated adaptive regimes [75].

Defensive traits across the Romaleidae radiation

The other lubber that has been most studied, *T. eques*, is polyphagous and gregarious, and exhibits the common pattern of becoming more aposematic with age: young nymphs are black with faint red/yellow lines as nymphs, the yellow colouration expands as they mature leading to striking warning colouration. Adults are flightless, and have been described as very sluggish, easily captured by hand (hence the name lubber). They exude a noxious smell, suggestive of strong chemical defense. When disturbed, the adults raise their forewings to display red hindwings and make a hissing sound from the spiracles [26]. By contrast, although *Romalea*

microptera and *Brachystola magna* are also polyphagous and flightless as adults, *R. microptera* nymphs are gregarious and aposematic and the adults exhibit broad variation in colour, whereas *B. magna* nymphs and adults are both solitary and cryptic [76]—see Table 1.

The romaleid toolkit of traits associated with chemical defense thus includes various different ontogenetic trajectories potentially linked to polyphagy vs oligophagy and associated effects of plant chemicals on metabolism, activity rate and development (see Table 1). Table 1 suggests a possible pattern among species with aposematic (and presumably distasteful) nymphs: some give rise to aposematic, flightless adults with noxious secretions (e.g. *T. eques*, *R. microptera*, native to the United States) whereas others (the *Tropidacris* and *Chromacris* spp of South America) mature into flighted adults with flash colouration. Little is known about the diet-breadth or distastefulness of the species with the latter strategy [28,43], but this table suggests that flight could be an important factor in adult defensive strategies. In general, romaleid defensive strategies appear very effective, as field studies record never witnessing even predation attempts [8,11,25,28].

Complex suites of traits associated with chemical defense have evolved more than once amid Orthoptera [77]. The Pyrgomorphidae (the gaudy grasshoppers) exhibit various combinations of the following traits; large size, polyphagy, flightlessness, warning colouration and nymphal gregariousness [7,78,79]. For example, *Zonocerus variegatus* nymphs are polyphagous, aposematic, gregarious and slow-growing (nymphal development typically 100–120 days) [36]. Their slow growth rate has been linked to high losses to respiration rate, suggested to be related to the ability to feed on and sequester toxic plants [80]. Feeding by *Z. variegatus* nymphs is facilitated by grouping and grouped nymphs grow faster than isolated individuals, particularly in the early instars, as later developmental stages show stronger tolerance of host plant compounds [80]. Similarly, *Phymateus leprosus* are aposematic, polyphagous and gregarious as nymphs. They develop very slowly (10 instars over an entire year [81]) and eventually metamorphose into very large, aposematic, polyphagous adults that are weak flyers and possess glands for defensive secretions [7,79]. Aposematism is thought to have evolved independently twice among the Pyrgomorphidae [7], and the genetic basis for feeding on toxic plants is being unraveled [79], making them an excellent parallel system to the Romaleidae for the study of defensive suites of traits.

Some Acridids are also aposematic, and some exhibit a remarkable density- and host-plant dependent aposematism, whereby nymphs that feed on chemically defended plants develop aposematic colouration at high density [71]. Similarly, desert locusts appear to switch from a solitary cryptic morph to an aposematic gregarious one according to population density [82]. This facultative aposematism has been linked to heightened costs of conspicuousness associated with aggregation on host plants that make crypsis impossible [57], in a mechanism similar to that proposed here. The Orthoptera thus include at least three families (Romaleidae, Pyrgomorphidae, Acrididae) in which aposematism appears to be linked to variable suites of traits and to follow different ontogenetic trajectories. Our results support previous work suggesting that defensive suites of traits depend not only on top-down selection pressures imposed by predators, but also on bottom-up forces from host plants, including responses to the toxicity of plant compounds.

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References

1. Boege K, Agrawal AA, Thaler JS. Ontogenetic strategies in insect herbivores and their impact on tri-trophic interactions. *Current Opinion in Insect Science*. 2019 Apr 1; 32:61–7. <https://doi.org/10.1016/j.cois.2018.11.004> PMID: 31113633
2. Booth CL. Evolutionary significance of ontogenetic colour change in animals. *Biological Journal of the Linnean Society*. 1990; 40:125–63.
3. Caro T, Sherratt TN, Stevens M. The ecology of multiple colour defences. *Evol Ecol*. 2016 Oct 1; 30(5):797–809.
4. Higginson AD, Ruxton GD. Adaptive changes in size and age at metamorphosis can qualitatively vary with predator type and available defenses. *Ecology*. 2010; 91(9):2756–68. <https://doi.org/10.1890/08-2269.1> PMID: 20957968
5. Bulbert MW, White TE, Saporito RA, Kraus F. Ontogenetic colour change in *Oreophryne ezra* (Anura: Microhylidae) reflects an unusual shift from conspicuousness to crypsis but not in toxicity. *Biol J Linn Soc*. 2018 Jan 1; 123(1):12–20.
6. Kohler G, Samietz J, Wagner G. Field observations on the bush locust, *Phymateus leprosus* (Fabricius, 1793), in the Great Karoo, South Africa (Acridoidea: Pyrgomorphidae). *Opuscula Zoologica Fluminensia*. 2001; 27:1–15.
7. Mariño-Pérez R, Song H. Phylogeny of the grasshopper family Pyrgomorphidae (Caelifera, Orthoptera) based on morphology. *Systematic Entomology*. 2018; 43(1):90–108.
8. Whitman DW, Vincent S. Large Size as an Antipredator Defense in an Insect. *Journal of Orthoptera Research*. 2008; 17(Journal Article):353–71.
9. Hatle JD, Spring JH. Inter-individual variation in sequestration (as measured by energy dispersive spectroscopy) predicts efficacy of defensive secretion in lubber grasshoppers. *Chemoecology*. 1998 Jul 1; 8(2):85–90.
10. Schowalter TD. Biology and Management of the Eastern Lubber Grasshopper (Orthoptera: Acrididae). *J Integr Pest Manag [Internet]*. 2018 Jan 1 [cited 2019 Dec 3]; 9(1). Available from: <https://academic.oup.com/jipm/article/9/1/10/4938808>
11. Whitman DW, Orsak LJ. Biology of *Taeniopoda eques* (Orthoptera: Acrididae) in Southeastern Arizona. *Annals of the Entomological Society of America*. 1985; 78(Journal Article):811–25.
12. Song H, Mariño-Pérez R, Woller DA, Cigliano MM. Evolution, Diversification, and Biogeography of Grasshoppers (Orthoptera: Acrididae). *Insect Syst Divers [Internet]*. 2018 Jul 1 [cited 2020 Feb 25]; 2(4). Available from: <https://academic.oup.com/isd/article/2/4/3/5052737>
13. Pocco ME, Guzmán N, Plischuk S, Confalonieri V, Lange CE, Cigliano MM. Diversification patterns of the grasshopper genus *Zoniopoda* Stål (Romaleidae, Acridoidea, Orthoptera) in open vegetation biomes of South America. *Systematic Entomology*. 2018; 43(2):290–307.
14. Whitman DW. Thermoregulation and daily activity patterns in a black desert grasshopper, *Taeniopoda eques*. *Animal Behaviour*. 1987 Dec 1; 35(6):1814–26.
15. Stauffer TW, Hatle JD, Whitman DW. Divergent egg physiologies in two closely related grasshopper species: *Taeniopoda eques* versus *Romalea microptera* (Orthoptera: Romaleidae). *Environmental entomology*. 2011; 40(1):157–166. <https://doi.org/10.1603/EN10200> PMID: 22182625
16. Lhano MG, Serrano LAL, Vidal-Neto F, Dias-Pini N. *Tropidacris collaris* (Orthoptera: Romaleidae) expands its damage in orchards of the dwarf cashew, *Anacardium occidentale* (Anacardiaceae). *Entomological Science*. 2019 01; /04; 22(2):151–6.
17. Carbonell CS. Revision of the Neotropical genus *Tropidacris* (Orthoptera, Acridoidea, Romaleidae, Romaleinae). *Proceedings of the Academy of Natural Sciences of Philadelphia*. 1986; 138:366–402.
18. Hunter-Jones P. The life-history of the Eastern Lubber Grasshopper, *Romalea microptera* (Beauvois), (Orthoptera: Acrididae) under laboratory conditions. *Physiological Entomology*. 1967; 42:18–24.

19. Bright KL, Bernays EA, Moran VC. Foraging patterns and dietary mixing in the field by the generalist grasshopper *Brachystola magna* (Orthoptera: Acrididae). *Journal of Insect Behavior*. 1994; 7(Journal Article):779–93.
20. Carbonell CS. The genus *Zoniopoda* (Acridoidea, Romaleidae, Romaleinae). *Journal of Orthoptera Research*. 2007; 16:1–33.
21. Campos De Domenico F. Nymphal Development of *Xyleus discoideus discoideus* (Serville 1831) (Acridoidea, Romaleidae, Romaleinae) in the Laboratory. *Journal of Orthoptera Research*. 2005; 14(Journal Article):127–35.
22. Rowell CHF. The Central American Genus *Rhacnoderma* (Orthoptera, Romaleidae, Bactrophorinae, Bactrophorini) and Some Closely Related New Taxa. *Journal of Orthoptera Research*. 2012; 21:1–24.
23. Mathieu JM. Biological Studies on *Chromacris colorata* (Orthoptera: Romaleinae). *Journal of the Kansas Entomological Society*. 1970;262–269.
24. De Jesús-Bonilla VS, Meza-Lázaro RN, Zaldívar-Riverón A. 3RAD-based systematics of the transitional Nearctic-Neotropical lubber grasshopper genus *Taeniopoda* (Orthoptera: Romaleidae). *Molecular Phylogenetics and Evolution*. 2019 Aug 1; 137:64–75. <https://doi.org/10.1016/j.ympev.2019.04.019> PMID: 31018164
25. Jones CG, Hess TA, Whitman DW, Silk PJ, Blum MS. Effects of diet breadth on autogenous chemical defense of a generalist grasshopper. *Journal of Chemical Ecology*. 1987; 13(Journal Article):283–97. <https://doi.org/10.1007/BF01025888> PMID: 24301807
26. Alcock J. Observations on the behaviour of the grasshopper *Taeniopoda eques* (Burmeister), Orthoptera, Acrididae. *Animal behaviour*. 1972; 20(2):237–242. [https://doi.org/10.1016/s0003-3472\(72\)80042-3](https://doi.org/10.1016/s0003-3472(72)80042-3) PMID: 4644153
27. Venci FV, Srygley RB. Enemy targeting, trade-offs, and the evolutionary assembly of a tortoise beetle defense arsenal. *Evolutionary Ecology*. 2013; 27(2):237–252.
28. Bidau CJ. Curious Orthopterological Errors of a Great Naturalist: William Henry Hudson. *Journal of Orthoptera Research*. 2012; 21:255–60.
29. Roberts HR, Carbonell CS. A revision of the grasshopper genera *Chromacris* and *Xestotrachelus* (Orthoptera, Romaleidae, Romaleinae). *Proceedings of the California Academy of Sciences* [Internet]. 1982 [cited 2020 May 6]; Available from: <https://agris.fao.org/agris-search/search.do?recordID=US19840032510>
30. Costa JT. *The Other Insect Societies*. Cambridge USA: Belknap Press; 2006.
31. Delgado-Vásquez C, Couturier G, Anteparra M. PRINCIPALES FITÓFAGOS DE LA COCONA *Solanum sessiliflorum* Dunal (SOLANACEAE) EN LA AMAZONÍA PERUANA. *Folia Amazónica*. 2011 Dec 31; 20(1–2):45–51.
32. Otte D, Joern A. On Feeding Patterns in Desert Grasshoppers and the Evolution of Specialized Diets. *Proceedings of the Academy of Natural Sciences of Philadelphia*. 1976; 128:89–126.
33. Cigliano MM, Braun H, Eades DC, Otte D, Cigliano, M.M., H. Braun, et al. [Internet]. *Orthoptera Species File*. Version 5.0/5.0. 2020. Available from: <http://Orthoptera.SpeciesFile.org>
34. Despland E, Simpson SJ. Surviving the change to warning colouration: density-dependent polyphenism suggests a route for the evolution of aposematism. *Chemoecology*. 2005; 15:69–75.
35. Bernays EA, Bright K, Howard JJ, Raubenheimer D, Champagne D. Variety is the spice of life: frequent switching between foods in the polyphagous grasshopper *Taeniopoda eques* Burmeister (Orthoptera: Acrididae). *Animal Behaviour*. 1992; 44(4):721–731.
36. Chapman RF, Page WW, McCaffery AR. Bionomics of the variegated grasshopper (*Zonocerus variegatus*) in West and Central Africa. *Annual Review of Entomology*. 1986; 31(1):479–505.
37. Despland E, Gervais D, Morcos L. Double allomimesis of advancing and retreating individuals maintains cohesion in exploring groups of nomadic caterpillars. *Behavioral ecology and sociobiology*. 2017; 71(3):56.
38. Despland E, Simpson SJ. Resource distribution mediates synchronization of physiological rhythms in locust groups. *Proceedings of the Royal Society B: Biological Sciences*. 2006; 273(1593):1517–1522. <https://doi.org/10.1098/rspb.2006.3471> PMID: 16777746
39. Cocroft RB. Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *Proceedings of the Royal Society B: Biological Sciences* [Internet]. 2005 May 20 [cited 2019 Sep 11]; Available from: <https://royalsocietypublishing.org/doi/abs/10.1098/rspb.2004.3041>
40. Fordyce JA. Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. *Oecologia*. 2003; 135:250–7. <https://doi.org/10.1007/s00442-003-1177-8> PMID: 12698347
41. Krischik VA, Goth RW, Barbosa P. Generalized plant defense: effects on multiple species. *Oecologia*. 1991; 85(4):562–571. <https://doi.org/10.1007/BF00323769> PMID: 28312504

42. Williams IS. Slow-growth, high-mortality—a general hypothesis, or is it? *Ecological Entomology*. 1999; 24:490–5.
43. Amedegnato C. Microhabitat of forest grasshoppers in the Amazon. In: Bassett Y, Miller S, Novotny V, editors. *Arthropods of Tropical Forests: Spatio-Temporal Dynamics and Resource Use in the Canopy*. Cambridge, UK: Cambridge University Press; 2003.
44. Joron M. Chapter 10—Aposematic Coloration. In: Resh VH, Cardé RT, editors. *Encyclopedia of Insects (Second Edition)* [Internet]. San Diego: Academic Press; 2009 [cited 2020 Feb 24]. p. 33–8. Available from: <http://www.sciencedirect.com/science/article/pii/B9780123741448000102>
45. Bidau CJ. Patterns in Orthoptera biodiversity. I. Adaptations in ecological and evolutionary contexts. *Journal of Insect Biodiversity*. 2014; 2(20):1–39.
46. Sword GA. Tasty on the outside, but toxic in the middle: grasshopper regurgitation and host plant-mediated toxicity to a vertebrate predator. *Oecologia*. 2001 Aug 1; 128(3):416–21. <https://doi.org/10.1007/s004420100666> PMID: 24549911
47. Brown KS. Chemistry at the Solanaceae/Ithomiinae Interface. *Annals of the Missouri Botanical Garden*. 1987; 74(Journal Article):359–97.
48. Raussi S, Jauhainen L, Saastamoinen S, Siivonen J, Hepola H, Veissier I. A note on overdispersion as an index of behavioural synchrony: a pilot study in dairy cows. *animal*. 2011; 5(3):428–432. <https://doi.org/10.1017/S1751731110001928> PMID: 22445409
49. Crawley MJ. *The R book*. John Wiley & Sons; 2012.
50. Simpson SJ, Raubenheimer D, Chambers PG. The Mechanisms of Nutritional Homeostasis. In: Chapman RF, de Boer G, editors. *New York: Chapman and Hall; 1995. p. 251–78. (Regulatory Mechanisms in Insect Feeding)*.
51. Kursar TA, Coley PD. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology*. 2003; 31(Journal Article):929–49.
52. Ruxton GD, Allen WL, Sherratt TN, Speed MP. *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry*. Oxford University Press; 2019.
53. Denno RF, Benrey B. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology*. 1997; 22:133–41.
54. Fiorentino VL, Murphy SM, Stoepler TM, Lill JT. Facilitative effects of group feeding on performance of the saddleback caterpillar (Lepidoptera: Limacodidae). *Environmental entomology*. 2014; 43(1):131–138. <https://doi.org/10.1603/EN13144> PMID: 24472205
55. Rhoades DF. Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. *The American Naturalist*, 125: 205–238. 1985;(Journal Article).
56. Tsubaki Y, Shiotsu Y. Group feeding as a strategy for exploiting food resources in the burnet moth *Pryeria sinica*. *Oecologia*. 1982; 55(1):12–20. <https://doi.org/10.1007/BF00386712> PMID: 28309896
57. Karban R, Agrawal AA. Herbivore Offense. *Annu Rev Ecol Syst*. 2002; 33:641–64.
58. Arab A, Alves MN, Sartorato A, Ogasawara DC, Trigo DC. Methyl Jasmonate Increases the Tropane Alkaloid Scopolamine and Reduces Natural Herbivory in *Brugmansia suaveolens*: Is Scopolamine Responsible for Plant Resistance? *Neotropical Entomology*. 2012; 42(Journal Article):2–8.
59. Bernays EA, Angel JE, Augner M. Foraging by a generalist grasshopper: the distance between food resources influences diet mixing and growth rate (Orthoptera: Acrididae). *Journal of Insect Behavior*. 1997; 10(6):829–840.
60. Bernays EA, Minkenberg OPJM. Insect herbivores: different reasons for being a generalist. *Ecology* 78: 1157–1169. 1997;(Journal Article).
61. Coxwell CC, Bock CE. Spatial variation in diurnal surface temperatures and the distribution and abundance of an alpine grasshopper. *Oecologia*. 1995; 104(4):433–439. <https://doi.org/10.1007/BF00341340> PMID: 28307658
62. Greenfield MD, Shelly TE, Gonzalez-Coloma A. Territory selection in a desert grasshopper: the maximization of conversion efficiency on a chemically defended shrub. *The Journal of Animal Ecology*. 1989;761–771.
63. Zalucki MP, Malcolm SB, Hanlon CC, Paine TD. First-instar monarch larval growth and survival on milkweeds in southern California: effects of latex, leaf hairs and cardenolides. *Chemoecology*. 2012; 22(2):75–88.
64. Ghent AW. A study of the group-feeding behaviour of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. *Behaviour*. 1960;110–148.
65. Wilmoth LW, Fordyce JA. Does group feeding by toxic prey confer a defensive benefit? Aristolochic acid content, group size and survival of first-instar pipevine swallowtail (*Battus philenor* L.) larvae.

- Ecological Entomology [Internet]. 2019 [cited 2019 Aug 30];0(0). Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/een.12753>
66. Bernays EA, Bright KL. Mechanisms of dietary mixing in grasshoppers: a review. *Comp Biochem Physiol* 104A: 125–131. 1993;(Journal Article).
 67. Raubenheimer D, Bernays EA. Patterns of feeding in the polyphagous grasshopper *Taeniopoda eques*: a field study. *Animal Behaviour*. 1993; 45(1):153–167.
 68. Jones CG, Whitman DW, Compton SJ, Silk PJ, Blum MS. Reduction in diet breadth results in sequestration of plant chemicals and increases efficacy of chemical defense in a generalist grasshopper. *Journal of chemical ecology*. 1989; 15(6):1811–1822. <https://doi.org/10.1007/BF01012268> PMID: 24272184
 69. Mason PA, Singer MS. Defensive mixology: combining acquired chemicals towards defence. *Functional Ecology*. 2015; 29(4):441–450.
 70. Despland E, Simpson SJ. Food choices of solitary and gregarious locusts reflect cryptic and aposematic antipredator strategies. *Animal Behaviour*. 2005 Feb 1; 69(2):471–9.
 71. Sword GA. Density-dependent warning coloration. *Nature*. 1999; 397(6716):217–217.
 72. Jacobsen DJ. Plant-insect interactions shape plant resource allocation and herbivore responses in Solanaceous species. *University of Indiana*; 2016.
 73. Bowers MD. The evolution of unpalatability and the cost of chemical defense in insects. *Insect chemical ecology: an evolutionary approach* Chapman and Hall, New York. 1992;216–244.
 74. Chambers P, Sword G, Angel JE, Behmer S, Bernays EA. Foraging by generalist grasshoppers: two different strategies. *Animal Behaviour*. 1996; 52(1):155–165.
 75. Despland E. Diet breadth and anti-predator strategies in desert locusts and other Orthoptera. *Journal of Orthoptera Research*. 2005; 14(2):227–233.
 76. Bright KL, Bernays EA, Moran VC. Foraging patterns and dietary mixing in the field by the generalist grasshopper *Brachystola magna* (Orthoptera: Acrididae). *J Insect Behav*. 1994 Nov 1; 7(6):779–93.
 77. Song H, Foquet B, Mariño-Pérez R, Woller DA. Phylogeny of locusts and grasshoppers reveals complex evolution of density-dependent phenotypic plasticity. *Scientific Reports*. 2017 Jul 1; 7:6606. <https://doi.org/10.1038/s41598-017-07105-y> PMID: 28747803
 78. Rowell CHF. Experiments on aggregations of *Phymateus purpurascens* (Orthoptera, Acrididae, Pyrgomorphinae). *Journal of Zoology*. 1967; 152(2):179–93.
 79. Yang L, Ravikanthachari N, Mariño-Pérez R, Deshmukh R, Wu M, Rosenstein A, et al. Predictability in the evolution of Orthopteran cardenolide insensitivity. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2019 Jul 22; 374(1777):20180246.
 80. McCaffery AR. A difference in the acceptability of excised and growing cassava leaves to *Zonocerus variegatus*. *Ent exp & appl*, 32:111–115. 1982;(Journal Article).
 81. Kohler G, Roth S, Reinhardt K. Ten instars in the leprous grasshopper *Phymateus leprosus* (Fabricius, 1793) (Caelifera: Pyrgomorphidae): Maximum number recorded in the Acridoidea. *Bonner zoologische Beiträge*. 2008; 56(1):17–24.
 82. Sword GA, Simpson SJ, El Hadi OTM, Wilps H. Density-dependent aposematism in the desert locust. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 2000; 267(1438):63–68. <https://doi.org/10.1098/rspb.2000.0967> PMID: 10670954