Fragmenta entomologica, 50 (2): 83-86 (2018)

eISSN: 2284-4880 (online version)
pISSN: 0429-288X (print version)

Short scientific note
Submitted: September 8th - *Accepted*: November 28th, 2018 - *Published*: December 31st, 2018

Lab observations on prolonged chrysalis diapause of *Brahmaea* (*Acanthobrahmaea*) *europaea* Hartig (Lepidoptera: Brahmaeidae)

Renato SPICCIARELLI

School of Agricultural, Forest, Food and Environmental Sciences (SAFE), University of Basilicata - Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy - renato.spicciarelli@unibas.it

Abstract
Brahmaea (*Acanthobrahmaea*) *europaea* Hartig, 1963, is an Italian endemic species exhibiting a very restricted geographic distribution (Basilicata and Campania regions, Grotticelle di Monticchio Nature Reserve and neighbouring areas), and developing as larvae on a few Oleaceae (*Fraxinus angustifolia* subsp. *oxycarpa*, *Ligustrum vulgare* and *Phyllirea latifolia*). From spring 2015 to spring 2017 a series of bioethological observations on the moth were conducted, as well as surveys aimed to study the distribution of its host plants in the habitats of SAC "Grotticelle di Monticchio". In this short communication a prolonged diapause of *B. europaea* chrysalis kept in the laboratory is documented. In spring 2018, four successfully adult eclosions occurred from two years old pupae, a case never previously reported in literature records for this rare and threatened taxon.

Key words: *Brahmaea* (*Acanthobrahmaea*) *europaea*, Brahmaeidae, Lepidoptera, chrysalis, prolonged diapause, Italy.

In 1963, *Brahmaea* (*Acanthobrahmaea*) *europaea* Hartig was discovered by Federico Hartig (Hartig 1963, 1966) in Basilicata (Southern Italy). In 1965, the moth was reared by Bilek starting from 8 eggs received from Hartig, by feeding them with privet leaves. This first successful rearing allowed an accurate description of the larval instars and the pupal stage. (Bilek 1965). The species is an Italian endemic exhibiting an exceedingly restricted geographic distribution (van Schepdael 1967; Rougeot 1971, 1975; Parenzan 1978; Bertaccini et al. 1995; Spicciarelli 2004, 2013, 2014, 2018a, 2018b; Mosconi et al. 2015). In 1970, Dufay reported a first bioethological account on this moth. In 1971, on proposal of the same Hartig, a protected site was established for the species (Hartig 1997): the Nature Reserve of "Grotticelle" (Basilicata Region) (Audisio et al. 2012; Spicciarelli 2013). Then, the Reserve area was incorporated into the perimeter of the Special Area of Conservation (SAC) "Grotticelle di Monticchio", an area surrounded mostly by watercourses: the "Torrente Refezzella", the "Fiumara di Atella", the Ofanto River, famous for flooding known to hystory (Spicciarelli et al. 2011). In 1997, for the first time, a group of *B*. (*A*.) *europaea* caterpillars was found in nature on *Phyllirea latifolia* L., broad-leaved phillyrea (Spicciarelli 1997). Previously, their rearing was possible only catching adults with light traps, and collecting eggs after mating in lab conditions. During research conducted in 2013-2014, an egg cluster of *B. europaea* was observed for the first time. Additional information was acquired about the mobility of the caterpillars during their development. Palatability tests allowed to attest *Fraxinus angustifolia* subsp. *oxycarpa* (Willd.), *Ligustrum vulgare* L. and *Phyllirea latifolia* L. as food plants of the moth among those present in the Reserve, plants that are mostly located in SAC between 300 and 400 meters above sea level (Spicciarelli 2018a).

From spring 2015 to spring 2017 other bioethological observations on *B*. *europaea* were conducted in the habitats of "Grotticelle di Monticchio". During 2016, ten egg clusters and numerous groups of caterpillars have been identified on 12 different trees. In 2017, an egg cluster was observed, and other groups of larvae were found. These findings allowed the observations on other details on egg-laying and behaviour of the caterpillars, until pupation. In addition, a map of local habitat 91B0 "Thermophilous *Fraxinus angustifolia* woods" has also been obtained (Spicciarelli 2018b).

Over the several decades since its discovery, the moth has been almost continuously reared in the laboratory starting from eggs laid by females captured by light traps and usually using the privet, as a food plant, a species available in early spring in city parks (Bilek 1965; Sauter 1967; Stella 2003).

Some authors provided information about rearing methods (Bilek 1965, 1967; Stella 2003; Spicciarelli 2013). Usually in *B. europaea* life-cycle, the larval molts are four (and the larval instars are five). Enrico Stella observed a higher number of instars in some caterpillars (personal communication, 2002), while Bilek (1967) defined

the main variabilities in this moth's liveries. The adopted materials and protocols change little and have in common the hibernation induction: in the autumn (approximately in end of November) the box containing the chrysalis is transferred to the refrigerator (3-4 °C). The pupae are generally moistened by water sprays every week. The chrysalids remain in these conditions until a couple of weeks before the emergence (end of March).

The present note focuses on an event recently noticed in the laboratory during the rearing of *B. europaea*. The foliage used for rearing activities was obtained from young plants of *Fraxinus angustifolia* subsp. *oxycarpa*. The rearing began on April 10th, 2016 with twenty-five eggs, and twenty-three individuals pupate from the 18th to the 20th of May 2016.

After pupation, they were placed inside a box 20 x 25 cm, above and in adherence to a layer of five centimeters of soil collected in protected area (Fig. 1). For wintering, they were not kept in a refrigerator, but in closed boxes outdoor, in climatic conditions similar to those present in the Nature Reserve "Grotticelle". On average, water was sprayed every month on the chrysalises and on the ground surface, a condition of humidity significantly lower than that normally maintained in previous rearing.

In the first days of April 2017, 10 adults emerged from the pupae. Of the others, 10 were still heavy, and 3 appeared light and empty.

Fig. 1 – In the left picture, the chrysalis of *Brahmaea europaea* into the rearing box during the 2016-2017 wintering. On the right, a *B. europaea* chrysalis (enlarged image).

The chrysalis remained in the box and in the same environmental conditions. Every two weeks the soil was wet, just under the limit of its saturation. In a check carried out on December 4th, 2017 many chrysalises were still heavy and 2 of them clearly showed signs of vitality. The same day, the box with the pupae were placed in the refrigerator and the soil inside was irrigated every week. The chrysalis remained in these conditions until March 12th, 2018, when the southern ash in the reserve was already close to sprouting (even if a few days later there was a return of cold with an abundant snowfall).

On April 16th, 2018, an adult emerged, and others followed on April 19th, 20th, 23rd, for a total of 4 (3 female, 1 male). Two moths were very vital without problems in eclosion, while the other 2 had difficulty in eclosion: their wings remained cramped until death.

Currently the six remaining chrysalises are in the same container and the same site, waiting for further emergences that may occur in the future.

B. europaea is considered a species with an obligatory winter diapause in the pupal stage, a strategy that can be used to overcome an adverse season, synchronize the release of many adults and so optimize the possibility of finding a mate when the food plants are about to become available (Danks 1987; Ellers & Van Alphen 2002).

The events described were never reported in the literature before, not even with personal communications among whose with previous experience in the rearing of *Brahmaea europaea*.

Long-term pupal diapause is documented at least since the end of the nineteenth century (Dyar 1891; Riley 1892). Butterlies species showing prolonged or multiple years of diapause are not uncommon in some families: Pieridae, Papilionidae, Nymphalidae and Lycaenidae (Scott 1979; Powell 1987). In different species the stage of prolonged diapause can occur either as a larva or a pupa (Scott 1979). Prolunged diapause is most frequent in butterfly species adapted to areas with extremely variabile precipitation (Scott 1979, 1986).

The lycaenid butterfly pupae of genus *Euphilotes*, particularly the ones adapted to annual buckwheats can diapause up to 5 years possibly waiting for the appropriate precipitation (Pratt & Ballmer 1987; Pratt 1988). *Euchloe falloui* Allard (Pieridae) has been observed to remain one to fifteen years in pupal stage in the Negev desert in Israel. In this area, pupal diapause is a common strategy in Papilionidae, Pieridae, and Lycaenidae, because without rain there is no or adequate host-plant supply available and the long-term diapause is a clear survival strategy (Benyamini 2008). In Palestine, prolonged pupal diapause in the pupae of *Papilio alexanor* Esper is again a strategy to survive in an arid climate (Nakamura & Ae 1977). A similar situation exists in North America: *Anthocharis cethura pima* Edwards emerged after nine years while *Papilio zelicaon* Lucas and *Papilio coloro* Wright are known to diapause for up to six years (Powell 1987). In Europe, *Tomares bal-*

lus (Fabricius) is an endemic Lycaenid species of Western Mediterranean occurring in the Maghreb, in the Iberian Peninsula (central Portugal and central-N Spain) and in France along the Mediterranean coast from the Rhône estuary to the Maritime Alps. During a rearing experiment done from 2009 to 2016, the emergence of adults occurred sequentially, year after year, and a part of the pupae remained in diapause for up to seven years (Obregón et al. 2017).

In the present case, the prolonged diapause of *B. europaea* may have been determined by the lower humidity the chrysalises were kept during the first wintering (2016-2017), compared to what occurred in previous rearings. Why do we observe such a prolonged pupal diapause of *Brahmaea europaea* in its habitat, in a population of a species considered a Miocene relict (Hartig 1963; Sauter 1967; Racheli & Zilli 1985)? Indeed, Hartig suggested that the species belongs to Asian or African, tropical or sub-tropical fauna domains of the Tertiary. It has likely preserved its characteristics and its eco-etological adaptations thanks to the favorable conditions still maintained in the current habitat (van Schepdael 1967). Like the other brameids, it is a species of hot, very humid and rainy areas, as was the climate in southern Europe during most of the Miocene (Dufay 1970).

On the basis of these last assessments, and on what was reported by some authors on the behavior of other species in conditions of rain shortage (Scott 1979, 1986; Nakamura & Ae 1977; Pratt & Ballmer 1987; Pratt 1988; Benyamini 2008; Obregón et al. 2017), it can be assumed that the prolonged diapause is a response of the species to environmental dryness conditions during the winter period before the spring eclosion.

This can be considered another useful survival strategy of the unique Brahmaeid present in Europe in its very limited shelter habitat.

References

Audisio P., Boitani L., Malaspina A., Mason F., Spicciarelli R., Vigna Taglianti A. (eds) 2012. Piano di gestione della Riserva Naturale Orientata Grotticelle. Ufficio per la Biodiversità, Corpo Forestale dello Stato, Rome, Technical Report, 146 pp + CD-ROM.

Benyamini D. 2008. Is *Euchloe falloui* Allard, 1987 (Pieridae) the butterfly with the longest diapause? Nota lepidopterologica, 31 (2): 293–295.

Bilek A. 1965. Die raupe von *Brahmaea europea* [sic!] Hartig 1963, und deren Aufzucht aus dem Ei. Bollettino dell'Associazione Romana di Entomologia, 20 (1): 5–8.

Bilek A. 1967. Das Ergebnis meiner Zucht von *Acanthobrahmaea europaea* Hartig, 1963. En Beitrag zur Variationsbreite dieser Art (Lep., Brahmaeidae). Zeitschrift der Wiener entomologischen Gesellschaft, 52: 88–90.

Danks H.V. 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Ottawa, 439 pp.

Dufay C. 1970. L'éthologie d'*Acanthobrahmaea europaea* (Hartig) (Lep. Brahmaeidae). Alexanor, 6 (6): 249–260.

Dyar H.G. 1891. Preparatory stages of *Pheosia dimidiata* H. S. Psyche, 6: 194–196.

Ellers J. & Van Alphen J.J.M. 2002. A trade-off between diapause duration and fi tness in female parasitoids. Ecol. Entomol., 27: 279–284.

Hartig F. 1963. Per la prima volta una *Bramaea* [sic!] in Europa. Bollettino dell'Associazione Romana di Entomologia, 18 (1): 5–7.

Hartig F. 1966. Auf *Brahmaea*–Fang in der Basilicata (Lep. Bombycidae). Nachrichtenblatt der Bayerischen Entomologen, 15 (11/12): 102–105.

Hartig F. (†) 1997. Promemoria per la creazione di una zona di riserva assoluta delle cosiddette Grotticelle in Comune di Rionero del Vulture. Bollettino dell'Associazione Romana di Entomologia, 51 (1/4) (1996): 69–73.

Mosconi F., Zilli A., Spicciarelli R., Maurizi E., Vigna Taglianti A., Audisio P. 2015. An overview on the most outstanding Italian endemic moth, *Brahmaea (Acanthobrahmaea) europaea* Hartig, 1963 (Lepidoptera, Brahmaeidae). Fragmenta Entomologica, 46 (1/2) (2014): 1–9.

Nakamura I. & Ae S.A. 1977. Prolunged pupal diapause of *Papilio alexanor*: arid zone adaptation directed by larval host plant. Annals of the Entomological Society of America, 70: 481– 484.

Obregón R., Haeger J.F. and Jordano D. 2017. Adaptive significance of the prolonged diapause in the western Mediterranean lycaenid butterfly *Tomares ballus* (Lepidoptera: Lycaenidae). European Journal of Entomology, 114: 133–139.

Powell J.A. 1987. Records of prolonged diapause in Lepidoptera. Journal of Research on the Lepidoptera, 25 (1986): 83–109.

Pratt G.F. 1988. The evolution and biology of *Euphilotes* biotypes. Unpublished doctoral dissertation, University of California, Riverside, 653 pp.

Pratt G.F., Ballmer G.R. 1987. The phenetics and comparative biology of *Euphilotes enoptes* (Boisduval) (Lycaenidae) from the San Bernardino Mountains. J. Res. Lepidoptera, 25: 121–135.

Racheli T. & Zilli A. 1985. Modelli di distribuzione dei Lepidotteri nell'Italia meridionale. Biogeographia. The Journal of Integrative Biogeography, 11 (1): 165–194.

Riley C.V. 1892. The yucca moth and yucca pollination. 3rd Annual Report of the Missouri Botanical Garden, 3: 99–159.

Rougeot P.C. 1971. Bombycoïdes (Lepidoptera-Bombycoïdea) de L'Europe et du Bassin Méditerranéen. Tome 1. Lemoniidae, Bombycidae, Brahmaeidae, Attacidae, Endromididae. Faune de L'Europe et du Bassin Méditerranéen 5. Masson et Cie, Paris, 159 pp.

Rougeot P.C. 1975. *Acanthobrahmaea europaea* (Lep. Brahmaeidae), le papillon du volcan Vulture (Italie du sud). L'Entomologiste, 31 (4-5): 145–149.

Sauter W. 1967. Zur systematischen Stellung von *Brahmaea europaea* Hartig (Lep. Brahmaeidae). Mitteilungen der Schweizerischen entomologischen Gesellschaft, 40 (1/2): 125–129.

Schepdael J., van 1967. Un événement capital en Entomologie: découverte d'une *Brahmaea* en Italie, *Brahmaea europaea* Hartig. Endémisme ou relique miocènique? Linneana Belgica, 3 (5): 91–103.

Scott J.A. 1979. Hibernal diapause of North American Papilionoidea and Hesperioidea. J Res Lepidoptera, 18: 171–200.

Scott J.A. 1986. The butterflies of North America, a natural history and field guide. Stanford University Press, Stanford, 583 pp.

Spicciarelli R. 1997. Primi reperti di larve di *Acanthobrahmaea europaea* (Hrtg.) (Lepidoptera: Brahmaeidae) su *Phyllirea latifolia* L. Entomologica, 31: 191–195.

Spicciarelli R. 2004. La Psiche del Frassino. Consiglio Regionale della Basilicata, Finiguerra Arti Grafiche, Lavello (Pz), 176 pp.

Spicciarelli R. 2013. La Riserva Naturale Orientata di Grotticelle 1963-2013. 50 anni dalla scoperta della Bramea. Corpo Forestale dello Stato, Unità Territoriale per la Biodiversità, Potenza, 157 pp.

Spicciarelli R. 2018a. Bioethological observations on Brahmaea (Acanthobrahmaea) europaea (Lepidoptera: Brahmaeidae) in its dedicated Nature Reserve of "Grotticelle" (Basilicata, Southern Italy). Pp. 360–375. In: Zilli A. (ed.), "Lepidoptera research in areas with high biodiversity potential in Italy 2". Natura Edizioni Scientifiche, Bologna.

Spicciarelli R. 2018b. New bioethological observations on *Brahmaea* (*Acanthobrahmaea*) *europaea* and its host plants in Special Area of Conservation "Grotticelle di Monticchio" (Basilicata, Southern Italy) (Lepidoptera: Brahmaeidae). Fragmenta Entomologica, 50(1): 43–52.

Spicciarelli R., Autera M., Battafarano R., Gerardi G., Lavinia C., Lazzari M., Lorusso L., Mancino G. 2011. Misure di Tutela e Conservazione "Grotticelle di Monticchio" (SIC IT9210140), Rete Natura 2000, Unione Europea - Regione Basilicata. Final Technical Report, 243 pp.

Stella E. 2003. Bramea. Nel 40° Anniversario della sua scoperta. Provincia di Potenza. Finiguerra Arti Grafiche, Lavello (Pz), 63 pp.