

Contribution to the biology, ecology and taxonomy of *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Part II¹: An experimental hybridisation of *P. (L.) c. gennargenti* × *P. (L.) c. nufrellensis*

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Abstract: *Polyommatus coridon* (PODA, 1761) is represented by endemic subspecies on the Mediterranean islands of Corsica (*nufrellensis* SCHURIAN, 1977) and Sardinia (*gennargenti* LEIGHEB, 1987). The latter taxon is considered a separate species by some authors, but evidence for reproductive isolation is lacking. In order to check for isolating mechanisms we carried out hybridisation experiments. Source material for these experiments were ♀♀ which had been caught for oviposition in 2007. We achieved synchronous hatching and development of larvae by keeping them under long-day conditions and high temperatures. With the help of the “semi-artificial method” we obtained 6 matings between ♀♀ of *P. c. nufrellensis* and ♂♂ of *P. c. gennargenti*, whereas the reciprocal copulation was only achieved once. 4 of the ♀♀ laid 155 eggs altogether, of which 79% proved fertile. Resulting caterpillars were reared under the above-mentioned laboratory conditions using *Hippocrepis comosa*, and from mid-November, after about 6 weeks, developed into adults of the F₁ generation (18 ♂♂, 22 ♀♀, 24 pupae died, 1 adult crippled). Almost all hybrids appear intermediate between the parents. We did not find evidence for reproductive isolation barriers because mortality rates of the hybrid breedings did not differ considerably from pure breedings.

Beitrag zur Biologie, Ökologie und Taxonomie von *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Teil II: Experimentelle Hybridisierung von *P. (L.) c. gennargenti* × *P. (L.) c. nufrellensis*

Zusammenfassung: *Polyommatus coridon* (PODA, 1761) ist auf Korsika (*nufrellensis* SCHURIAN, 1977) und Sardinien (*gennargenti* LEIGHEB, 1987) mit endemischen Unterarten vertreten. Die letztgenannte Unterart wird von einigen Autoren als eigene Art betrachtet, wenngleich es keine Belege für eine reproduktive Isolation gibt. Um zu überprüfen, ob Isolationsmechanismen zwischen beiden Taxa existieren, führten wir Hybridisierungsexperimente durch. Als Ausgangsmaterial verwendeten wir ♀♀, die im Sommer 2007 auf den Inseln gefangen wurden. Wir synchronisierten Eischlupf und Raupenentwicklung durch Zucht unter Langtagbedingungen und Wärme. Mit Hilfe der „semiartifizialen“ Methode erzielten wir 6 Paarungen zwischen ♂♂ von *P. c. gennargenti* und ♀♀ von *P. c. nufrellensis*, aber nur eine reziproke Verbindung. Vier ♀♀ legten zusammen 155 Eier, von denen sich 79% als fertil erwiesen. Die Raupen wurden unter den oben genannten Bedingungen aufgezogen, mit *Hippocrepis comosa* gefüttert und ergaben nach wiederum etwa 6 Wochen ab Mitte November die F₁-Hybridgeneration: 18 ♂♂ und 22 ♀♀, 24 Puppen waren abgestorben, ein Falter verkrüppelt. Fast alle Hybridfalter stehen in ihrer Färbung und Anordnung der Ozellen in der Mitte zwischen den Elterntieren. Wir konnten keine Hinweise auf reproduktive

Isolationsmechanismen finden, da sich die Mortalitätsraten der Hybridzuchten nicht deutlich von denen reiner Zuchten unterschieden.

Contribution à la biologie, l'écologie et la taxonomie de *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Partie II: Une hybridation expérimentale de *P. (L.) c. gennargenti* × *P. (L.) c. nufrellensis*

Résumé: En Corse ainsi qu'en Sardaigne *P. coridon* est représenté par des sous espèces endémiques. La sous-espèce *P. c. gennargenti* est considérée par certains auteurs comme une bonne espèce mais il n'y a aucune preuve que son isolat soit un obstacle à la reproduction justifiant de la considérer comme une espèce. Afin de vérifier cela, nous avons procédé à l'hybridation des deux taxons. Le matériel utilisé était constitué de ♀♀ capturées sur les deux îles durant l'été 2007. Nous avons réussi à obtenir une émergence synchrone des chenilles en les plaçant dans des conditions artificielles consistant en un éclairage constant et des températures élevées. En recourant à la méthode “semi-artificielle” 6 accouplements entre des ♀♀ de *P. c. nufrellensis* et des ♂♂ de *P. c. gennargenti* ont été conduit avec succès alors que l'accouplement inverse n'a fonctionné qu'une seule fois. Au total, 4 ♀♀ ont pondu ensemble 155 œufs dont 79% se sont avérés fertiles. Les chenilles ont été élevées dans des conditions de laboratoire sur *Hippocrepis comosa* à partir de la mi-novembre. Après 6 semaines environ, des imagos F₁ ont été obtenus (18 ♂♂ et 22 ♀♀), 24 chrysalides n'ont pas éclos et 1 était abîmée. Presque tous les hybrides présentèrent des caractères intermédiaires entre *P. c. gennargenti* et *P. c. nufrellensis* au niveau de la coloration des ailes et de la forme des ocelles. Nous n'avons pas trouvé de preuve concernant l'existence d'une barrière reproductive, notamment si l'on considère que le taux de mortalité des hybrides n'est pas différent de celui observé lors de l'élevage de chacune des souches.

Contributo alla biologia, ecologia e tassonomia dei *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Parte II: Ibridazione sperimentale di *P. (L.) c. gennargenti* × *P. (L.) c. nufrellensis*

Riassunto: In Corsica e in Sardegna *Polyommatus coridon* è presente in due sotto-specie endemiche. La sotto-specie *P. c. gennargenti* è considerata da certi autori come buona specie, ma non ci sono prove che questa farfalla non si potrebbe riprodurre con altri *P. coridon* se non fosse isolata. Per verificare questo fatto abbiamo provato di ibridare queste due specie. Il materiale utilizzato consisteva in due ♀♀ campionate sulle isole durante l'estate 2007. Siamo riusciti

¹ Part I: see SCHURIAN et al. (2006).

ad ottenere un'emergenza sincrona dei bruchi mettendogli in condizioni artificiali con illuminazione costante e temperatura elevata. Ricorrendo a questo metodo «semi-artificiale» sono state ottenute sei accoppiamenti tra ♀♀ di *P. c. nufrellensis* e ♂♂ di *P. c. gennargenti*; l'inverso è successo soltanto una volta. In totale 4 ♀♀ hanno prodotto 155 uova, tra quali 79% erano fertili. I bruchi sono stati elevati in condizioni di laboratorio su *Hippocrepis comosa* a partire dalla metà di novembre. Dopo circa 6 settimane sono nati gli adulti F₁ (18 ♂♂ e 22 ♀♀). 24 bruchi sono morti, uno era deformato. Quasi tutti gli ibridi mostravano caratteri intermedi tra *P. c. gennargenti* e *P. c. nufrellensis* al livello di colorazione delle ali e forma degli ocelli. Non abbiamo trovato nessuna prova di una barriera riproduttiva tra le due specie. Perfino abbiamo trovato che la percentuale della mortalità degli ibridi non era affatto più alta di quella osservata in non-ibridi.

Introduction

Polyommatus (Lysandra) coridon (PODA, 1761) is a widespread and often common blue butterfly in most of Europe up to 55° N (KUDRNA 2002), but was unknown from the Mediterranean islands until 1975, when local populations were discovered on the Tyrrhenian islands of Corsica (ssp. *nufrellensis* SCHURIAN, 1977) and Sardinia (ssp. *gennargenti* LEIGHEB, 1987). Later the latter taxon was raised to species level by several authors (KUDRNA 1999, LAFRANCHIS 2000, KUDRNA 2002, JUTZELER et al. 2003) due to morphological differences, but this status is not universally accepted (e.g. TOLMAN & LEWINGTON 2008, DIRINGER 2009, 2010) and also not clearly supported by available genetic studies (MARCHI et al. 1996, WIEMERS 2003 and unpublished data).

Crossing experiments which have been carried out in several *Lysandra* species (SCHURIAN 1989a, 1989b, 1990) have proved helpful to infer the species status of questionable populations, because they can clarify the existence of prezygotic and especially postzygotic isolating mechanisms. Therefore the first three authors intended to also carry out such studies on the populations from Corsica and Sardinia.

However, for more than a quarter of a century after its discovery the Corsican subspecies *nufrellensis* had not been observed until it was rediscovered (SALA et al. 2005). In recent years it has been recorded several times, and the complete life cycle was documented (SCHURIAN et al. 2006).

Due to the fact that *nufrellensis* is only found in a few locations and sometimes only in low numbers, it is not surprising that, on a visit in 2005, the first author only found a few ♀♀ which could be used for breeding. On another visit in 2006 the search also did not succeed, while H. DESCIMON (Marseille) observed only a single ♂ of *nufrellensis* (in litt. 28. XI. 2006). The search for the original larval foodplant on Corsica, which is still unknown, was also not successful. In the years 2005, 2006, 2007 and 2008 the authors carefully checked the known sites for possible foodplants without being able to discover any.

Although it is easily possible to obtain eggs from ♀♀ of *nufrellensis* collected in the wild and breed them on the substitute foodplant *Hippocrepis comosa* L. (Horseshoe vetch), the pairing of these butterflies under lab conditions was not successful, even though these specimens were almost equal in size to the specimens in the wild and did not show any abnormal behaviour.

The related Sardinian ssp. *gennargenti*, however, was intra-subspecifically paired and bred a few times (JUTZELER et al. 2003, SCHURIAN et al. 2006 and unpublished data).

Material and methods

Collecting ♀♀ from the wild

The aim of KGS' and AW's visit to Sardinia and Corsica in 2007 was to collect ♀♀ of *gennargenti* and *nufrellensis* in order to obtain breeding stock for hybridisation experiments. To minimize the risk of failure, breeding experiments were carried out by both authors who separated the two forms and had them lay eggs separately. Breeding was then also conducted separately.

A third breeding process was carried out by YD, who could also collect ♀♀ of *nufrellensis* on the western side of the main Muvrella ridge and bring them to oviposition.

Habitats of *P. (L.) coridon gennargenti*

In Sardinia, there are several sites where *P. (L.) coridon gennargenti* can be found. We found the lycaenids at the localities Perda e Liana and the Monte Novo San Giovanni (Figs. 1, 2). The butterfly is found only in habitats on limestone, since only here the foodplant *Hippocrepis comosa* grows. Only a few meters beyond these areas no more butterflies were found. Both at Perda as well as in San Giovanni in the hot midday we observed ovipositing ♀♀ (Fig. 5). We did not find any caterpillars during the period end of July to the beginning of August. We dug out some small plants for the onward journey to Corsica and thus had enough fresh plants to enable the oviposition of *nufrellensis*.

Habitats of *P. (L.) coridon nufrellensis*

In Corsica, we have so far confirmed only two locations where the butterfly can be found. One of them is near the village of Haute Asco (SALA et al. 2005) and the other one on the west side of Muvrella (SCHURIAN 1977, SCHURIAN et al. 2006) (Figs. 3, 4). At these locations we found ♀♀ (and ♂♂) in the years 2005, 2007 and 2008, which we used for breeding experiments (Figs. 6–9).

Methodology of oviposition

Before starting their journey AW and KGS prepared several breeding glasses: The bottom of 1-litre-glass jars was first covered with two layers (2 cm) of cellulose tissue so that excess moisture would be absorbed. Then a styrofoam plate was put inside with two wells for roll

edge glasses to be fitted. One 30 ml glass contained a concentrated solution of sugar water absorbed in a roll of cellulose, the other water and small branches of *H. comosa* from Germany (Fig. 11).

In Sardinia fresh shoots of the local *Hippocrepis* plants were used for the oviposition of *gennargenti*.

The glass was closed with small-meshed gauze. The glasses with the ♀♀ were placed outside in the morning and afternoon sun with some shade, as it had been observed that these were ideal times to maximize oviposition.

According to our experience, the ♀♀ of *nufrellensis* – despite seemingly optimal conditions – vary in the number of eggs that are laid and need more intensive care than those of *gennargenti* or *coridon* from Central Europe. While ♀♀ of *coridon* of the habitat Mainzer Sand (environs of Mainz-Mombach, Rhineland-Palatinate, Germany) could remain unobserved in receptacles over many days during oviposition and produced a large number of eggs, the *nufrellensis* would sometimes only survive a few hours under suboptimal conditions until they died. This was previously only observed in the eastern Anatolian *Polyommatus (Lysandra) dezinus* (DE FREINA & WITT, 1983), see SCHURIAN (1993), SCHURIAN & FIEDLER (1994). Upon closer observation of caged ♀♀ of *nufrellensis* and of *coridon* or *gennargenti* the following differences were identified:

- *P. coridon* + *P. gennargenti* ♀♀: several specimens in a storage vessel hardly interfere with each other in oviposition.
- *P. nufrellensis* ♀♀: several specimens in a storage vessel interfere intensively during their egg-laying behaviour. The ♀♀ behave in a restless manner, flutter around and waste a lot of energy. The butterflies die quickly, if they do not “automatically” come into contact several times a day with a food source in order to be able to take nectar.
- *P. coridon* + *P. gennargenti* ♀♀ fly towards the top when in contact with other ♀♀ and almost never fall down.
- *P. nufrellensis* ♀♀ when they come in contact fall to the bottom of the storage jars, where they end up under the cellulose tissue and easily perish.

The *nufrellensis* ♀♀ only laid a third or even fourth of the number of eggs laid by *coridon* or *gennargenti* ♀♀. To obtain an optimal number of eggs therefore solitary confinement of the ♀♀ is essential.

One method of YD to place the ♀♀ of *nufrellensis* in shallow plastic receptacles for oviposition proved to be very successful. The animals were thus permanently in contact with the host plants as well as the food source, and consequently the number of eggs obtained this way was significantly greater than that with the method used by KGS and AW.

Four caged ♀♀ of AW laid a total of 155 eggs, while three ♀♀ of YD laid 200 eggs. These were mostly found on the plant parts, but also on the glass (plastic walls with

YD) and the vessel gauze. For better preservation and observation the eggs were moistened by AW, carefully removed and placed in the small rim glasses.

Synchronization and recording of events for the breeding

To carry out the planned hybridisations, breeding of *gennargenti* and *nufrellensis* had to be exactly synchronized. In order to achieve this, hatching time of the larvae had to be manipulated because under field conditions *gennargenti* have a prolonged hatching period from late September until the end of October (D. JUTZELER in litt. 23. v. 2001). This behaviour is known from almost all univoltine taxa of the *Lysandra* group and so far it was not known how to prevent it (DANIELS 2004).

The following method to accelerate hatching of *gennargenti* proved successful for almost all the eggs, hatching after 14–21 days:

Each test glass of 30 ml with about 20–30 eggs was kept under long-day conditions (17 h light, 7 h darkness) and at temperatures of 25–33 °C at daytime and 25–26 °C at night. After 9. VIII. 2007 temperature for *gennargenti* was increased (see below).

Larvae of *nufrellensis* behave differently. They hatch spontaneously (SCHURIAN et al. 2006), which is an important distinction from the other representatives of the subgenus *Lysandra*, a behaviour which usually only occurs in bivoltine species of the subgenus.

After about 6–9 days the eggs changed to grey which showed that a larva had developed. Now a branch of fresh foodplant was added, which lead to spontaneous hatching of some of the caterpillars. These were placed in a separate test glass to allow them to feed. It is essential for the food to be removed after approximately 24 h, otherwise mildew occurs and no further caterpillars will hatch. The process of adding food was repeated after 2 days, during which the eggs were kept under dry conditions, until all the fertile eggs had hatched.

With YD the first larvae of *nufrellensis* hatched on 6. VIII. 2007, with AW and KGS on 7. VIII. 2007.

While in *nufrellensis* 106 larvae hatched until 9. VIII. 2007 (43 on 7. VIII., 60 on 8. VIII., 3 on 9. VIII. 2007), in *gennargenti* only two hatched up to this point. The conditions in *gennargenti* were then modified so that the daytime temperature was raised to 30–33 °C, and the nighttime temperature to 30 °C. Subsequently until 19. VIII. a total of 238 larvae of *gennargenti* had hatched.

The small larvae of the two forms were initially reared in the 30 ml test glasses, with 10–15 caterpillars in each. Fine holes were cut into the lids for ventilation. To prevent infections, the test glass has to be cleaned daily and fresh food (*Hippocrepis comosa*) supplied.

Method of YD: During L₁–L₂, the larvae were reared in very small round plastic boxes (1 cm diameter; the boxes

were the ones in which eggs were placed for hatching). During these first stages, 20–30 larvae were reared together.

In L_3 – L_4 , the larvae were put in rectangular (5 cm × 3 cm × 1 cm) or round (5/6 cm diameter) plastic boxes. Like in the first instars, 20–30 larvae were kept in one box.

In L_5 , the larvae were placed in even larger plastic boxes (15 cm × 15 cm × 8 cm) and a maximum of 40 larvae were reared together.

At each stage food was changed daily in the morning, and in L_4 – L_5 even twice a day in order to avoid cannibalism. Sometimes it is necessary to change the food three times a day in L_5 in order to have big and healthy pupae. The boxes have to be washed daily to prevent mould and virus diseases.

For removing the young larvae from old food, a fine brush, like one for watercolour painting, was used. Those brushes are very soft and flexible and don't cause any damage to larvae. With this brush, larvae could be removed without any problems. Obviously, in L_3 – L_5 it is possible to remove the larvae with a bigger brush or even by hands or with a flexible pair of tweezers.

When larvae stopped feeding and were ready to pupate, they were transferred to another plastic box in which we put sand and some absorbent paper.

Despite careful nurturing of *nufrellensis* larvae we lost a total of 37 caterpillars by 1. ix. 2007 due to cannibalism or other unknown causes, although the small caterpillars had been dispersed into an ever increasing number of test glasses. Apparently with AW too many caterpillars were in one vessel, since with YD – with a different method, see above – no cannibalism occurred. The richness of the diet seemed to play a role, too, since when food is available in large quantities less cannibalism has been observed in tube breeding.

Food choice experiments using alternative Fabaceae species were carried out by AW with *P. (L.) coridon nufrellensis* using a larger Petri dish with 20 caterpillars. Apart from *Securigera* (= *Coronilla*) *varia* (L.) LASSEN and *Hippocrepis comosa*, the following additional potential foodplants were tested: *Colutea arborescens* L., *Genista tinctoria* L., *Medicago sativa* L., *Onobrychis viciifolia* SCOP., *Vicia cracca* L., *Astragalus glycyphyllos* L. and *Lotus pedunculatus* CUV. None of the plants offered in the food choice experiments was accepted, apart from *Securigera varia* and *Hippocrepis comosa*, which are the only regular foodplants of *P. coridon* on the European mainland.

Even with *gennargenti* cannibalism and susceptibility to diseases were observed so that here, too, the number of breeding glasses had to be increased.

During growth the larvae were distributed to bigger glasses, especially those already used for oviposition (1-litre jars) in which now pupation took place.

Some of the larvae in L_3 and L_4 were fed with *Securigera varia*. This plant has the advantage over *Hippocrepis comosa* that it provides more leaf material, and with such a large number of larvae this may be important for successful breeding. In addition, the cultivation of this plant is much easier, since it is not dependent on calcareous soil. However, we found that the caterpillars of *nufrellensis* preferred *Hippocrepis comosa* over *Securigera varia* and developed better on the former plant. Therefore, the vast majority of the larvae were fed exclusively with *H. comosa* while YD also used *Hippocrepis glauca* (TEN.) BONNIER & LAYENS, which was also well accepted.

The larval period of *gennargenti* and *nufrellensis* lasted almost exactly 4 weeks under long-day conditions.

Results and discussion

Morphology of larvae

The larvae of the *Lysandra* group differ only slightly among different species. However, they exhibit considerable variation among individuals and populations.

The larvae of the parental taxa *nufrellensis* and *gennargenti* were already illustrated in SCHURIAN et al. (2006: 186–187).

Rearing results

KGS and AW attained 55 pupae of *nufrellensis* and 142 of *gennargenti*. YD received about 200 pupae of *nufrellensis* of which he made available 25 pupae to KGS for his planned crossbreeding experiments (these are not listed below).

26 ♂♂ and 22 ♀♀ of *nufrellensis* emerged (3 pupae did not hatch) and 63 ♂♂ and 52 ♀♀ of *gennargenti* (from 22 pupae no butterflies emerged). An additional number of pupae delivered crippled butterflies: 4 specimens of *nufrellensis* and 5 of *gennargenti*.

The first adult *nufrellensis* emerged on 23. ix. 2007. It was with these that the first author initially tried intraspecific copulae (*nufrellensis* × *nufrellensis*). Despite the availability of sufficient adult butterflies of both sexes and good weather conditions, these tests remained unsuccessful because the ♂♂ showed no mating behavior. This is in line with the experience from the years 2003 (with attempts by KGS with *nufrellensis* material from M. GASCOIGNE-PEES, Stones Field, England) and 2005 (KSG collecting and breeding). Again, despite intensive attempts the mating of the butterflies failed. We can only speculate about the reasons. The failure could be due to the unsuitability of the larval host plant or of the food for the butterflies (BECK 2007), although the larvae of *gennargenti* were fed with the same plants and the butterfly sugar solutions were given in the same concentration. Moreover, it is conceivable that the extremely condensed time of development has an influence on the fertility of *nufrellensis*, but here the conditions were also identical with those used for *gennargenti*.

Beginning on 22. ix. 2007, adult *gennargenti* emerged and during a period of fine weather the crossing attempts started (Tab. 1, Fig. 10).

To reduce the risk of loss during egg-laying and breeding of the subsequent F₁ generation, the paired ♀♀ were again shared between AW and KGS.

Oviposition

Due to time constraints this could only be accurately recorded for a few ovipositions (Text-Fig. A).

The breeding of the F₁ generation

From 155 eggs (Fig. 12) of the pairing *gennargenti* ♂ × *nufrellensis* ♀, we received a total of 122 larvae. The hatching of these caterpillars under the above conditions occurred spontaneously within 5 days (7. x.-11. x. 2007), which corresponds almost exactly to our experience with the ♀ parents (*nufrellensis*).

Under laboratory breeding conditions only 3 larval moults were observed:

1st moult	10.-11. x. 2007
2nd moult	14.-15. x. 2007
3rd moult	17.-19. x. 2007

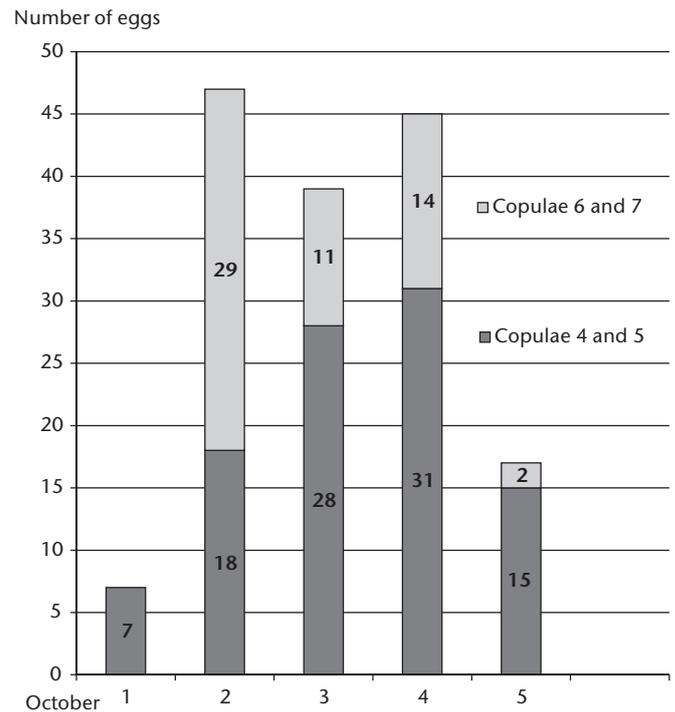
Through cannibalism primarily among the L₁/L₂ caterpillars, as well as infections, the number of caterpillars with AW and KGS diminished continuously; about 28% of them died before pupation (Text-Fig. B).

Appearance of the hybrid larvae in comparison with those of the parental taxa *nufrellensis* and *gennargenti*

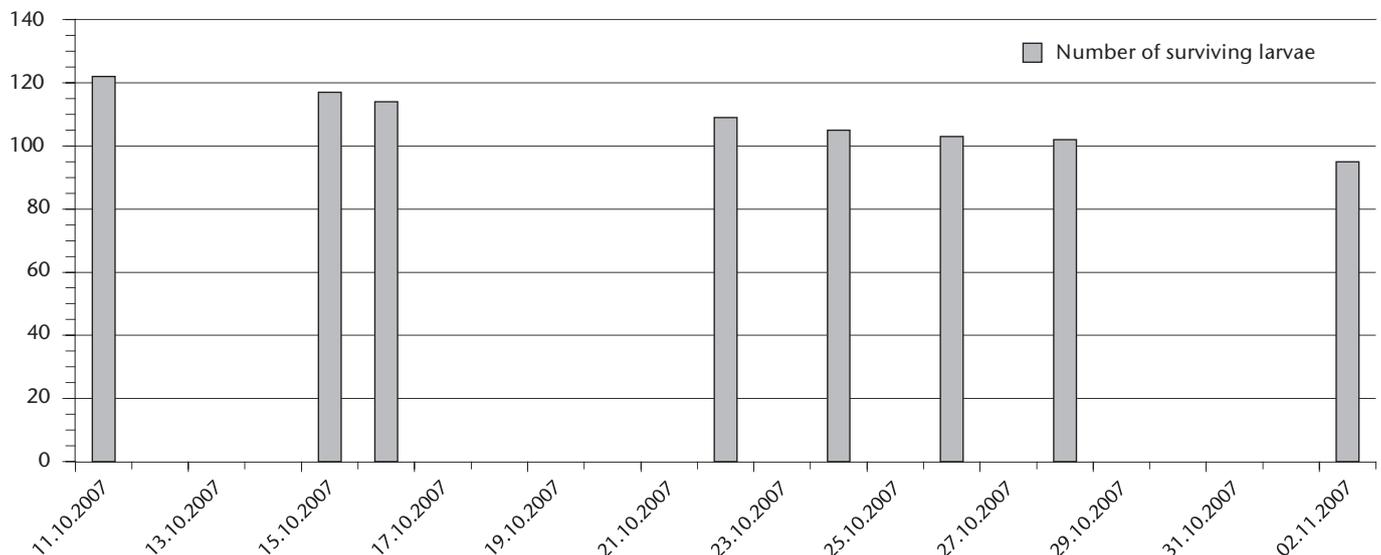
The larva of the adult F₁-hybrids (Figs. 13-14) is neither similar to that of *nufrellensis* nor to the larva of *gennargenti*. Instead, it is most similar to the caterpillar of the nominotypical continental *coridon* (compare SBN 1987, WEIDEMANN 1995, SCHURIAN et al. 2006). For the com-

Tab. 1: Cross pairing copulas for F₁ hybrids between *gennargenti* and *nufrellensis*, pairing supported by the semiartificial method.

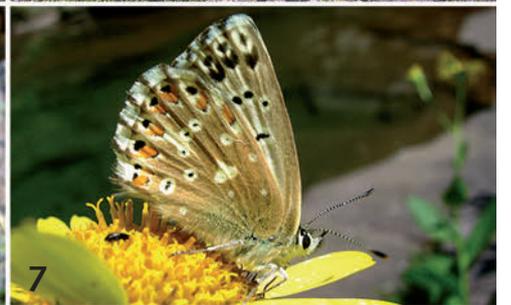
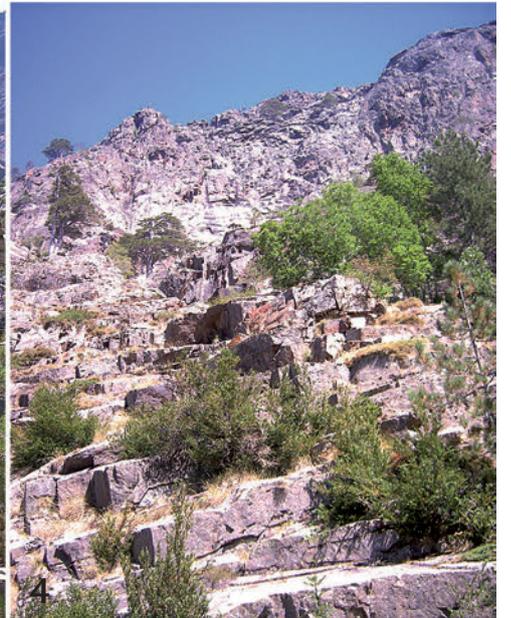
No.	Date (2007)	Cross pairing copula	Time	Duration [min.]
1	26. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	14:05-15:15	70
2	26. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	14:49-16:52	123
3	26. ix.	<i>nufrellensis</i> ♂ × <i>gennargenti</i> ♀	15:52-16:40	48
4	30. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	12:55-15:05	130
5	30. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	15:07-16:15	68
6	30. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	15:14-16:30	76
7	30. ix.	<i>gennargenti</i> ♂ × <i>nufrellensis</i> ♀	15:24-16:15	51
Average duration of the pairings				81



Text-Fig. A: Oviposition of *P. (L.) coridon nufrellensis* ♀♀ which had been mated with ♂♂ of *P. (L.) coridon gennargenti* on 30. ix. 2007. (Total number of eggs received: 155.)



Text-Fig. B: Mortality of F₁ hybrid larvae of *P. (L.) coridon gennargenti* (♂) × *P. (L.) coridon nufrellensis* (♀).





Figs. 1–2: Biotopes of *Polyommatus (Lysandra) coridon gennargenti* on Sardinia. Fig. 1: Perda e Liana, 23. vii. 2007. Fig. 2: Monte Novo San Giovanni, 24. vii. 2007. — Figs. 3–4: Biotopes of *P. c. nufrellensis* on Corsica, western side of Muvrella, 28. vii. 2007. — Fig. 5: Ovipositing ♀ of *P. c. gennargenti* at Monte Novo San Giovanni, 24. vii. 2007. — Figs. 6–9: Specimens of *P. c. nufrellensis* at Muvrella, most on 31. viii. 2008. Fig. 6: ♀, Fig. 7: ♀, Fig. 8: ♂. Fig. 9: Aberrant ♀ of *P. c. nufrellensis*, on the finger-tip of one of the authors, 1. viii. 2007. — Fig. 10: Pairing of *P. c. gennargenti* ♂ × *P. c. nufrellensis* ♀ on 30. ix. 2007. — Fig. 11: Different glasses and vials for egg-laying and rearing of caterpillars. Left: 1 l glass jar; middle: honey glass; right: 30 ml test vial. — Figs. 12–16: Preimaginal instars of F₁-hybrid *P. c. gennargenti* × *P. c. nufrellensis*. Fig. 12: Eggs, 2. x. 2007. Fig. 13: Adult larvae, 7. xi. 2007. Fig. 14: Adult and younger larvae, 7. xi. 2007. Fig. 15: Fresh pupae, 15. xi. 2007. Fig. 16: Moulting of F₁-hybrid larvae to pupa, 9. xi. 2007. — Figs. 17–20: Freshly hatched specimens (with emergence dates) of F₁-hybrid *P. c. gennargenti* ♂ × *P. c. nufrellensis*. Fig. 17: ♂, 15. xi. 2007. Fig. 18: ♂, 15. xi. 2007. Fig. 19: ♀, 21. xi. 2007. Fig. 20: ♀, 21. xi. 2007. — Figs. 21–24: Set specimens of F₁-hybrid *P. c. gennargenti* ♂ × *P. c. nufrellensis*. Figs. 21–22: ♂, 22. xi. 2007; ups./uns. Figs. 23–24: ♀, 24. xi. 2007; ups./uns.

parison with the latter work, SCHURIAN et al. (2006: fig. 4 on plate 2) should be used, but not fig. C1 (on plate 3 of SCHURIAN et al. 2006). These photos, apart from fig. 11 all by D. JUTZELER, show the different stages of the larvae of *coridon*, *nufrellensis* and *gennargenti* in very good quality. Unfortunately, our colleague D. JUTZELER illustrates an unusual colour form of the larva of *coridon* in fig. C1 (cf. SBN 1987: 396, WEIDEMANN 1995: 268), and, therefore, this figure is not suitable for comparative purposes.

Pupation

In early November (3. XI.) the first larvae pupated: We received a total of 65 pupae of the F_1 generation (Figs. 15–16).

Hatching of the butterflies of the F_1 generation

The emergence of the F_1 generation began in mid-November with a ♂ (15. XI. 2007, Figs. 17–18). Until 24. XI. we received a total of 17 ♂♂ and 4 ♀♀. Because of a holiday trip of the first author the remaining pupae were kept cool by AW (to delay the butterflies' hatching and so to spare them for further hybrid crossings later), and until early December, we received an additional ♂ and 18 ♀♀ of the F_1 generation (Figs. 17–20). 23 pupae did not emerge, probably due to infection during the larval stage, and 2 butterflies were crippled.

Description of the F_1 generation imagines

♂, **upperside:** The wing upperside of most ♂♂ is intermediate between the parental taxa (Figs. 17, 21). The ground colour is a shimmering turquoise. On the hindwings the submarginal spots are slightly smaller than in *nufrellensis* and are thus similar to those of the ♂ parent. The checkering of the fringes, in *gennargenti* either absent or hardly noticeable, is quite similar to that of *nufrellensis*.

♂, **underside:** The underside is similar to that of the ♀ parent. The basic colour of the forewing is a whitish grey, while the hindwings are a bright greyish brown (Figs. 18, 22). The shapes of the ocelli are similar to those of *nufrellensis*. In *gennargenti* the ocelli often have almost disappeared or have a strongly reduced white ring, unlike any of our hybrids. While both sexes of *nufrellensis* have a wedge-shaped white streak at vein 3, this feature is missing completely in *gennargenti*, and in the hybrid butterflies it is only weakly visible, but always present. The hood-shaped submarginal spots have an intermediate form compared to the parents. While *nufrellensis* has clearly marked orange caps on the edge of the hindwing spots, those in the hybrids are small and not elongated; in *gennargenti* this feature is missing or hardly visible.

♀, **upperside:** The basic colour (turquoise green) is also present in the ♀ and intermediate to the parents (Figs. 19, 23). The ♀♀, however, have a peculiar character in the region of the costal vein and along the main veins

of the forewing where there are dark brown scales. The resulting clearer prominent veins tend to remind of *gennargenti*. The black spots along the margin of the hindwing are intermediate compared to the parents.

♀, **underside:** Here the overall impression is that the butterflies resemble *nufrellensis* rather than *gennargenti* (Figs. 20, 24). The forewing base colour is whitish-grey, but darker than the ♂♂, with a brownish tone, the hindwing a light coffee brown, darker than *nufrellensis* and *gennargenti*. The ocelli are more similar to *nufrellensis*, those of the hindwings do not possess such a strong white ring as in *gennargenti*. The white streak at vein 3 is only weakly developed but still present, and the orange caps on the marginal spots are always clearly visible and rounded.

Conclusions

The above results show that *gennargenti* and *nufrellensis* are closely related taxa. The experimental crossing of the two taxa did not provide any evidence for barriers of reproductive isolation. The ♂ of *gennargenti* could copulate with the fixed ♀ of *nufrellensis* without difficulties. The duration of the copulation of 48–123 min. (average: 81 min.) corresponds with that of the parents (*gennargenti* and *nufrellensis*) and was also observed in other *Lysandra* taxa (SCHURIAN 1989a). The number of eggs laid by the 6 paired ♀♀ with an average of 38.7 eggs per ♀ was very small. One could assume that this is partly due to methodological shortcomings, such as artificial light or the diet of the butterflies. But we have now evidence that under these laboratory conditions massive egg-laying may occur, as a ♀ hybrid *coridon* × *nufrellensis* (F_1 × F_1) laid a total of 433 eggs within 17 days (in January 2009).

The number of larvae obtained from the eggs (78.7% of the eggs proved to be fertile) seems to us a relatively good result, but we have no exact figures from other *Lysandra* taxa, although we know from previous breeding attempts in the *Lysandra* group that a certain proportion of the eggs usually does not hatch. During the larval time only 3 moults could be observed, as was the case in the parents' generation. Therefore the extreme developmental acceleration for breeding purposes apparently lead to the skip of one moult, something which has also been observed in other Lycaenidae taxa (FISCHER & FIEDLER 2001).

The results presented here are in line with the low level of genetic differentiation as indicated by the molecular data (MARCHI et al. 1996, WIEMERS 2003 and unpublished data), leading to the assumption that the two isolated insular lycaenid taxa *nufrellensis* and *gennargenti* are probably not differentiated to the level of species rank. Further crossing experiments (including F_2 hybrid generations) should be carried out to verify whether postzygotic isolating barriers are absent between these two taxa.

Acknowledgements

The authors would like to thank a number of colleagues. Without their help parts I and II of this work could not have been carried out. Paolo CASULA (Cagliari) for precise indications on the presence of *gennargenti* and technical discussions, Wolfram EHRHARDT (Queloh) for his help in the preparation of graphs in Part I of the work, David JUTZELER (Effretikon) for his cooperation in Part I and fine colour slides, to the late Ronny LEESTMANS (Beersel), unfortunately deceased far too early, for his tireless efforts at the production of Part I, J. GAMISANS (Toulouse) for his information on the distribution of *H. comosa* in Corsica. Konrad FIEDLER (Vienna) for professional advice to both Parts I and Part II. Clwyd OWEN (Hermannsburg) for some help to improve the English text of an early version. Andrea GRILL for the Italian summary.

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Received: 23. VIII. 2010, 26. IX. 2010

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Nachrichten des Entomologischen Vereins Apollo](#)

Jahr/Year: 2010

Band/Volume: [31](#)

Autor(en)/Author(s): Schurian Klaus G., Westenberger Alfred, Diringer Yvan, Wiemers Martin

Artikel/Article: [Contribution to the biology, ecology and taxonomy of *Polyommatus \(Lysandra\) coridon nufrellensis* \(Schurian, 1977\) \(Lepidoptera: Lycaenidae\), Part II1: An experimental hybridisation of *P. \(L.\) c. gennargenti* × *P. \(L.\) c. nufrellensis* 177-186](#)