

LIFE HISTORY AND PLURIVOLTINISM OF *Scolitantides orion* (PALLAS 1771), (LEPIDOPTERA, LYCAENIDAE) FROM ROMANIAN SOUTHERN DOBROGEA, IN CAPTIVE BREEDING ON *Hylotelephium telephium*, SOZOLOGICAL IMPLICATIONS

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Abstract. *Scolitantides orion* (Pallas, 1771) (Lepidoptera, Lycaenidae), is a Palaearctic species, spanning across Eurasia, in localized, locally threatened populations. It has a characteristic biology, being stenophagous on a few Crassulacean species, and facultative myrmecophytes. In Europe, it is declining, severely in some areas - mainly due to habitat loss, being red listed and legally protected in Fennoscandia and Central Europe. In Romania, it is not listed and endangered, but some of its populations are sharply declining due to multiple causes. Besides its own conservation concerns, it is interesting as a model for ethological, physiological and ecological studies. As it shares the food-plant with *Parnassius apollo* - another critically endangered species in some places, any knowledge gained about its biology and ecology could prove essential for conservation. The knowledge about the phenology and voltinism of this species -from other parts of Europe is scarce and contradictory, and no data are available about its ecology, phenology or voltinism in Romania. The aim of the experimental work presented herein was to investigate the life history and voltinism of this species under conditions in Southern Romania in captive breeding, with the larger scope of gaining knowledge about the biology and logistics involved in the conservation, (re)introduction and propagation of endangered lepidopteran species which use Crassulaceae as host/food plants. Under our conditions, and feeding exclusively on live *Hylotelephium telephium* (Crassulaceae), *Scolitantides orion* typically developed 4 generations/year, with an average generation turnover of 42-55 days, and a diapausal length of about 185 days. Such a long diapause and other personal data describing anomalous 5th generations more frequent during the last years, with unusually warm and excessively long autumns, suggested the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers for (re)introduction conservation programs.

Keywords: developmental plasticity, *Hylotelephium telephium*, *Scolitantides orion*, conservation, life history.

Rezumat. Ciclul de viață și plurivoltinismul la *Scolitantides orion* (Lepidoptera, Lycaenidae) din Dobrogea Sudică Română, crescut în captivitate pe *Hylotelephium telephium*, implicații zoologice. *Scolitantides orion* (Pallas, 1771) (Lepidoptera, Lycaenidae), este o specie Palaearctică, cu distribuție Eurasiacă, în populații localizate, local amenințată cu o biologie caracteristică, stenofagă pe câteva specii de Crassulaceae, este facultativ myrmecofilă. În Europa, este în declin - sever în unele regiuni - în principal, datorită degradării habitatului, fiind trecută pe listele roșii și legal protejată în Fennoscandia și Europa Centrală. În România, specia nu este trecută pe listele roșii și nici periclitată, dar unele populații sunt în declin abrupt datorită unor multiple cauze. În afara propriilor probleme de conservare, specia este interesantă ca model pentru studii etologice, fiziológice și ecologice. Cum împărtășește planta gazdă cu *Parnassius apollo* - altă specie critic periclitată în unele locuri, orice cunoștințe obținute despre biologia și ecologia ei pot fi esențiale pentru conservare. Datele privind fenologia și voltinismul acestei specii, din alte părți ale Europei, sunt puține și contradictorii, iar cele despre ecologia, fenologia sau voltinismul în România, lipsesc. Scopul acestui studiu experimental este investigarea ciclului de viață și a voltinismului acestei specii în condițiile din sudul României în creșterea în captivitate, cu scopul mai larg de a obține cunoștințe despre biologia și logistică implicate în conservarea, (re)introducerea și propagarea de specii de lepidoptere periclitante care folosesc Crassulaceae, ca plante gazdă. În condițiile noastre, în hrănire exclusivă pe *H. telephium* (Crassulaceae) viabil, *S. orion* dezvoltă tipic 4 generații/an, cu un turnover de 43-54 zile/generație, și o durată a diapauzei de circa 185 de zile. O asemenea diapauză lungă și alte date personale inedite care arată o a 5-a generație anormală mai frecventă în ultimii ani cu toamne neobișnuite de calde și îndelungate, au sugerat perspectiva unei culturi în masă mai eficientă a acestei specii de lepidoptere și a altora similare pentru obținerea unui număr mai mare de adulți pentru programe de (re)introducere pentru conservare.

Cuvinte cheie: plasticitatea dezvoltării, *Hylotelephium telephium*, *Scolitantides orion*, conservare, ciclu de viață.

INTRODUCTION

Scolitantides orion (Pallas, 1771) (Lepidoptera, Lycaenidae), is a Palaearctic species, spanning across Europe and Asia, in localized populations confined to patchy habitats where it can find its specialized food plants, and is locally threatened in some areas (KUDRNA, 2002).

According to COULONDRE (1994) on such a vast range with multiple (meta)-populations, several subspecies are accepted as valid. In Europe, 3 subspecies are present; *S. orion parvula* (De Sagara 1926) in the Pyrenees and Iberian Peninsula, *S. orion ultraornata* (Verity 1937) = *S. o. wahlgreni* (Bryk, 1946) in Fennoscandia, while the populations from Central, South-Eastern Europe and Turkey being assigned to the nominotypical subspecies *S. orion*.

According to the IUCN evaluation from 2010 (VAN SWAAIJ ET AL., 2010), still valid through 2018, *S. orion* is reported extinct in the European part of Turkey while “Strong decline in distribution or population size of more than 30% has been reported from Germany, Norway, Poland and Ukraine” and a “decline in distribution or population size of 6-30% has been reported from Austria, Romania, Russia, Slovakia and Sweden”. In Finland, the species is protected under the Nature Conservation Decree (IUCN class Vulnerable; RASSI ET AL., 2001). Throughout Fennoscandia the distribution is patchy in disjunct (meta)-populations, in Finland the species existed in only 5 -10 patches (SOMERMA, 1997) but tends to somewhat recover following an apparently successful action of habitat restoration at one site

(MARTTILA et al., 2000). While the species was always been rare, the current steeper decline seems to be caused by shrub/forest succession after woodland pastures abandonment, scarcity of natural forest fires, residential development and quarrying of stone (MARTTILA et al., 2000).

The current threat status in Europe is LC (Least Concern) and according to European Red list of butterflies; (VAN SWAAY et al., 2010) NT (near threatened) in the European Union. In Sweden, due to steep population decline in the last 30 years, *S. orion* was classified as EN (Endangered) (Red list of Swedish species; Swedish Species Information Centre 2012).

Knowledge about *S. orion* in Romania is scarce at best, and it is considered not endangered (although declines between 10-30% have been reported, IUCN) and it is not explicitly protected. No ecological data from Romania were available at the beginning of this study and the situation did not improve in recent years.

S. orion is a stenophagous species throughout its distribution range, the larvae feed exclusively on a few species in a couple of Crassulacean genera, e.g. *Hylotelephium* in Finland (SAARINEN, 1995; MARTTILA et al., 2000; KOMONEN et al., 2008), Sweden (ELMQVIST, 2011; CARLSSON & ELMQUIST, 2009), Norway (ENDRESTØL et al., 2009), Germany (TRAENKNER & NUSS, 2005). In Romania, *S. orion* feeds on *Hylotelephium telephium* together with *Aizobius sedi* (BÂRCĂ & NICULAE, 2011), *Sedum*, *Jovibarba* and *Sempervivum* (BÂRCĂ V. unpublished data) and therefore its distribution conforms with that of its host-plants (also see BÂRCĂ & NICULAE 2005, 2006, BÂRCĂ 2016a, 2016b; NICULAE & BÂRCĂ, 2005; 2006). Due to its narrow stenophagy it faces the same threats as its host-plants, both in Romania and elsewhere (ARBUNE et al., 2009).

The species is considered as facultative myrmecophilous, according to TRAENKNER & NUSS, (2005); being attended by several ant species from at least 4 genera (*Camponotus*, *Formica* (*Serviformica*), *Lasius* (*Lasius*) and *Tetramorium*), but does not depend on association with ants for completing its life cycle.

The ecology of *S. orion* is quite complex, in Central and southern Europe the species inhabiting patchy habitats with xero-thermophilous rocky landscapes in close proximity with taller shrubs and forest margins. In Finland in Linnansaari National Park, at the Northern range limit of the species, it inhabits small unforested exposed bedrock areas occurring patchily within a true island network, where they feed on *H. telephium* (KOMONEN et al., 2008).

The phenological data available are controversial and suggest a remarkable phenological plasticity for this species, which shows differing numbers of generations per year in different areas. Thus, it has one generation per year over most of Europe TOLMAN & LEWINGTON, 1998, according to KUDLA, 1951, for Moravia, REINHARDT & KINKIER, 2004, for Rhineland-Palatinate, ELMQUIST & CARLSSON, 2009; CARLSSON & ELMQUIST, 2013 for Sweden, ENDRESTØL et al., 2009, for Norway, KOMONEN et al., 2008, for Finland.

In warmer regions it has 2 or 3 generations per year: in Switzerland (TOLMAN & LEWINGTON, 1998; TRAENKNER & NUSS, (1994, 2005); in Czech Republic Central Bohemia (SRDINKO, 1912), in Italian South Tyrol (HUEMER, 2004). In Germany, 2 generations were reported in Saxony (REINHARDT, 2003) and in Thuringia (BERGMANN, 1952), where also a partial second generation occurs, while TRAENKNER & NUSS, (2005) reported 3 generations.

In Central Europe, TOLMAN & LEWINGTON (1998) report *S. orion* has one generation per year while FORSTER & WOHLFAHRT (1955) reported two generations, with one at higher elevation in the Alps.

There is a wider consensus though on the fact that pupae of all generations could enter diapause and only hatch the next or the after-next year. No data are available about intraseasonal dormancy/diapause between generations of the same year.

MATERIAL AND METHODS

Insects: The *Scolitantides orion* insect female progenitors were collected from the vicinity of the village Sipotele, Constanța County in Southern Dobrogea, in April, and placed in “oviposition cages” where they laid eggs that were further used in the subsequent experimental breeding of larvae that produced the next generations.

Plants: In the field, under natural conditions, *S. orion* feeds on several genera of Crassulaceae, but our previous experience showed that the most biomass-efficient food-plant species in seminatural conditions is *Hylotelephium telephium*, so we chose this plant species as food source.

The food/host plant for *S. orion* was *Hylotelephium telephium* stock plants cultivated intensively from plant progenitors were collected during the previous years at the same site where the *S. orion* adults were collected. For the breeding experiments the plants were transplanted in small plastic bags approximately 10x10 cm, tied around the main stem(s) too avoid larval access into the bags. This allowed easy placement in the cages and easy replacement maneuvers with minimal disturbance to the larvae.

Captive breeding was performed in relatively smaller “rearing cages” and the resulting pupae were retrieved by hand and used for further observations.

To provide adults with more room for pairing and flight, the retrieved pupae were transferred, and adults left to emerge in relatively larger “oviposition cages” where they copulated and oviposited freely, and the eggs were retrieved daily.

Because our previous experience showed that the neonate and un-burrowed first instar larvae are more sensitive to environmental conditions and much more prone to getting lost in the litter, the eggs were placed in “hatching containers” where they were kept as the whole first instar developed.

The second instar larvae were transferred to the "rearing cages" where the development of the rest of larval stage and the pupation occurred.

Cages:

The "oviposition cages" consisted of parallelepipedic tent with the approximate side dimensions 1.5x2x1m, made of plastic/fiberglass screen 10 mm mesh placed over a supporting frame of plastic construction PVC pipes. The bottom of the cage was made of a plastic sheet with margins raised 15 cm from the ground and fastened with velcro to the "cover section", allowing relatively easy access to the interior of the cage and "insulating" the interior from the access of insect predators. Previous experience showed that the "insulation" is very important as spiders and wasps readily catch the adults and can ruin the experiment. Our setup was very efficient at keeping the predators and water out of the cage and at keeping the adults and even the neonates inside the cage.

The "rearing cages" consisted of cylindrical tubes made of 1x1.5 m sheets of the same plastic screen mesh sewn on a side, pulled over 2 metal wire circles and tied at the ends after the plant with the insects was placed inside. These cages were hung from a cord above ground to avoid access of *Forficula* and *Periplaneta* which readily eat the late instars larvae.

The "hatching containers" consisted of 2 approximately cylindrical 3L plastic food containers with tightly fitting lids custom-fashioned to allow reversed stacking one on top of the other, fitted with a laptop fan to allow adequate ventilation. The second, reversed container was only placed on top of the first one after the oviposition took place.

The plants on which the adults were to lay the eggs were placed in the lower container of the "hatching containers" and 4 of these containers with oviposition plants were placed in the "oviposition cage".

Every 2 days the eggs were removed together with segments pinched off the leaves or stems or together with the flowers to which they were attached and placed in containers on fresh food-plants so that the neonates could burrow into fresh leaves.

All the pupae that we could find were promptly transferred in the oviposition cages until the imagines hatched. The pupae were placed in plastic yogurt 1L containers with tight lid fitted with the same screen mesh, and having inside a rectangular metal screen mesh rolled into a cylinder onto which the imagines could climb and hang to extend their soft wings after hatching from the pupae.

The egg stage begun when the first eggs were found until the last eggs were found when inspecting the plants and the rest of the oviposition cage.

The larval stage begun when we could observe the first eggshells or eggshell marks on the leaves, or when observing neonates (but normally the neonates readily burrow into the leaf parenchyma so they could have easily been overlooked), and lasted until no more viable eggs remained. (The nonviable eggs change appearance after a couple of days, so they could easily be distinguished from viable eggs).

The pupal stage begun when we found the first pupae in the rearing cages and lasted until no more pupae or larvae could be seen in the rearing cages. (The pupae were readily removed from the cages every day).

The imago stage begun when we found the first imagines in the containers and lasted until the last day an imago was found. (The containers were checked every morning and evening and the newly hatched adults were released from the containers in the oviposition cage, but between inspections the containers were closed). All generations produced diapausing pupae, which were removed from the containers after 30 days and stored separately in a larger container similarly fitted with a hatching net made of metal screen mesh. Some adult hatched erratically after a short diapause, before autumn and these were not counted towards any generation.

RESULTS AND DISCUSSIONS

The 6 females introduced in the oviposition cage produced 58 eggs over a period of 8 days. Of this egg batch of the first generation 9 eggs were found dead and 49 produced neonates of the first generation. The females of the first generation oviposited on the upper leaves and stems down to the 3rd internode, the females of the second generation oviposited on the upper leaves and stems with a few down to the 3rd internode, and also on the inflorescences of SM food-plants, while the females of the subsequent generation oviposited on the top leaves and stems down to the 2nd internode, with a higher proportion of eggs on the upper leaves (data not shown, more thorough experimental work was needed to evaluate the significance and causes of the apparent preference differences shown by the females of different generations, and the results are reported elsewhere).

These were the founders which developed in the later stages giving 4 generations, with various lengths of developmental stages, as shown in Table 1. The absolute duration of each of the developmental stages of the 4 generations are shown in the chart in Figure 1, and the life history of *S. orion* as revealed by our present experimental study with the relative length of the developmental stages and their placement in the life cycle is depicted in the chart in Figure 2.

First generation. The first eggs were found on April 9, the eggs needed 12-16 days for full development and the first larvae hatched on April 20. The larvae needed 27-30 days for full development and the first pupated after 27 days on May 18. The hatching pupal stage lasted 14 days and the first imagines of the first generation emerged from pupae on June 01. Some pupae entered diapause, of which 3 normal-looking imagines hatched erratically and were not

counted. These erratic emergences of imagines outside the “normal” life cycle of the majority of their generation suggest that under natural conditions the adult populations on wing at a certain time would comprise also individuals from other generations. The imagines of this first generation hatched synchronous over 4 days and started feeding the first day after wing expansion and started ovipositing the second day, and oviposition lasted for 12 days, while the last adult died after 12 days. The whole first generation lasted 69 days but the first oviposition started after 55 days.

Table 1. Absolute durations of each *Scolitantides orion* developmental stage of all generations in one year (days).

Developmental Stage	Egg	Larva	Pupa	Imago	Generation length
				a	b
First Generation	12	27	14	16	69
2nd Generation	9	20	11	14	54
3rd Generation	10	21	10	15	56
4th Generation	11	25	185	16	237
Dev Stage Sum	42	93	220*	61	365
					365

a- generation length including whole flight period of imagines
b- generation length until the day the first eggs were oviposited
*- pupal stage including the diapause
Dev Stage Sum = Summary of duration of each Developmental Stage n the life cycle; e.g. duration of all 4 Egg stages of one year life cycle

Second generation. The first eggs of the second generation were found on June 3rd, the eggs needed 9 days for full development and the first larvae hatched on June 12. The larvae needed just 20-24 days for full development and the first pupated after 20 days on July 12. The active pupal stage lasted 11 days and the first imagines of the first generation emerged from pupae on July 11. This generation also, some pupae entered diapause, of which 5 normal-looking imagines hatched erratically and were not counted. The imagines of this 2nd generation hatched synchronous over 3 days and started feeding the first day after wing expansion and started ovipositing the second day, and oviposition lasted for 13 days, while the last adult died after 14 days. The whole 2nd generation lasted 54 days but the first oviposition started after 42 days.

Typical Life Cycle of *S. orion*, durations of stages (days)

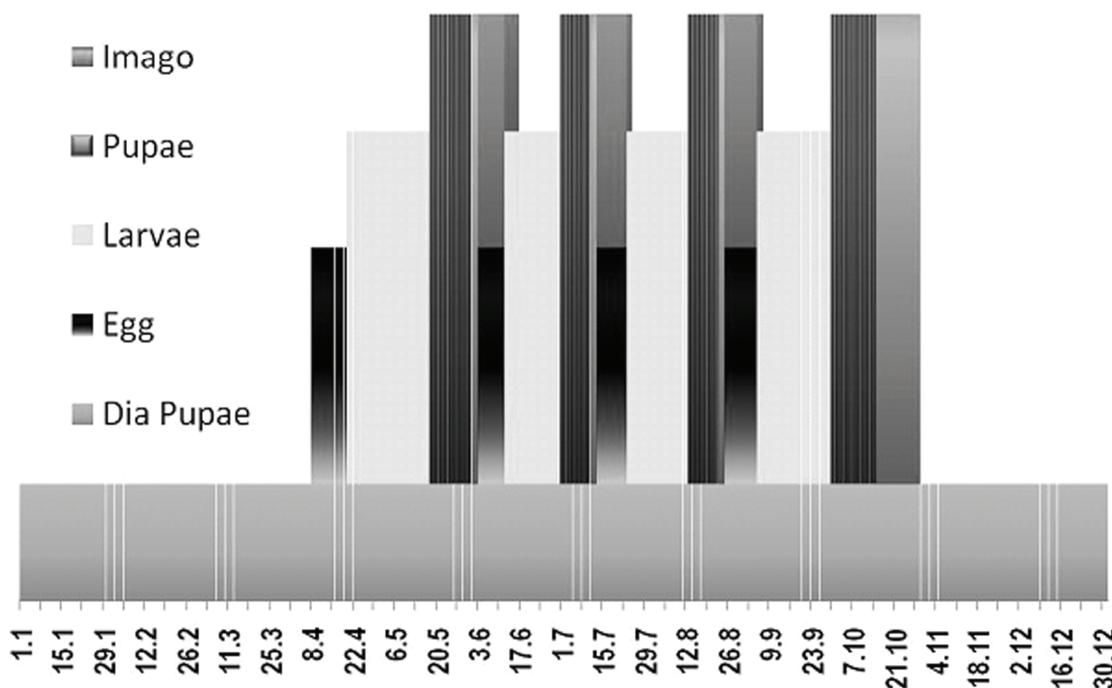


Figure 1. The absolute duration of each of the developmental stages of the four *S. orion* annual generations.

Third generation. The first eggs of the third generation were found on July 13, the eggs needed 10 days for full development and the first larvae hatched on July 23. The larvae needed 21-23 days for full development and the first pupated after 21 days on August 13. The active pupal stage lasted 10 days and the first imagines of the first generation emerged from pupae on August 23. This generation also, some pupae entered diapause, of which 2 normal-looking imagines hatched erratically and were not counted. The imagines of this 3rd generation hatched synchronous over

2 days and started feeding the first day after wing expansion and started ovipositing the second day, and oviposition lasted for 14 days, while the last adult died after 15 days. The whole 3rd generation lasted 56 days but the first oviposition started after 48 days.

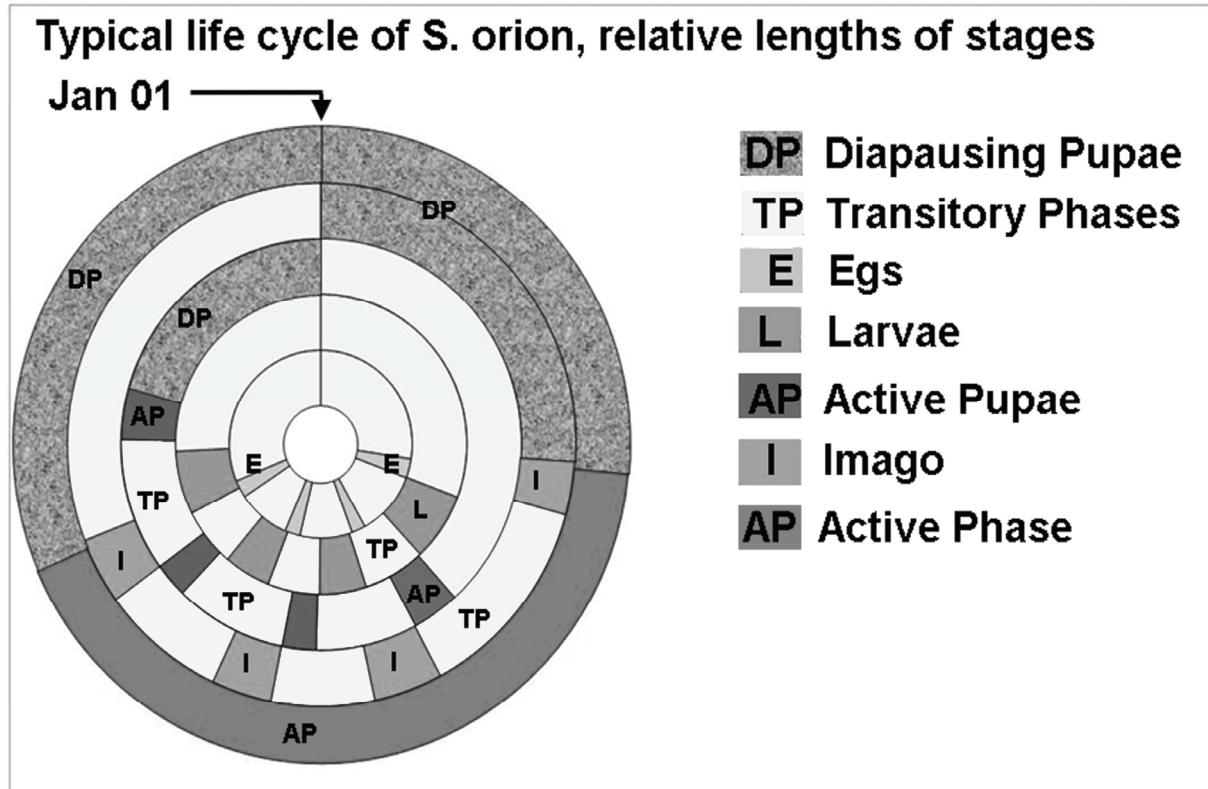


Figure 2. Life history of *S. orion* as revealed by our present experimental study with the relative length of the developmental stages and their placement in the life cycle.

Fourth generation. The first eggs of the third generation were found on August 25, the eggs needed 11 days for full development and the first larvae hatched on September 5. The larvae needed 25-29 days for full development and the first pupated after 25 days on September 30. The pupae entered diapause, of which 6 normal-looking imagines hatched erratically and were not counted. These diapausing pupae generated the imagines of the first generation of the subsequent year, after overwintering. The whole fourth generation needed 36 days to reach pupation of the first individuals.

Normally, the duration of the overwintering normally could only be inferred from previous observations. In our case, the pupae were washed, disinfected briskly with 5% bleach, kept outside on moss in a single layer in a cardboard box sheltered from direct sun and rain until December when they were checked and transferred to and stored in a refrigerator at 5°C for overwintering. 10 pupae were placed outdoors for overwintering in the same cardboard box and the 7 live remaining pupae after overwintering were transferred to a hatching container placed outdoors, where 5 of them hatched between April 3rd and April 8. Judging from the emergence of these imagines, the diapause of the 4th generation lasted cca. 185 days.

Our results showed that *S. orion* life cycle in captive breeding on *H. telephium* under climatic conditions in Southern Romania consisted of 4 generations per year, with an effective generation turnover between 46 and 59 days between larval eclosion and oviposition, the last generation entering diapause and giving the imagines of the first generation of the subsequent year. This did seem normal to us but later, checking the published data of other authors we noticed that the largest number of generation per year reported was of 3 generations, in Southern Germany, by TRÄNKNER & NUSS (2005) with a comprehensive discussion in (REINHARDT, 2003). This discrepancy could be explained by differing climatic conditions, especially with regard to temperature. In our experiments, the generation turnover shortened, so one more generation could develop over a year, possibly also due to a longer period with favorable temperatures.

It also need to be noted that our experiment gave the larvae ad-libitum access to good quality food resources and provided shelter both from inclement weather and predator, and such optimal conditions may prove difficult to find under natural conditions. Also, during this experiment, after the hatching of the larvae of the first generation we did not count the survival rate of the larvae, so it is possible that cannibalism occurred and so a certain degree of selection and pressure towards faster development might have also occurred.

These facts suggest a developmental plasticity, allowing the individuals to adapt their phenology to the climatic condition of the moment and so exploit windows of opportunity open by favorable climatic (or nutritive) conditions.

Another interesting fact was a relatively good synchronization of developmental stages, the developmental stage length variation falling within 2-3 days for the vast majority of individuals. This made for a very clear-cut separation of generations on wing, while our observations in the field on multiple populations in various locations in Romania and Macedonia showed much more continuous flight periods with potentially overlapping generations.

The observed synchronization in our experiment might be a result of cannibalism and/or could be explained by a higher degree of genetic uniformity in our population than under natural conditions, factor that should not be neglected as it has been shown that inbreeding is an actual possibility and could represent a serious threat in small butterfly populations (SACCHERI et al., 1998). Moore experimental work is needed to check for this fact.

Some individuals of all generations entered diapause and some hatched erratically throughout the summer and autumn without any explanation of the triggers and mechanisms involved in selective inducement of diapause and then for the erratic “escape” from diapause exhibited by only some individuals, as reported by TRÄNKNER & NUSS (2005) and discussed by MÜLLER, 1992. These erratic emergences of imagines not complying with the “normal” life cycle of the majority of their generation suggest that under natural conditions the adult populations on wing at a certain time would comprise also individuals from other generations.

This also points to a lack of hormonal inhibition of reproduction in later generations as observed in some migratory Sphingidae species whose last generation developing at more northern latitudes, even if reached maturity and imagines emerged, they seem to be sterile and do not reproduce. Our findings seem to support such hypothesis and warrant more thorough experimental research towards its empirical validation or rejection.

The presumed voltinism elasticity and lack of hormonal inhibition of reproduction in late generations could, coupled with a very long diapause lasting almost half a year or more and other personal data describing anomalous 5th generations more frequent during the last years, with unusually warm and excessively long autumns, suggest that the active life cycle could be prolonged and more generations could be pushed into the diapause period of the year, provided optimal conditions are given for longer period of time, even if the active life cycle could not be accelerated and no more generations could be compressed in the natural life cycle by shrinking the developmental stages into shorter generation turnover. This opens the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers for (re)introduction conservation programs.

CONCLUSIONS

a) - Captive breeding of *S. orion* on *H. telephium* under climatic conditions in Southern Romania is possible and could prove a viable option for generation of larger numbers of adults in artificial conditions.

b) - *S. orion* life cycle in captive breeding on *H. telephium* under climatic conditions in Southern Romania has 4 generations per year, with an effective generation turnover between 46 and 59 days our findings differ from other published experimental data reporting only 3 generations, in Southern Germany, by TRÄNKNER & NUSS (2005). This discrepancy could be explained by several factors:

i - presumably more favorable climatic conditions in our experiment;

ii - optimality of other ecological conditions may prove difficult to find under natural settings;

iii - a certain degree of selection and pressure towards faster development, cause by e.g. cannibalism, behavioral reproductive depression due to confinement;

c) - These discrepancies regarding voltinism and length of developmental stages under different environmental conditions suggest a developmental plasticity, allowing the individuals to adapt their phenology to the climatic condition of the moment and so exploit windows of opportunity opened by favorable climatic (or nutritive) conditions as suggested by HUNTER & MCNEIL (1996) and HOPPER (1999).

d) - A relatively good synchronization of developmental stages was observed, resulting in very distinct flight periods of subsequent generations on wing, contradicting our multiple observations in the field which showed much more continuous flight periods with potentially overlapping generations. The observed synchronization in our experiment might be a result of cannibalism and/or could be explained by a higher degree of genetic uniformity in our population than under natural conditions. Moore experimental work is needed to check for this fact.

e) - This also point to a lack of hormonal inhibition of reproduction in later generations as observed in some migratory Sphingidae species whose last generation developing at more northern latitudes, even if reached maturity and imagines emerged, they seem to be sterile and do not reproduce.

f) - The presumed voltinism elasticity and lack of hormonal inhibition of reproduction in late generations could, coupled with a very long diapause lasting almost half a year or more suggest that the active life cycle could be prolonged and more generations could be pushed into the diapause period of the year, provided optimal conditions are given for longer period of time, even if the active life cycle could not be accelerated and no more generations could be compressed in the natural life cycle by shrinking the developmental stages into shorter generation turnover. This opens the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers

for (re)introduction conservation programs. Our findings generated many hypotheses and prompted more thorough experimental research towards their empirical validation or rejection which will be reported elsewhere.

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