

A successful habitat patch creation for *Zerynthia cassandra*

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Abstract

Zerynthia cassandra (Geyer) (Lepidoptera Papilionidae) is a butterfly species endemic to Italy, which was recently split from the Habitats Directive species, *Zerynthia polyxena* (Denis et Schiffermuller). The distribution of *Z. cassandra* is fragmented, limited to microhabitats where the larval food plants (*Aristolochia* spp.) can grow. The creation of new habitat patches can increase habitat availability and connectivity, thus improving the chances of survival of this species. This study relates on the creation of a new habitat patch in a 320,000 m² park in the province of Bologna (Northern Italy), 8-15 Km distant from natural patches occupied by *Z. cassandra*. Fifty rootstocks of the local larval food plant, *Aristolochia rotunda* L., were introduced in 1982 in a 5 × 30 m belt along the edge of a pond. From the 1990s onwards, the management of the park became more conservation-oriented and favoured the spread of *A. rotunda*, that is now abundant. In 2016, the new habitat patch was spontaneously colonized by *Z. cassandra*. The number of adults and the areas where eggs and larvae were found increased in 2017 and 2018, the presence of 67 adults was estimated in 2018, so *Z. cassandra* appears to be now well established in the park. In the light of this experience, criteria for the selection and management of sites for the creation of new habitat patches are suggested and discussed.

Key words: *Zerynthia cassandra*, Italian festoon, *Aristolochia rotunda* introduction, habitat patch creation, unaided colonisation, conservation action.

Introduction

The Italian festoon, *Zerynthia cassandra* (Geyer), (Lepidoptera Papilionidae) has long been considered as a subspecies, endemic to Italy, of the southern festoon, *Zerynthia polyxena* (Denis et Schiffermuller), a species distributed over a wide range in central and southern Europe. Due to relevant morphological and genetic differences, *Z. cassandra* was recently reinstated as a species, with a range of distribution comprising peninsular Italy (approximately south of the Po river) Sicily and Elba (Dapporto, 2010; Zinetti *et al.*, 2013; Cini *et al.*, 2019). *Z. cassandra* is listed as Least Concern in the Red List of Italian Butterflies (Balletto *et al.*, 2015; Bonelli *et al.*, 2018), but is protected under the Habitats Directive 92/43/EEC (Annex IV) and the Bern Convention (Appendix II) as *Z. polyxena* sensu lato.

Z. cassandra is a univoltine species, with adults flying in spring, larvae pupating in late spring or early summer, and overwintering pupae, with some variation depending on the region and microclimatic conditions (Descimon, 1996; Vovlas *et al.*, 2014; Ghesini *et al.*, 2018). As is the case with other *Zerynthia* species (Descimon, 1996; Ćelik, 2012; Vovlas *et al.*, 2014), *Z. cassandra* is distributed in metapopulations, as a consequence of the localized distribution of larval food plants, birthworts of the genus *Aristolochia*. In the northern portion of *Z. cassandra* range, *Aristolochia rotunda* L. is the only food plant. On the Tyrrhenian side of Italy, *Aristolochia lutea* Desf. is also present (Nardi, 1984) and serves as a food plant (Cini *et al.*, 2019). *Aristolochia clematitidis* L., which is found in northern and central Italy and is a food plant for *Z. polyxena* in Eastern Europe (Batáry *et al.*, 2008, Örvössy *et al.*, 2014; Slancarova *et al.*, 2015) and Austria (Trattning and Gepp, 1992), is eaten only occasionally by late instar larvae (Ghesini *et al.*, 2018).

The province of Bologna (Northern Italy) is one of the areas where *Z. cassandra* is more abundant (Boldreghini *et al.*, 1998; Marini, 1998a). In this region, the larval food plant is *A. rotunda*, a geophyte that vegetates in spring and loses its aboveground parts in early summer. On the hills to the southwest of Bologna, *A. rotunda* is present in small isolated patches, while in the plain to the northeast of the town habitat patches are larger and better connected (Ghesini *et al.*, 2018). However, most of the territory of the plain is cultivated, and intensive agricultural practices such as deep ploughing and the use of herbicides, greatly limit the availability of suitable areas where wild plants can grow. *A. rotunda* is found nearly exclusively on the edges of drainage canals, and patches are often several kilometres distant from each other. This fragmented habitat is challenged by canal maintenance activities and agricultural practices (including the use of pesticides). In addition, *Z. cassandra* populations can be threatened due to the collection of adults and larvae. In this context, the creation of new habitat patches in selected areas of the matrix could improve the chances of survival of the local population, by increasing habitat availability and connectivity.

In the 1980s, in the region considered, one of the few areas with low disturbance and with controlled access was the park of ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale, Italian Institute for Environmental Protection and Research, formerly INBS), which is situated in the plain to the east of the town of Bologna, and surrounded by cultivated fields. Within an 8 km range, both *A. rotunda* and *Z. cassandra* were absent, but several *Z. cassandra* populations existed outside this range. The park could work as a suitable for *Z. cassandra*, but at that time the larval foodplant (*A. rotunda*) was missing: its introduction was therefore needed.

This paper relates on the creation of a suitable habitat patch in the park of ISPRA, which led, after 34 years, to the unaided colonization of the park by *Z. cassandra*. Characteristics that should be taken into account when selecting sites for the creation of new habitat patches and management criteria are also discussed.

Materials and methods

The study was carried out at the ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) section located in Ozzano dell'Emilia (Bologna, Northern Italy), comprising about 320,000 m² of land and three ponds, the first one created in the early 1970s and the other two in the late 1980s. The ground level slopes slightly and almost uniformly northwards, from 41 to 38 m a.s.l. Until the end of the 1980s, most of the grounds of ISPRA were cultivated. Afterwards, the management of the area became more conservation-oriented: cultivated sections were progressively abandoned, and hedges - mainly of *Prunus spinosa* L. and *Crataegus* sp. - were planted. Fields were consequently colonized by spontaneous vegetation. In 2010 and 2011, fields downstream of the ponds were flooded.

The grounds of ISPRA and the surrounding areas were inspected in 1981, during the *A. rotunda* vegetative season (spring and early summer), in order to verify if any plants were present. None were found, either in the park or in surrounding areas. For the introduction of *A. rotunda* in the park, rootstocks were collected from the patches nearest to ISPRA (8-15 km distant), in order to avoid the introduction of plants genetically distant from the local population. In particular, rootstocks that came to the surface of the ground during canal edge renovation activities were collected and kept in plastic pots (20 × 12 × 12 cm) containing a 1:1:1 mixture of sand, clay and peat, until replanting. In February 1982, 50 rootstocks were planted 20 cm deep in the ground, in a 5 × 30 m belt along the western edge of the pond (figure 1). The survival and spreading of *A. rotunda* was occasionally verified in the following years.

From 2010 to 2015, the presence of adults was checked yearly, surveying the open areas of the park 2-3 times during the flying period. After adults of *Z. cassandra* were first seen in the park of ISPRA in 2016, the area was inspected twice per week from late March to June, to check which areas hosted eggs and larvae. In 2016-2018, the number of adults was evaluated by counting the individuals that were identified during surveys that were conducted twice per week, from March to May. In order to avoid to count the same individual more than once, butterflies were marked on the upper side of a wing with a permanent marker. In 2018, a mark-recapture survey was also carried out in the period of maximum presence of adults, between the 19th and the 24th of April (27 captures over four marking days), in order to estimate the abundance of adults. Each survey was carried out by 3 persons, who walked paths and fields in the whole area of the park, approximately from 10:00 to 14:00. Butterflies were marked with a dot on the upper side of a wing (using an individual combina-

tion of position and colour of the dot), and released immediately. For each capture or recapture, the individual marking pattern was recorded together with site and time of capture. Population abundance was estimated using the software Mark 9.0 (White and Burnham, 1999) using the POPAN parametrization of the Jolly-Sieber method, and choosing the model with the lowest corrected AIC value (which had time-independent probabilities of survival and recapture).

The presence of eggs and larvae was checked during weekly surveys carried out from late April to early June. Eggs and early instar larvae were looked for by examining all the stems in small *A. rotunda* patches (less than about 50 plants) and at least 20% of the stems in larger patches. The identification of areas with late instar larvae did not normally require a close inspection of the stems, as the aposematic colouration of these larvae and the damage they caused to leaves and stems made them clearly visible even from some distance.

Results

Characteristics of the selected area

Entomological observations carried out in the park of ISPRA in 1981 showed that it possessed some desirable characteristics, which made it a suitable site for the creation of a habitat patch for *Z. cassandra*:

- the park extended over a vast and diverse area (herbaceous, scrubby and woody, wet and dry, sunny and shady);
- some nectar plants, such as *Bellis perennis* L., *Salvia pratensis* L. and *Taraxacum* sp., were present;
- a large pond provided a constantly available source of water;
- access to the area was limited to authorized personnel, so that the risk of butterfly collection was negligible;
- the park was not too distant from known *Z. cassandra* natural habitats (8-15 km), so that a spontaneous colonization was possible.

The main drawback was the absence of the larval food plant, *A. rotunda*, which had to be introduced.

In the first years after its introduction, *A. rotunda* spread along the banks of the pond. Until the end of the 1980s, the pond was surrounded by cultivated fields, so *A. rotunda* could not spread further. After cultivation was abandoned, *A. rotunda* progressively spread over much of the park. Its colonization of a vast area downstream of the ponds was probably boosted by the flooding of some of the fields in 2010 and 2011; moist fields are a favourable environment for *A. rotunda* (Nardi, 1984) and its seeds can be transported by water. Mammals (roe deer, porcupine) and birds that live in the park, as well as human activities such as the use of mowing equipment, could also have contributed to seed dispersal, also upstream of the ponds. To date, *A. rotunda* is widespread in the park, colonizing nearly all the areas free from thick shrubs: grassy areas in open grounds, edges of shrubs, hedges, paths and ditches. More than a thousand mature plants were counted in 2018.

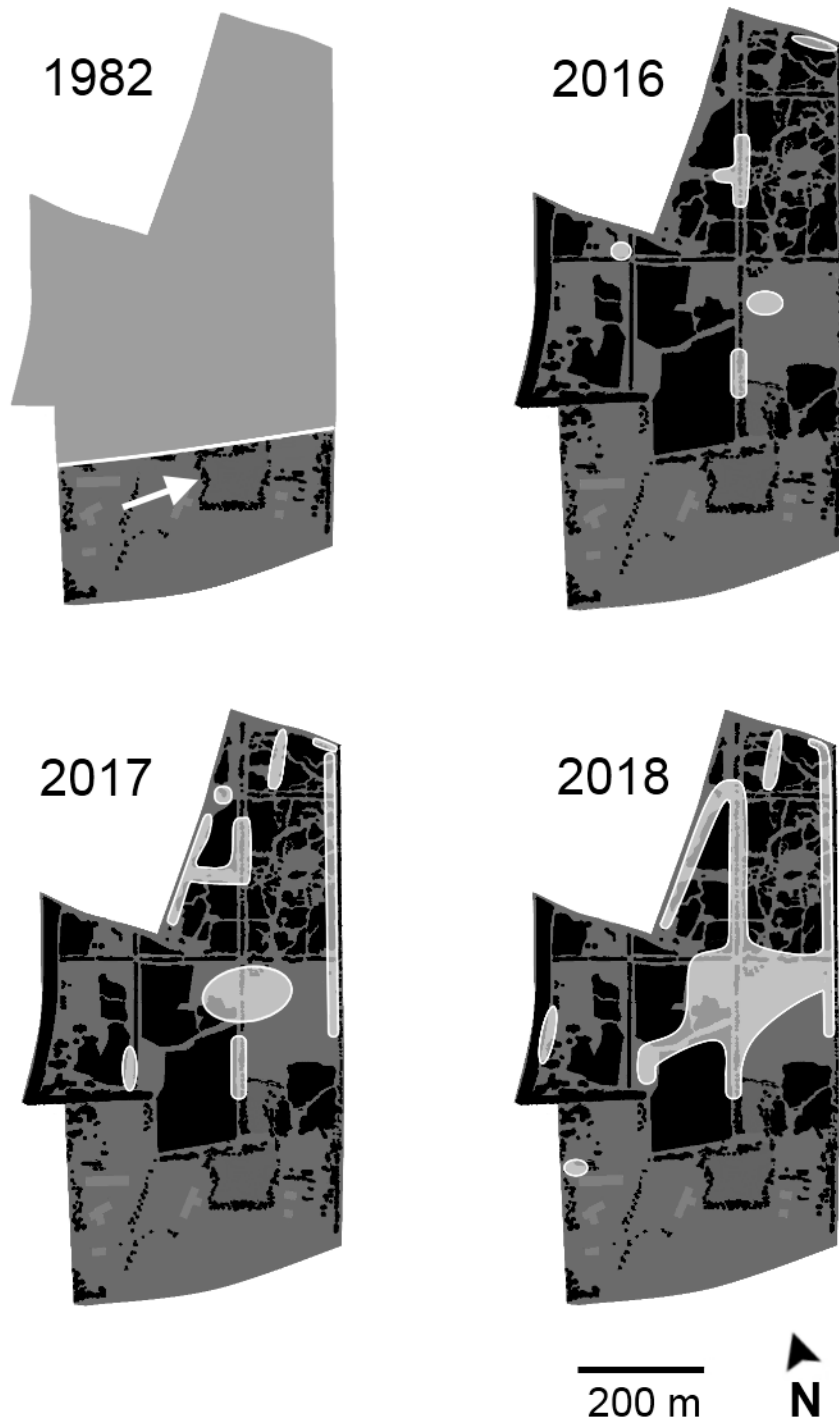


Figure 1. Maps of the park of ISPRA in 1982, 2016, 2017 and 2018. 1982: the white arrow indicates the area next to the pond where *A. rotunda* was initially introduced; the light grey area above the white line shows the extension of cultivated fields. 2016-2018: woody vegetation (mainly *P. spinosa*) is indicated in black; areas where eggs and/or larvae of *Z. cassandra* were found are shaded in white.

In addition to the natural spreading of the larval food plant, some other environmental conditions progressively improved after the end of the 1980s, when the management of the park became more conservation-oriented, aiming to favour the re-naturalization of the area and the creation of a wetland. The main purpose of the new management was to favour bird biodiversity. However,

the improved environmental heterogeneity also turned out to be beneficial for butterflies. In particular, after the abandonment of cultivations, nectar plants became progressively more abundant. *Ajuga reptans* L., *Bellis perennis* L., and *Lychnis flos-cuculi* L. are now quite abundant in open grounds and along the pathways, where *Z. cassandra* adults have been seen while feeding on them.

Colonization of the new habitat patch by *Z. cassandra*

Until 2016, no eggs, larvae, or adults of *Z. cassandra* were observed in the grounds of ISPRA. Adults of *Z. cassandra* were seen for the first time in 2016. Three and 15 adults were observed respectively in 2016 and 2017. In 2018, 27 adults were marked, and 6 (22.2%) were recaptured. An unmarked adult was seen two weeks later, after a rainy period that had prevented the execution of further surveys. A population of 67.0 ± 22.5 adults could be estimated (95% confidence interval: 35.3-127.2). The maximum recapture distance was 640 m. An adult marked on the first marking date was recaptured on the last marking date.

In 2016, eggs and larvae were found in a few small areas, covering approximately 9,500 m². Their presence became progressively more widespread in 2017 and 2018 (about 33,000 m² and 52,000 m², respectively) (figure 1).

The increasing number of adults found in the last three years, as well as the expansion of the area occupied by eggs and larvae, indicates a consolidation of *Z. cassandra* population in the park. It can be expected that the presence of *Z. cassandra* will persist in the years to come, provided favourable conditions are maintained.

Discussion

In regions where *Z. cassandra* is present, but limited to a few isolated habitat patches, the introduction of food plants in suitable areas is a practical conservation action, that can improve the long-term chances of survival of the species. This study shows that a new habitat patch can be created also with very little investment and also in areas intended for other uses, such as parks and gardens, with slight modifications to their management.

The new habitat patch created in the park of ISPRA was spontaneously colonized by *Z. cassandra* 34 years after *A. rotunda* was introduced. The causes of this delay are probably to be found in the relatively long distance between the park and the nearest occupied patches and in the small number of *A. rotunda* plants present until 2010, that was probably insufficient to be attractive to butterflies from long distances.

It is not possible to identify with certainty the origin of the individuals that colonized the park. The nearest habitat patches occupied by *Z. cassandra* are 8-9 km distant and situated in the hills to the southwest of the park. Several other occupied patches exist in the plain to the northeast of the park at a distance of 15-20 km. Patches in the plain are much larger than those in the hills and host larger *Z. cassandra* populations (Ghesini *et al.*, 2018). In addition, while patches in the hills are quite isolated, the plain is crossed by a web of canals and ditches that can serve as dispersal pathways for *Z. cassandra*. Moreover, prevailing spring winds in the considered area blow from the southwest (ARPA, 2013) and could have carried *A. rotunda* volatiles toward the plain, thus attracting butterflies. For these reasons, a colonization originating from the plain seems more probable. *Zerynthia* spp. are considered sedentary butterflies, with normal flying ranges up to a few hundred

meters (Verity, 1947; Marini, 1998b; Ćelik, 2012; Vovlas *et al.*, 2014). However, flying ranges of a few kilometres are not uncommon, and some adults are likely to cover distances of at least 10 km (Šlancarová *et al.*, 2015).

At present, the main threat to the new habitat patch in the park of ISPRA is the invasion of open areas by shrubs, composed mainly of *P. spinosa*. A similar situation was recently reported for another area in northern Italy, where the colonization of butterfly habitat by shrubs, mainly *P. spinosa*, was the main cause of the extinction of *Z. cassandra* (Camerini *et al.*, 2018). The expansion of shrubs was controlled in recent years by the mowing of meadows and paths once or twice a year. It is important that this control activity be maintained. Ploughing selected areas, with the aim of eliminating the roots of shrubs, could also be beneficial.

As the diversity of nectar plants in the park is limited, the introduction of some other plant species of benefit to adults, such as *Bellevalia romana* (L.) and *Lamium purpureum* L. (Ghesini *et al.*, 2018), is also planned.

In the light of the experience related above and of the literature cited below, some criteria for the creation and management of new habitat patches for *Z. cassandra* can be suggested:

- i The selected site should comprise areas with different microclimates and vegetation. In fact, suitable habitats for butterfly species include several resources, such as larval hostplants, nectar plants for adults, suitable microclimatic conditions, sites for mate location, resting and escape from predators (Dennis *et al.*, 2006; Dennis *et al.*, 2014). In particular, as *Z. cassandra* food plants grows in sites where water is constantly available in the surface layers of the soil, sites should include some source of water. The selected area needs not to be vast, as long as it provides a diverse environment.
- ii The selected site must contain a sufficient number of mature larval food plants and provide favourable conditions to their survival. In fact, patches with less than 50 mature food plants rarely host *Z. cassandra*, and females do not normally lay eggs on young plants (Ghesini *et al.*, 2018). If a suitable *Aristolochia* species is not present in the selected site, it may be introduced either by planting rootstocks or by sowing seeds. The second option is easier to implement and less impacting on the donor site, but requires more time before plants are mature enough to host *Z. cassandra*. Micropropagation has also been proposed for multiplying *A. rotunda* (Gatti and Vecchi, 2016), but the convenience of this method appears to be limited to special cases, as *A. rotunda* can easily be reproduced by conventional methods, and the introduction of clones in natural environments could decrease the genetic variation of natural populations.
- iii Nectar plants that flower in spring are necessary and, if not present, have to be introduced. Adults of *Z. cassandra* can feed on a large variety of nectar plants, but some species, such as *A. reptans*, *B. romana* and *Lamium* spp. are particularly favoured (Ghesini *et al.*, 2018).

- iv If unaided colonisation is desired, the re-created habitat should not be too far from areas where natural populations exist.
- v As *Z. cassandra* is endangered by collection (Descimon, 1996), limited access or surveillance of the area of the new habitat patch is recommended. Not only private, but also public areas, such as urban parks and schoolyards could provide at least some degree of protection against collection. Involving local communities in the creation and maintenance of *A. rotunda* patches in urban areas could be a successful strategy, as shown by the conservation project for another birthwort-feeding butterfly, the Australian birdwing *Ornithoptera richmondia* (Gray) (Sands, 2008).
- vi Management of the created habitat patch should aim to maintain habitat heterogeneity and improve conditions favourable to larval food plants and nectar plants. In fact, heterogeneously managed habitats are favourable to butterflies (Shreeve and Dennis, 2011; van Halder *et al.*, 2017). While the presence of some trees and bushes can be beneficial to butterflies, as it provides shelter from excessive heat, the colonisation of open spaces by woody plants is recognized as one of the main threats to *Zerynthia* spp. (Trattng and Gepp, 1992; Descimon, 1996; Höttinger, 2003; Bonelli *et al.*, 2011; Camerini *et al.*, 2018), as it reduces the availability of larval food plants and nectar plants. Moreover, females of *Z. cassandra* preferentially lay eggs on plants growing in open areas, and normally avoid plants in full shadow (Vovlas *et al.*, 2014; Camerini *et al.*, 2018). Open spaces can be maintained by mowing once or twice a year. Mowing is not detrimental to *Z. cassandra* and other butterflies, provided it is scheduled outside its period of activity and carried out properly (Humbert *et al.*, 2009; 2010; Čížek *et al.*, 2012; Vovlas *et al.*, 2014).

Acknowledgements

The Authors wish to thank Nicola Baccetti, Research Manager of ISPRA-Ozzano, for favouring this project; Mario Spagnesi, former Research Manager of ISPRA-Ozzano, for his willingness to host the introduction of *A. rotunda* in the park of the Institute; and Eddie John for the English revision of a previous version of the manuscript.

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Received March 5, 2019. Accepted September 19, 2019.