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Host plant selection and differential survival on two *Aristolochia* L. species in an insular population of *Zerynthia cassandra*

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Abstract

Understanding host plant preference and the relative quality of resource provided by cooccurring host plants is a key step to predict butterfly species abundance and responses to environmental changes, and, consequently, to plan management measures. Zerynthia cassandra is an Italian endemic species strongly dependent on the availability of its host plants, Aristolochia rotunda and A. lutea. The insular population occurring on Elba island (Tuscan Archipelago) is highly threatened, because of limited host plant distribution, small population size and apparent lack of gene flow with the mainland. In 2017, we carried out field surveys and rearing experiments to i) identify the characteristics of the host plants (vegetative status) and the site characteristics (aspect, irradiation, distance from other patches) correlated with the number of eggs occurring on individual plants, ii) compare larval growth, food-conversions rate and larval and adult survivorship on the two host plants species. Egg occurrence depends on patch irradiation, the number of leaves and flowers occurring on individual plants and the occurrence of nearby patches. These findings allowed to identify the optimal Aristolochia patch features for egg laying and development. Laboratory rearing success was higher than 50% and although plant species did not show a significant effect on oviposition, we found that larval and adult survival was higher on A. rotunda. Our results suggest habitat management aimed at increasing resource availability for Z. cassandra and possible ex-situ conservation actions aimed at recovering the population in case of potential catastrophic events.

Key words: Zerynthia cassandra; Aristolochia; egg laying; laboratory rearing; Elba island.

Introduction

A key challenge in conservation biology is to understand which factors affect species survival and abundance and to develop evidence-based frameworks to predict how species will respond to environmental changes (Redford et al. 2011). Most applied conservation actions take place locally, at the population scale, making crucial to understand the small-scale factors, such as habitat quality, affecting population survival. Availability and quality of food sources is a defining feature of habitat quality, as resource supply sets an upper limit on carrying capacity (Fleishman et al. 2002; Krauss et al. 2005; Dennis 2010). Butterflies lay eggs near to, directly on, primary larval resource (the host/s). Thus, the presence, abundance and quality of larval resource has a clear impact on survival and abundance of butterfly populations (Curtis et al. 2015). Understanding host plant preference and the relative quality of resource provided by potential host plants, is thus a crucial step to predict species abundance and reaction to environmental changes, and, consequently, plan management measures.

Zerynthia cassandra (Geyer, 1828) (Lepidoptera, Papilionidae) is an endemic Italian festoon, recently separated from *Zerynthia polyxena* (Denis & Schiffermüller, 1775) based on morphological, genetic and life history differences (Dapporto 2009; Zinetti et al. 2013; Vovlas et al. 2014). Before the separation of *Z. cassandra*, the entire taxon *Z. polyxena*, has been listed in the Annex IV of the Habitats Directive (43/92/EEC), in the Appendix II of the Bern Convention, and in many local red-lists (Sforzi and Bartolozzi 2001). *Zerynthia cassandra* is declining in many areas and at least 15 populations are known for having become extinct during the past 50 years (Bonelli et al. 2011; Vovlas et al. 2014).

The critical resource limiting the occurrence and the persistence of the sedentary and oligophagous *Z. cassandra* is the availability of a sufficient number of host plants (Vovlas et

al. 2014; Curtis et al. 2015; Camerini et al. 2018; Ghesini et al. 2018). Interestingly, Z. cassandra also shows a drastic difference in the main host plants selection: while Z. polyxena feeds on several Aristolochia L. species, including the most widespread A. clematitis L., Z. cassandra has been only reported to feed on Aristolochia rotunda L. and Aristolochia lutea Desf., although A. clematitis is widespread in its range (Vovlas et al. 2014; Ghesini et al. 2018). Z. cassandra also shows a marked plant selection as not all the host plants occurring in a given area are used for oviposition. Recent studies demonstrated that a combination of individual plant features and site features has a fundamental importance in determining plant selection. In fact, Z. cassandra usually prefers tall plants with a large number of leaves and flowers and which grow at high density in sunny areas (Vovlas et al. 2014; Camerini et al. 2018; Ghesini et al. 2018). However, this evidence is only based on the study of a single host plant species, Aristolochia rotunda, thus disregarding the importance of the other food plant, A. lutea which occurs over large areas of the distribution of Z. cassandra (Nardi 1984). Whether A. lutea represents an equivalent larval resource for Z. cassandra, however, has never been investigated. Indeed, different Aristolochia species might provide Zerynthia individuals with rather different food qualities (Jordano and Gomariz 1994). These authors demonstrated that Zerynthia rumina caterpillars feeding on Aristolochia longa grew faster and converted food into biomass more efficiently than those feeding on A. baetica. These differences are mirrored by a lower abundance of Z. rumina in sites where larvae use A. baetica.

Aristolochia rotunda and *A. lutea* prefer different (micro-) environments, with *A. lutea* being more common in hill-mountain areas (Nardi 1984). In some areas they can coexist, thus being potentially equally available to *Z. cassandra*. The availability of both hostplants is the case also for the *Z. cassandra* population on Elba island (Tuscan Archipelago), where the two plants co-occur is a small area (4 square km) on the southern facing slope of Mount Capanne (1019m). The Elba population of *Zerynthia cassandra*, has been recorded in 1932 (Bryk. 1932) and it has been re-discovered in 2008 (Dapporto et al. 2017). This population shows a different mitochondrial signature compared to Tuscan populations (Dapporto et al. 2017), which indicates that there has been no gene-flow with mainland probably since the last glacial maximum when the island was connected to the mainland (Fattorini 2009).

Elba is among the most important islands for butterfly conservation not only among Italian islands (Dapporto and Dennis 2008) but also among all European islands (Dennis et al. 2008) and *Z. cassandra* represents a prominent element determining its endemicity and genetic uniqueness (Dapporto et al. 2017). *Z. cassandra* survival on Elba is likely to be under several threats, such as habitat modifications due to human activities and fires as well as climate change (Dapporto et al. 2017), worsened by its restricted distribution.

Understanding the relative quantity and quality of the two host plants as larval resource, as well as the host preference of *Z. cassandra* is thus crucial to plan any evidence-based conservation actions. We thus performed field surveys to investigate host plant preference of *Z. cassandra* on Elba focusing on micro-environmental conditions, plant species and individual plant features. We then assessed, through controlled laboratory rearing, the influence of plant species on larval growth and on larval and adult survival. The results can provide important insights in planning conservation actions to preserve the populations of this species over the Italian Peninsula and to take specific measures to preserve the endangered population living on Elba island.

Material and Methods

The study area

The study has been carried out on Elba island in an area of about 4km² in the municipality of Campo nell'Elba. This area is part the south facing slope of Mount Capanne (1019 m) and ranges in altitude between 250 and 450 metres a.s.l. As done in previous studies (Ghesini et al. 2018), the exact location is not provided to protect the population from collectors, but it can be provided upon request. The vegetation is composed of a mosaic of abandoned pastures, Mediterranean shrubs and pine plantations. Three main seasonal streams occur in this area creating narrow areas of hygrophilous vegetation. This area represents the only location on Elba where the two main host plants of *Zerynthia cassandra* (*A. lutea* and *A. rotunda*) co-occur (Nardi 1984).

Field work

From April 19th to April 24th 2017 we searched for *Aristolochia* shoots in the study area. Whenever a patch of plant shoots was found, we measured, according to previous studies (Vovlas et al. 2014; Ghesini et al. 2018), the following parameters of the area of 1 m radius around the plants: i) number of host plants in the patch; ii) percentage of coverage of iia) herbaceous, iib) shrubby and iic) arboreal plants; iii) a variable describing irradiation (from completely shady (1) to fully irradiated (4)); iv) occurrence of other patches in the area of 10 m radius. Moreover, the following parameters of single host plants have been measured on an average of ten randomly chosen plants per patch representative of the size variation: i) number of *Z. cassandra* eggs laid on the plant; ii) plant height; iii) number of leaves; iv) number of blooming flowers. Finally, we collected 53 larvae (within the third instar) from the two host plants (26 on *A. lutea* and 27 on *A. rotunda*) and brought them to the laboratory for rearing experiments.

Laboratory experiments

To assess larval growth, food conversion rate and survival of immature and adult stages, we performed common-garden rearing experiments by breeding the larvae collected in the field on the two host plant species (see above) under standard laboratory conditions. According to (Jordano and Gomariz 1994), the larvae were reared singly in 5cm diameter Petri dishes until pupation. Leaves were weighted and placed in the Petri dishes with the petioles inserted in wet paper and inserted in Eppendorf vials filled with water to maintain leaf turgor. Larvae, unconsumed plant material and new leaves provided were weighted every three days. The Petri dishes were left in natural light in a non-heated room and were rearranged each time when the measurements were done to prevent position effects. When larvae were close to pupation (loss of weight, reduced mobility and food intake), they have been moved to plastic glasses with their host plants (treated as stated above) and a wooden stick to facilitate pupation. The pupae were weighted and left in the glasses until the next spring in an unheated room with open windows and they have been weekly provided with vaporized water to maintain humidity.

At least five hours after the emergence, the adults were gently marked with a two-colour code on the ventral side of both hindwings taking care of not damaging the wings. Then they were moved into the rearing-cages (100x70x100 cm³). Five to ten adults were simultaneously placed in each of the two cages. A wall of the cages was made of glass, whereas others were made of fine mesh net, thus providing ventilation. Different nectar sources were provided, such as flowering plants like *Ranunculus* sp. and *Lamium purpureum* (selected on the basis of personal observations) and a petri dish with a sugary liquid, together with some plants of *Aristolochia rotunda*. Survival of adults was daily checked and one of the authors (LPa) observed the behaviour of the butterflies in the central hours of the day (10 am-3 pm) to detect mating and egg-laying.

Statistical analyses

We assessed the oviposition preference of adults in nature through a Generalized Linear Mixed Models multimodelling approach. Number of eggs laid were used as response variable, adopting a negative binomial distribution that is well suitable for such overdispersed count data using the (using the glmmadmb function of the glmmADMB R package). The opportunity to add a zero-inflated component in the model was assessed and discarded, through model comparison using Akaike Information Criterion (AIC) as the proper random component accounting for the hierarchical structure of the data, selecting the patch as random effect factor accounting for spatial correlation of the observations.

The environmental variables collected in the field were used as explanatory variables, including: i) number of host plants in the patch; percentage of coverage of ii) herbaceous, iii) shrubby and iv) arboreal plants (transformed via the arcsin transformation); v) irradiation (used as ordinal variable ranging from 1, complete shadow to 4, complete sun); vi) occurrence of other host plant patches in the area of 10m radius, vii) plant height; viii) number of leaves; ix) number of open flowers and x) species of *Aristolochia* (i.e. *A. rotunda* vs *A. lutea*).

To avoid model overfitting and to provide a better parametrization of variables, we used the framework of multi-model inference of Generalized Linear Mixed Models through Information-Theoretic Approach (Burnham and Anderson 2002) to select a set of "best models". This approach allows to select the best combination of predictors from the global model including all possible combinations. The model comparisons were performed adopting the corrected Akaike Information Criterion (AICc), and the model choice was done basing on Δ AICc (which represents the difference between each model and the most parsimonious model). We selected all the models with a Δ AICc with values < 4, considered to be equally parsimonious (Burnham and Anderson 2002). According to this procedure only a small

subset of predictors was selected as significantly affecting the response variable, and the correlation coefficients of each predictor were averaged among the selected best-fitting models and significance of the estimated coefficient was calculated with a z wald test.

The proportion of larvae that reached the adult stage between the groups of larvae reared on *A. rotunda* and *A. lutea* have been compared using the Chi Square test. The growth of larvae was compared by using a quadratic growth model by modelling larval weight in a mixed general linear model (using the glmmPQL function of the MASS R package) against time (in day), squared time, sex and host plant. Individual has been inserted as a random factor. Time has been aligned among specimens as number of days before pupation.

We determined the conversion of food ingested between every two weighting measurements (t and t+1) in the following way

$$CFI_t = \frac{WL_{t+1} - WL_t}{WP_t - WP_{t+1}}$$

Were WL_{t+1} - WL_t represents the weight gained between t and t+1 and WP_t - WP_{t+1} the vegetal mass eaten by the larva as the weight difference between the plant provided in t and the unconsumed plant in t+1. The differences among sexes and host plants have been tested by using the same mixed model used for larval growth.

The differences in mortality of adults between males and females and between larvae reared on different plants have been compared using the non-parametric Kaplan-Meier statistic using the R survival package. Due to a small sample size, more complex Cox Regressions could not be used. All statistical analyses and graphics were generated using R version 3.5.0 (R Development Core Team 2018), with the additional packages glmmADMB vers. 0.8.3.3 (Fournier et al. 2012), MuMIn vers. 1.40.4 (Barton 2018) and survival vers. 2.43-1.

Results

We recorded oviposition and measured traits of 749 *Aristolochia* shoots from 98 patches. The multimodelling approach allowed to select a set of 17 out of 1024 possible models. Number of eggs laid on plants were affected significantly by a smaller subset of the considered environmental variables, such as the number of flowers, the irradiation and the number of leaves. Particularly, the number of lied eggs increased with the increase of both number of flowers and number of leaves and it was significantly higher in plants exposed to a high level of irradiation (coefficient of levels 3 and 4, Table 1). The occurrence of nearby patches was only marginally significant (p < 0.1, table 1) and showed a weak trend of an increase in egg laying in presence of neighbouring patches (this result has been obtained considering the full averaging of coefficients, while when considering the conditional averaging of the coefficients the effect was statistically significant with p=0.035).

Larval growth

The mixed GLM revealed a statistically significant curvilinear growth of the larvae since both time and time² had significant effects (Table 2). Females grew more than males (Table 2, Fig. 1a) but individuals reared on the two host plants did not show any significant difference (Table 2, Fig. 1b).

A similar result was returned by the analysis of the conversion of food ingested where we found that in a curvilinear relationship with time (Table 3) revealing that larger larvae have a lower rate of conversion (negative influence of time) and that males have a higher rate of conversion than females; individuals reared on the two plants did not differ (Table 3).

Mortality

Among 53 larvae, 10 died before pupation (18.87% larval mortality), 16 did not emerge from pupae (30.19% of the initial number) and 27 individuals emerged (50.94% of the initial number of larvae). Among the larvae that did not reach the pupal stage, 7 out of 10 were fed on *A. lutea*. Among the pupae that did not emerge (16), 9 were fed on *A. lutea*. The comparison of the fractions of individuals dead and emerged from *A. rotunda* (10 vs 17) and *A. lutea* (16 vs 10) although not statistically significant revealed a trend, with a higher proportion of larvae reaching the adult stage on *A. rotunda* than on *A. lutea* (Chi-squared = 3.182, df = 1, p-value = 0.074) (Fig. 2).

According to (Vovlas et al. 2014) the maximum adult lifespan recorded was 8 days (mean 5.14 ± 2.16 s.d.). For *Z. polyxena* the maximum recorded life-span was 20 days but the mean lifespan was similar to that measured in our lab study 4.4 (3.4-6.0 95% confidence interval) (Celik, 2012). Males and females did not differ in mortality (median lifespan, males = 6 days; females = 6 days, Kaplan-Meier test Chi-square = 0.100, d.f. = 1, P = 0.733); conversely, adults having fed on *A. lutea* showed a higher mortality than those which fed on *A. rotunda* (median lifespan on *A. rotunda* = 6 days; on *A. lutea* = 4 days; Kaplan-Meier test Chi-square = 6.9, d.f. = 1, p-value = 0.009, Fig. 3). We did not observe any mating event and only one egg has been laid on *A. rotunda* in the cages. The egg was probably unfertilized since it did not hatch.

Discussion

As found in continental populations of *Z. cassandra* (Vovlas et al. 2014; Camerini et al. 2018; Ghesini et al. 2018) the Elba population showed a significant host plant selection for egg-laying. The features of individual plants (number of flowers) and of their micro-environmental conditions (irradiation) together with the quantity of host plant resources in

the surroundings (presence of other patches) largely explained the quantity of eggs occurring on individual plants. Laboratory experiments showed that larvae of *Z. cassandra* tended to have a higher survivorship on *A. rotunda* and adults that fed on this plant had a longer lifespan (6 vs 4 days median, Fig 3). These results have important implications for the conservation of *Z. cassandra* and the management of its habitat on Elba island.

Limitations of our study.

Our study investigated larval growth and larval and adult survival under controlled laboratory conditions, to eliminate confounding variables and to allow a precise and regular measurement of larval growth and rate of food conversions. However, the success of individuals grown on different plant species should be evaluated also under field conditions, where abiotic (such as micro-habitat variables) and biotic factors (such as predators and parasites) could differentially affect individuals living on the two plant species. As an example, in the Alaskan swallowtail butterfly, *Papilio machaon aliaska*, larval survival differs between hosts according to predators presence, being higher on the novel Asteraceae hosts when predators are present, while higher on the ancestral Apiaceae hosts in the absence of predators (Murphy 2004). Further studies are also needed to shed light on the ultimate and proximate mechanisms which mediate this marked preference, such as nutritional quality and the visual and chemical cues involved in the choice of the most suitable host plant by *Z. cassandra* females (Thompson and Pellmyr 1991).

Implication for conservation in situ

As found in previous studies, plants in half and full sun (irradiation 3 and 4) are preferred for oviposition (Vovlas et al. 2014; Camerini et al. 2018; Ghesini et al. 2018), as well as plants in patches close to other patches and plants with more flowers (Ghesini et al. 2018).

The preference of more irradiated patches of plants could be explained by a faster development of immatures with warmer temperatures, as seen in other Papilionidae species, namely the larvae of *Papilio glaucus* (Scriber and Lederhouse 1983) and the pupae of *Parnassius mnemosyne* (Valimaki and Itamies 2005). In these species, and in *Z. polyxena* and *Z. cerysi* as well, a higher concentration of larvae occurs in sunlight areas (Slancarova et al. 2015).

The proximity of nearby patches is another pivotal factor determining the preference of oviposition, in fact, due to its sedentary behaviour and the strong dependence on the host-plant (Vovlas et al. 2014; Curtis et al. 2015; Camerini et al. 2018; Ghesini et al. 2018), *Z. cassandra* and *polyxena* tend to occupy areas with a higher density of plants than isolated patches (Celik 2012).

As reported by Vovlas and coworkers (2014), we observed that the first instar larvae are frequently found inside the *Aristolochia* flowers. The peculiar shape of *Aristolochia* flower probably offers an effective protection against predators and parasitoids. This may explain why the number of flowers emerged as the only plant variable influencing the occurrence of eggs. Another not mutually exclusive explanation is that flowers are composed by less though tissues. Jordano and Gomariz (1994) showed that newly hatched larvae of *Z. rumina* only feed on young leaves of *A. baetica* that are more easily assimilated. Future studies should assess whether flowers which are eaten by the larvae (personal observation) represent a higher quality food for larvae compared to leaves.

Based on these results, the conservation actions should be directed to reduce shrub and trees coverage in the very reduced areas where *Aristolochia* patches already exist, thus increasing the irradiation and the consequent availability of the plants for egg laying. The target patches for this action should be preferentially located in areas with a lower density of patches and a general low irradiation suitable patches and the removal of shrubs should be done in the late

winter, before the emergence of *Aristolochia* plantulae. Moreover, such a management of bushes occurring around plants could also give benefits to *Aristolochia* plants themselves, with better irradiation favoring plant growth. However, it is essential to maintain an overall variability in patch irradiation across the host plant distribution range, as this would help securing host plants availability even in case of early year droughts.

Implications for the conservation ex-situ

Captive breeding has been suggested as a conservation method for many threatened vertebrates, and it is increasingly being proposed as a valuable conservation strategy for invertebrates (Lewis and Thomas 2001). Our first efforts so far failed in establishing a laboratory line of Z. cassandra, as adults did not mate under our laboratory rearing conditions. The 50.94% of larvae which emerged as adult were apparently in a good health and probably the lack of mating was due to the lack of some environmental resources inside the cages or to some stressing factors existing in our captivity settings. Nevertheless, Z. cassandra females usually lay about 50/60 eggs (Vovlas et al. 2014) and in a stable population mortality is expected to be around 96%, in largest part at the expenses of the immature stages. Under this perspective, the high rearing success obtained in the laboratory allows to hypothesize that ex-situ actions can be undertaken. The decision to act with ex-situ actions should be taken with caution, since the rearing of larvae to the adult stage is a timeconsuming activity and the economic costs could be lower than the benefits only when the aim is to protect very small and declining populations of Z. cassandra. Moreover, potential risks associated to ex-situ conservation have also been identified in negative genetic consequences (such as an increase in homozygosity), behavioural adaptations to captive

conditions and accidental introductions of parasites and diseases back into the originary population (Lewis and Thomas 2001; Witzenberger and Hochkirch 2011).

Although *Z. cassandra* has been identified as least concern for conservation in Italy (Balletto et al. 2015), the reduced and genetically diversified population on Elba island deserves conservation efforts. On Elba, the population does not seem to be declining. However, among the main threats we identified, the change of land use (from agricultural landscape to Mediterranean shrubs) in the area with *A. rotunda* or a large fire involving most of the 4km² area, as occurred in the past in nearby areas, could potentially affect most butterfly population. In case of summer-autumn fires, pupae located on bushes are expected to be destroyed in large numbers, but *Aristolochia* tuberous rhizomes usually survive (Rausher and Feeny 1980). The collection of a relatively low number of eggs in each spring (around 100) from different sites to be representative of the existing genetic diversity could allow to preserve stocks of individuals reared in the lab. The release of adults in the successive spring could represent a crucial resource to rescue the population.

However, since the lifespan of adults is rather short (Celik, 2012; Vovlas et al 2014), the adults should be transferred immediately after emergence on the field. This problem could be possibly solved by relocating the pupae in the field in the last months before the emergence (February) by placing them back in their environment. Finally, the success from larva to adult on *A. lutea* was 38.5% while on *A. rotunda* it was 63.0% and the adults that fed on *A. rotunda* survived longer than those that fed on *A. lutea* (generally two days more all along the survival curve). Although the difference in mortality rate at the immature stage failed to reach the statistical significance, probably because of the relatively small sample size (53 larvae in total), we strongly suggest the use *A. rotunda* to rear the larvae in ex-situ conservation actions.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors. Any applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Specimens were collected and reared in the laboratory after permission from the Italian Ministero dell'Ambiente e della Tutela del Territorio e del Mare (Prot. 0012493/PNM 24/06/2015).

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Table 1. Full averaging of Generalized Linear Mixed Models coefficients among models
showing a delta $AICc < 4$. The number of eggs on individual plants have been explained
based on patch and plant features.

	Estimate	Std. Error	Adj. SE	Z	Р	N. models
Intercept	-5.694	1.014	1.016	5.607	< 0.001	17
N. flowers	0.367	0.073	0.073	5.041	0.000	17
Irradiation 2	0.878	0.798	0.799	1.099	0.272	17
Irradiation 3	1.855	0.821	0.822	2.256	0.024	17
Irradiation 4	2.472	0.807	0.809	3.058	0.002	17
N. leaves	0.157	0.075	0.075	2.092	0.036	16
Close patches	0.701	0.378	0.378	1.853	0.064	16
Grass coverage	-4.843	3.575	3.578	1.353	0.176	15
Tree coverage	0.067	0.054	0.054	1.237	0.216	13
N. plants	-0.001	0.003	0.003	0.315	0.753	5
Plant species	0.029	0.134	0.135	0.218	0.827	5
Height	0.000	0.003	0.003	0.051	0.960	3
Shrub coverage	0.000	0.001	0.001	0.002	0.999	3

	Value	Std.Error	DF	t-value	p-value	_
time	0.004	0.013	185	0.303	0.763	
time ²	-0.002	0.001	185	-2.573	0.011	
sexm	0.042	0.019	36	2.167	0.037	
plantR	0.008	0.019	36	0.394	0.696	

Table 3. Result of mixed GLM for conversion of ingested food.

	Value	Std.Error	DF	t-value	p-value
time	0.0651	0.0057	321	11.350	0.000
time ²	-0.0012	0.0002	321	-7.468	0.000
sexm	-0.0429	0.0126	36	-3.395	0.002
plantR	-0.0053	0.0127	36	-0.419	0.678

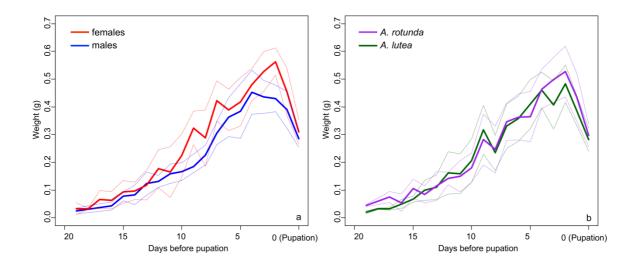


Figure 1. Average growth curves and standard deviation (dotted lines) for males and females larvae (a) and for larvae reared in *Aristolochia rotunda* and *A. lutea* (b).

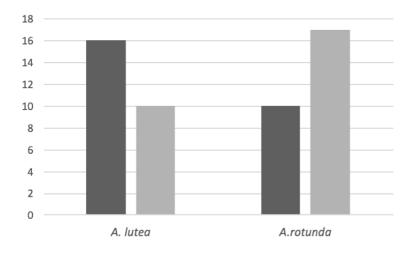


Figure 2 Number of larvae that emerged as adults (light grey) and number of larvae that did not reach the adult stage (dark grey) on *A. lutea* and *A. rotunda*.

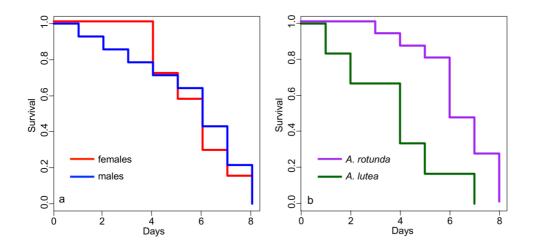


Figure 3. Survival curves of adult comparing males and females (a) and individual fed on *A*. *rotunda* and *A*. *lutea* (b).