

Contribution to the biology, ecology and taxonomy of *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Part III¹: An experimental hybridisation of *P. (L.) c. coridon* × *P. (L.) c. nufrellensis*

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Abstract: In order to clarify the species status of the Corsican endemic *Polyommatus (Lysandra) coridon nufrellensis* we carried out experimental hybridisations with the nominotypical subspecies from Germany, because these can be informative about the existence of reproductive isolating mechanisms. Additionally, molecular analyses were done to resolve the degree of differentiation between both taxa. Six *nufrellensis* females could be mated to males of the nominotypical subspecies with the semi-artificial method. These produced males and females of the F₁ hybrid generation. One mating could be achieved amongst these hybrid butterflies. This mated female laid a very large number of eggs (433), most of which turned out to be fertile and produced 24 males and 19 females of the F₂ generation. Adults of both the F₁ and the F₂ generation had characters of both parental taxa. An analysis of DNA sequences from the mitochondrial (COI) as well as the nuclear genome (ITS2) with statistical parsimony networks did not provide evidence for genetic differentiation of *nufrellensis* from the nominotypical subspecies. Our results point to a young and incomplete speciation process, and therefore we suggest keeping the current taxonomic treatment of *nufrellensis* as a subspecies of *P. coridon*. As an addendum to part II of our series on the *coridon* populations of the islands of Sardinia and Corsica we report the successful breeding of the F₁ hybrid generation of *P. c. gennargenti* × *P. c. nufrellensis* to the F₂ generation.

Beitrag zur Biologie, Ökologie und Taxonomie von *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Teil III¹: Experimentelle Hybridisierung von *P. (L.) c. coridon* × *P. (L.) c. nufrellensis*

Zusammenfassung: Um den strittigen Artstatus des endemischen Taxons *Polyommatus (Lysandra) coridon nufrellensis* aus Korsika zu klären, wurden zum einen experimentelle Hybridisierungen mit der nominotypischen Unterart aus Deutschland durchgeführt, die Aufschlüsse über das Vorhandensein reproduktiver Isolationsmechanismen geben können, zum anderen molekulare Untersuchungen zur Klärung ihres Differenzierungsgrads. Bei sechs *nufrellensis*-Weibchen gelang die semiartifizielle Paarung mit Männchen der Nominatunterart, wobei Männchen und Weibchen der F₁-Hybridgeneration erzielt wurden. Mit diesen Faltern konnte eine Paarung erzielt werden, bei der das Weibchen eine sehr große Anzahl Eier (433 Stück) ablegte, von denen sich die meisten als fertil erwiesen und 24 Männchen sowie 19 Weibchen einer F₂-Generation ergaben. Sowohl die Falter der F₁- als auch diejenigen der F₂-Hybridgeneration zeigten habituelle Eigentümlichkeiten beider Elterntaxa. Eine Analyse von DNA-Sequenzen aus dem mitochondrialen (COI) und dem Kerngenom (ITS2) mit Hilfe von Maximum-Parsimonie-Netzwerken zeigte keine Differenzierung zwischen beiden Taxa. Die Ergebnisse weisen auf einen sehr jungen und noch unvollständigen Speziationsprozeß hin, weshalb wir dafür plädieren, den subspezifischen

Status von *P. coridon nufrellensis* beizubehalten. Als Nachtrag zu Teil II der Serie über die *coridon*-Populationen auf Sardinien und Korsika berichten wir, daß mit Faltern der F₁-Hybridgeneration von *P. c. gennargenti* × *P. c. nufrellensis* erfolgreich eine F₂-Generation gezüchtet werden konnte.

Introduction

Polyommatus (Lysandra) coridon (PODA, 1761) is a widespread and often common blue butterfly in most of Europe up to 55° N (KUDRNA et al. 2011), but was unknown from the Mediterranean islands until 1975, when local populations were discovered in mountains on Corsica (ssp. *nufrellensis* SCHURIAN, 1977) and Sardinia (ssp. *gennargenti* LEIGHEB, 1987). The latter taxon was later raised to species status by JUTZELER et al. (2003), but this decision was not universally accepted (e.g. KUDRNA et al. 2011). Crossing experiments can be helpful to clarify the species status of questionable taxa by testing for reproductive isolation mechanisms.

Experiments which were carried out between both island taxa did not provide evidence for post-zygotic isolation mechanisms, because an F₁ generation could successfully be produced (SCHURIAN et al. 2011) as well as an F₂ (Addendum to Part II; this paper).

However, a recent study by TALAVERA et al. (2013) suggested that *P. c. nufrellensis* might be more closely related to mainland populations of *P. coridon* than to *P. c. gennargenti* from the neighbouring island of Sardinia. Therefore we carried out experimental hybridisation experiments between *P. c. nufrellensis* and a population of the nominotypical subspecies from Germany. Additionally, we re-evaluated the molecular evidence for the phylogenetic position of both island taxa by analyzing a highly enlarged set of *Lysandra* DNA sequences using Statistical Parsimony Networks, a method which is more appropriate than tree construction methods for populations of closely related taxa with a high amount of gene flow and hybridisation as is the case in the *P. coridon* complex (POSADA & CRANDALL 2001).

Material and methods

Breeding experiments

The parental material to produce F₁ hybrids of *P. (L.) coridon coridon* with *P. (L.) coridon nufrellensis* was obtained in the following way: ♀♀ of *P. (L.) coridon nufrel-*

¹ Part I: see SCHURIAN et al. (2006); Part II: see SCHURIAN et al. (2011).

Tab. 1: New material of *Lysandra* used for molecular analysis.

Voucher	Taxon	Sex	Country	Province	Location	Altitude	Date	COI acc. no.	ITS2 acc. no.
MW09030	<i>P. bellargus</i>	M	Romania	Alba	Colțești, Cetatea Colțești	600 m	31. v. 2009	KM972614	
MW09058	<i>P. bellargus</i>	M	Greece	Macedonia	Mt. Falakro, Falakro Ski Resort	1700 m	19. vii. 2009	KM972616	
MW09123	<i>P. bellargus</i>	M	Austria	Wien	Weidlingau, Wiental	200 m	26. ix. 2009	KM972619	
MW98303	<i>P. bellargus</i>	M	Turkey	Konya	Taşkent	1450 m	5. viii. 1998	KM972623	KM972638
MW99039	<i>P. bellargus</i>	M	Turkey	Erzincan	5 km E Çağlayan	1500 m	6. vii. 1999	KM972624	KM972639
MW99076	<i>P. bellargus</i>	M	Turkey	Artvin	Kiliçkaya, Yusufeli	1350 m	8. vii. 1999	KM972601	KM972640
MW99446	<i>P. bellargus</i>	M	Turkey	Hakkari	Dez valley, NE Hakkari	1500–1700 m	23. vii. 1999	KM972626	GQ166183
MT05039	<i>P. coridon caelestissimus</i>	M	Spain	Teruel	El Valecillo	1400 m	16. vii. 2005	KM972608	
MT06075	<i>P. coridon caelestissimus</i>	M	Spain	Teruel	El Valecillo	1400 m	16. vii. 2005	KM972610	
MT04010	<i>P. coridon coridon</i>	M	Italy	Macerata	Marche, Bolognola, Monti Sibillini	1200 m	21. viii. 2004	KM972606	
MT04011	<i>P. coridon coridon</i>	M	Italy	Macerata	Marche, Bolognola, Monti Sibillini	1200 m	21. viii. 2004	KM972607	
MW09050	<i>P. coridon coridon</i>	M	Greece	Macedonia	Mt. Olympus	1040 m	17. vii. 2009	KM972615	
MW09099	<i>P. coridon coridon</i>	M	Austria	Wien	Wien-Donaustadt, Donauinsel, KW Freudenau	150 m	27. vii. 2009	KM972617	
MW09115	<i>P. coridon coridon</i>	M	Austria	Nieder-österreich	Grossmittel, Steinfeld	250 m	23. viii. 2009	KM972618	
KS05002	<i>P. coridon gennargenti</i>		Italy	Sardinia	Monte Novo S. Giovanni, 15 km S Orgosolo	1250 m	2. viii. 2005	KM972602	KM972633
OK08001	<i>P. coridon gennargenti</i>		Italy	Sardinia	Orgosolo, M. Fumai, Nuoro	1100–1200 m	11. viii. 2008	KM972632	KM972644
KS05051	<i>P. coridon nufrellensis</i>	M	France	Corse	Calvi, Cirque de Bonifatu (Spasimata)	1250 m	26. vii. 2005	KM972603	KM972634
MW99043	<i>P. corydonius</i>	M	Turkey	Erzurum	Köşkköy, 25 km N Erzurum	1900 m	7. vii. 1999	KM972625	
MW99140	<i>P. corydonius</i>	M	Turkey	Iğdır	Badilli, Tuzluca	1800–2000 m	12. vii. 1999	(AY557055)	KM972641
MW99518	<i>P. corydonius</i>	M	Turkey	Iğdır	Gaziler, 10 km N Gaziler	1800 m	27. vii. 1999	KM972627	KM972642
MW99519	<i>P. corydonius</i>	M	Turkey	Iğdır	Gaziler, 10 km N Gaziler	1800 m	27. vii. 1999	KM972628	
MW99539	<i>P. corydonius</i>	M	Turkey	Erzurum	Köşkköy, 25 km N Erzurum	1900 m	28. vii. 1999	KM972629	KM972643
MW99540	<i>P. corydonius</i>	M	Turkey	Erzurum	Köşkköy, 25 km N Erzurum	1900 m	28. vii. 1999	KM972630	
MW99541	<i>P. corydonius</i>	M	Turkey	Erzurum	Köşkköy, 25 km N Erzurum	1900 m	28. vii. 1999	KM972631	
KS09003	<i>P. dezinus</i>	M	Turkey	Hakkari	Kirikdağ, Dez valley	2100–2200 m	12. vii. 2009	KM972604	
MT04008	<i>P. hispanus</i>	M	Italy	Liguria	Lac. Marano, Sarzana (SP)	200–300 m	25. viii. 2004	KM972605	
MT06068	<i>P. hispanus</i>	M	Spain	Barcelona	Mura, Bages	500 m	16. v. 2006	KM972609	KM972635
MT07037	<i>P. hispanus</i>	M	Italy	Parma	Varano de'Melegari, Emilia Romagna		5. vi. 2007	KM972611	
MT07038	<i>P. hispanus</i>	M	Italy	Parma	Varano de'Melegari, Emilia Romagna		5. vi. 2007	KM972612	
MW98130	<i>P. ossmar</i>	M	Turkey	Isparta	Dedegöl Geçidi, W Kurucuova	1700 m	21. vii. 1998	KM972620	KM972636
MW98155	<i>P. ossmar</i>	M	Turkey	Nevşehir	Zelve, N Ürgüp	1100 m	22. vii. 1998	KM972621	GQ166181
MW98187	<i>P. ossmar</i>	M	Turkey	Sivas	Gökpinar, Gürün	1700 m	25. vii. 1998	KM972622	KM972637
MT08007	<i>P. punctifera</i>	M	Morocco		Col du Zad, S Tamahdite, Moyen Atlas Central	1800–2250 m	1. v. 2008	KM972613	

lensis were collected on Corsica (France) in July and August 2008 and brought to oviposition (see SCHURIAN et al. 2006, 2011 for methods). ♀♀ of *P. (L.) coridon* were collected on 16. viii. 2008 at the “Mainzer Sand” (Rhineland-Palatinate, Germany). The offspring was raised separately and adults for cross-breeding experiments were obtained in October 2008. Flightcages were transferred to the High Taunus mountains due to better weather conditions (caused by an inversion with fog in the lowlands and sunny weather at higher altitudes). Matings were obtained with the semi-artificial method (SCHURIAN 1990) and the mated ♀♀ were used for further oviposition and breeding.

The adult butterflies resulting from these breedings were used for mating experiments to produce an F₂ generation in December 2008 to beginning of January 2009. Due to the suboptimal weather conditions during this time period with little sunshine, short day-length and low temperatures, artificial lights were used to improve the light regime and increase the temperature.

For egg-laying, a method developed by our colleague Yvan DIRINGER (Paris) was used: The ♀ was placed in a

plastic box of 5 cm height and 15 × 15 cm dimension. The bottom was covered with a layer of absorbent paper. Two wetted glasses each contained a small twig of both dry and fresh *Hippocrepis comosa* (Horseshoe Vetch, Fabaceae). A 50 Watts halogen light was used as additional light source during daytime, which produced a temperature of about 30°C during daytime, while nighttime temperature was about 24°C. Adults were fed with a sucrose solution with an additive mix of proteins, vitamins and minerals (“High Protein 90” from isostar®).

Fig. 1: Wild-caught ♂ of *Polyommatus (Lysandra) coridon nufrellensis*, Corsica, Calvi, vic. Cirque de Bonifatu (Spasimata), 1250 m NN, 26. vii. & 5. viii. 2005, leg. SCHURIAN, ups./uns. **Fig. 2:** Wild-caught ♀ of *P. (L.) coridon nufrellensis*, Corsica, Muvrella, western slope, 1400 m NN, 3. viii. 2008, leg. SCHURIAN. **Fig. 3:** ♂ F₁ hybrid *coridon* × *nufrellensis*, ex ovo, xii. 2008, cult. SCHURIAN. **Fig. 4:** ♀ F₁ hybrid *coridon* × *nufrellensis*, ex ovo, xii. 2008, cult. SCHURIAN. **Fig. 5:** ♂ F₂ hybrid *coridon* × *nufrellensis*, ex ovo, v.–vi. 2009, cult. SCHURIAN. **Fig. 6:** ♀ F₂ hybrid *coridon* × *nufrellensis*, ex ovo, v.–vi. 2009, cult. SCHURIAN; **6a:** brown morph; **6b:** blue morph; **6c:** partly blue morph. **Fig. 7:** ♀ *P. (L.) coridon manleyi* (DE LESSÉ, 1962), Spain, Sierra de la Peña, 800m NN, ex ovo 20. x. 1975, cult. SCHURIAN. **Fig. 8:** F₂ adult larva. **Fig. 9:** F₂ pupa, freshly pupated. **Fig. 10:** ♂ F₂ freshly emerged. **Fig. 11:** ♀ F₂ freshly emerged. **Fig. 12:** Hybrid *P. (L.) gennargenti* × *P. (L.) nufrellensis* F₂ ex ovo, 10. ii. 2008, cult. SCHURIAN.



Molecular analysis

For the molecular analysis of the mitochondrial gene “cytochrome-*c* oxidase I” (COI) and the nuclear marker “internal transcribed spacer” (ITS2) available sequences from GenBank and BOLD were combined with newly sequenced material of *P. c. nufrellensis* and 9 other *Lysandra* taxa (see Tab. 1). The material includes sequences published by WIEMERS (2003), KANDUL et al. (2004), WIEMERS & FIEDLER (2007), VODOLAZHISKY & STRADOMSKY (2008), WIEMERS et al. (2009), LUKHTANOV et al. (2009), DINCA et al. (2011), HAUSMANN et al. (2011), VILA et al. (2011), TALAVERA et al. (2012), SANUDO-RESTREPO et al. (2013) and TALAVERA et al. (2013).

Methods for DNA extraction, PCR, sequencing, and alignment were described in WIEMERS (2003). Statistical parsimony network analyses were performed with TCS 1.21 (CLEMENT et al. 2000). For the COI analysis, all COI barcodes with a length of less than 470 bp in the COI barcode section were discarded from the analysis. For ITS2, two analyses were performed, the first one counting gaps as missing character information and using all sequences with a minimum length of 430 bp, and a second one counting gaps as 5th character, retaining all sequences with a minimum length of 505 bp. In the latter analysis, 28 bp at the 3'-end of the ITS2 alignment were not included into the analysis, because these were missing in some sequences.

Results

Crossing of *P. (L.) c. coridon* × *P. (L.) c. nufrellensis*

The cross-mating experiments between *P. (L.) c. coridon* and *P. (L.) c. nufrellensis* resulted in the following 6 successful copulae (Tab. 2).

Tab. 2: Pairings in cross-mating experiments between *P. (L.) c. coridon* and *P. (L.) c. nufrellensis* 2008.

No.	Date	Mating	Time of day	Duration [min]
1	14. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	13:33–14:51	78
2	14. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	13:37–15:??	?
3	16. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	16:34–19:46	192
4	17. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	14:47–16:58	131
5	19. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	14:45–15:21	36
6	20. x.	<i>coridon</i> ♂ × <i>nufrellensis</i> ♀	12:33–13:31	58

The females no. 2 and 5 only laid few eggs and no larvae hatched of these. The other ♀♀ also laid only a small number of eggs, which turned out to be only partly fertile. Due to problems with cannibalism and diseases, the number was further reduced and only few adult butterflies of the F₁ generation hatched: 6 ♂♂ and 5 ♀♀ with the first author from 20. XII. to 27. XII. 2008 as well as 1 ♂ and 1 ♀ with Alfred WESTENBERGER.

Morphology of the F₁ generation

Males

(Fig. 1.)

All ♂♂ have a greenish colouration on the upperside and are therefore more similar to their ♂ parent *P. c. nufrellensis*. The colouration of the underside varies and is either more similar to the ♂ or ♀ parent. With a forewing length of 13.3–14.7 mm, the ♂♂ were slightly smaller than their ♂ parent.

Females

(Fig. 2.)

All ♀♀ have a brown upperside and are thus similar to their ♀ parent from “Mainzer Sand”. Only 2 of the 5 ♀♀ can be used for a more detailed comparison, because 1 ♀ is unusually small with aberrant wing markings on the underside and two others were strongly damaged during mating and egg-laying experiments. The two well-preserved ♀♀ are identical on the upperside with the ♀ parent (*P. c. coridon*), including the brown colouration and the basal markings in the submarginal area. The underside of one of the ♀♀ is more similar to *P. c. nufrellensis*, whereas the other one is more similar to *P. c. coridon*.

With a forewing length of 13.3–13.9 mm, they are also slightly smaller than the ♀ parent.

Breeding the F₂ generation

The mating experiments with the 11 adults which emerged with the first author only resulted in one copula:

F₁ hybr. (*coridon* × *nufrellensis*) ♂ × F₁ hybr. (*coridon* × *nufrellensis*) ♀

The ♀ laid a total of 433 eggs during the period 24. XII. 2008–8. I. 2009, which is the highest number of eggs ever obtained in laboratory experiments with *Lysandra* by the first author. The eggs were laid on the paper as well as on the foodplant, especially on dry twigs. Most eggs turned out to be fertile and the first larvae hatched on 3. I. 2009 (Fig. 6: larva, Fig. 7: pupa). Due to high losses mainly during the larval stage, only 43 adult butterflies of the F₂ generation emerged from the pupae, 24 ♂♂ and 19 ♀♀.

Morphology of the F₂ generation

Males

(Figs. 3, 8.)

The colouration of the upperside of the ♂ butterflies is intermediate with respect to the parental species, which have yellowish green (*coridon*) or bluish green colour (*nufrellensis*). The underside reminds more of *coridon*, because none of the hybrids have reduced ocelli, a feature typical for *nufrellensis*. Their undersides, however, are more greyish in colouration.

Their size, with a forewing length of 13.5–14.9 mm, corresponds to the size of wild-caught specimens of their parental species.

Females

(Figs. 4 a, b, c, 9.)

The ♀ F₂ hybrids can be divided into three fractions according to the colouration of their uppersides:

- uniform brown, as in *coridon*
- completely blue as in *nufrellensis* (adults obtained by Alfred WESTENBERGER)
- partly blue, especially the hindwings

The undersides also vary in colouration and markings. Some are within the variation of *coridon*, whereas others have reduced ocelli and therefore are very similar to *nufrellensis*. The ground colour ranges from grey brown (like *nufrellensis*) to yellowish brown (as in *coridon*).

With a forewing length of 12.2–14.5 mm, the size of the ♀♀ does not differ from those of wild-caught specimens of the parental species.

Molecular analysis

The alignment of COI barcodes consisted of 324 sequences with a maximum length of 658 bp. No indels were present in any of these sequences. 5 sequences had a length of less than 497 bp and were excluded from further analysis. The remaining 319 sequences consisted of 126 different haplotypes. The calculated maximum number of connection steps at 95% parsimony probability was 11. Two networks were constructed using this connection limit, one containing only the two sequences of *P. syriacus* (a single haplotype), and the other one containing all other *Lysandra* sequences.

The sequences of *P. c. nufrellensis* formed two haplotypes with a difference of only one step. One of them included two sequences of *P. c. coridon* from Italy (Abruzzi and Lazio) and the other one two *coridon*-sequences from S France and SW Germany (Saarland). Further closely related sequences with only one step difference also belong to *P. coridon* and were from Italy (MT04010, MT04011) and England ("*P. c. insulana*").

All sequences of *P. c. gennargenti* formed a single haplotype which is most closely connected to a sequence of *P. bellargus* (ROTTEMBURG, 1775) from Turkey (MW99039; 4 steps) and is also close (6 steps) to a common haplotype of *P. c. coridon*, but rather distant (8 steps minimum) to *P. c. nufrellensis*.

The ITS2 alignment consisted of 77 sequences with an aligned length of 574 bp. Nine of these had a length of less than 430 bp and were excluded from further analysis. Five further sequences had a length of less than 505 bp and were excluded from the second analysis which counted gaps as a 5th character. The calculated maximum number of connection steps at 95% parsimony probability was 11 in the first and 10 in the second analysis. The analysis with gaps counting as missing characters resulted in 24 different haplotypes which formed a single network. The second analysis with gaps counting as 5th character resulted in 33 different haplotypes which formed 6 networks. Most *Lysandra* sequences were

contained in network 1 with 20 haplotypes. Networks 2 and 3 only contained single sequences of *P. corydonius* (HERRICH-SCHÄFFER, 1852), network 4 all sequences of *P. ossmar* (GERHARD, [1851]) and several sequences of *P. coridon* from the Eastern part of its range (8 different haplotypes), network 5 the two sequences of *P. syriacus* (TUTT, [1910]), and network 6 all sequences of *P. bellargus* and one sequence of *P. corydonius* (MW99140).

In both analyses, the sequences of *P. c. nufrellensis* form a single haplotype, together with several sequences of *P. albicans* (HERRICH-SCHÄFFER, 1851) and *P. coridon* from Spain, Andorra, South France, the UK and one from Romania. In the first analysis, sequences of *P. c. caelestissimus* (VERITY, 1921) and *P. hispanus* (HERRICH-SCHÄFFER, 1851) are also included in this haplotype.

All sequences of *P. c. gennargenti* form a distinct haplotype in both analyses, with a distance of one step to the haplotype which includes *P. c. nufrellensis*. This haplotype also includes one sequence of *P. c. asturiensis* (SAGARRA, 1924) from NW Spain (La Rioja). – The haplotype also includes one sequence of *P. c. caelestissimus* (OK96022). However, results from COI and ITS2 analyses indicate that the sample of this specimen seems to have been mixed up with a sample of *P. c. gennargenti* (OK99001) before the analyses were conducted.

Conclusions

Polyommatus (Lysandra) coridon nufrellensis from the island of Corsica can be crossed with the nominotypical subspecies from Central Europe until at least the F₂ generation. The breeding difficulties and high losses can mainly be attributed to methodological problems and suboptimal breeding conditions, e.g. due to the adverse weather conditions during winter. Even though *Hippocrepis comosa* (Horseshoe Vetch) stays green in winter (unlike the second foodplant Crown Vetch, *Securigera varia*, both Fabaceae), the food quality is not perfect for the caterpillars during this season. Especially the first larval stages hesitate to feed on the old and hard leaves and therefore have a reduced growth rate. Overwintering the larvae or young larvae would be no good alternative, because this would lead to even higher losses.

According to their morphology, *P. c. coridon* and *P. c. nufrellensis* are quite similar, and some specimens cannot be reliably identified by their uppersides alone. The ♀♀ of *nufrellensis*, however, which are always blue on the upperside, are clearly different from *coridon*. Although the latter taxon also has a blue ♀ form (f. *syngrapha* KEFERSTEIN), this form is very rare in Central Europe and even completely absent in the Eastern part of its range, only found again in Greece.

According to the molecular analyses, *P. c. nufrellensis* does not appear well differentiated from other populations of *P. coridon*. COI barcodes are even identical to those found on the mainland (Italy, S France and SW Germany), which indicates a very recent origin. *P. c.*

gennargenti is more strongly differentiated from other populations of *P. coridon* and not especially closely related to *P. c. nufrellensis*. TALAVERA et al. (2013) differentiated a western and an eastern clade within the *coridon*-group of taxa, with *gennargenti* in the former and *nufrellensis* in the latter clade. However, the bootstrap values for these two clades were low. Our network analyses do not corroborate the existence of these two clades, or the attribution of *P. c. nufrellensis* and *P. c. gennargenti* to distinct clades. Instead, the west-east differentiation is gradual, and our much increased data set shows many exceptions, one of which (a sequence of *P. c. asturiensis* from NW Spain in the eastern clade) was already noted by TALAVERA et al. (2013). The cline appears to be mainly the result of differential introgression in the western and eastern part of the range. Whereas introgression in the west is mainly due to hybridisation with the Iberian taxa *P. hispanus* and *P. albicans*, introgression from *P. bellargus* is most common in eastern populations of *P. coridon*.

The results confirm previous assumptions (SCHURIAN 1989) that *Lysandra* is of relatively recent origin in evolutionary terms and some forms might even have a post-glacial origin. This is in accordance with TALAVERA et al. (2013), who postulate an origin of *Lysandra* about 4.5 Ma [Ma = Million years or 'Megaanni'] ago and a start of their radiation but 1.5 Ma ago.

We would like to stress that the reported hybridisation experiments were conducted under laboratory conditions. Even though the islands of Corsica and Sardinia are only separated by a narrow sea strait of about 15 km, gene exchange is prevented by the isolated occurrence of the blues high up in the mountains. It seems most probable that the islands were colonized from two different origins on the mainland. TALAVERA et al. (2013) already noticed a close relationship of *P. (L.) c. nufrellensis* with *P. (L.) c. narbonensis* from South France, and postulated a relatively recent immigration event from the continent. However, according to our results the origin of *P. c. gennargenti* and *P. c. nufrellensis* remains unresolved with available molecular data.

LEIGHEB (1991) postulated a Spanish origin for *L. c. gennargenti*: „la costanza di ♀♀ azzurre avvicina morfologicamente *gennargenti* alla popolazione spagnola“, but we do not subscribe to this point of view. In *P. c. caelestissimus*, blue ♀♀ (f. *deliciosa* DE SAGARRA) only exist in very rare cases, and this taxon is restricted to Central Spain and does not occur near the coast. If one would accept an origin of *gennargenti* from the Spanish mainland, the most probable origin might be *P. coridon manleyi* (DE LESSE, 1962), because this taxon contains populations with only blue ♀♀, a character which appears to be determined genetically (SCHURIAN 1976). These ♀♀ appear to be most similar to a typical *gennargenti* (see Fig. 8).

MARCHI et al. (1996) argued that *gennargenti* might have either originated in Spain (*P. c. caelestissimus*) or in Italy (*P. c. apennina*). In *P. c. apennina*, however, no blue ♀♀ are known to occur.

Differentiation of *nufrellensis* and *gennargenti* in molecular, morphological or ethological characters is certainly due to longer isolation on the respective island. Whether this isolation only dates back to the end of the last glacial maximum or to pre-glacial times (MARCHI et al. 1996), remains uncertain at present.

Threats

Although a new locality on Corsica was discovered lately (PARMENTIER & ZINSNER 2013), the habitats of *P. c. nufrellensis* are restricted to very few locations with occurrences of *Hippocrepis conradiae*, the only known natural foodplant of this taxon (GAMISANS et al. 2012). Further decimation of the reproductive sites by too intensive grazing could lead to the extirpation of its populations. Currently, *H. conradiae* appears to grow mainly on sites which are difficult to access such as screes, cliffs or between small conifer trees, and not on open plains, which are subject to intensive grazing regimes. The first author also discovered *H. conradiae* in the Spasimata valley in 2012, but the few plants were more or less dried out already. They were bearing feeding traces from the spring, but no eggs from the summer generation.

Island endemic taxa are often highly threatened, e.g. by the growing tourism industry, intensification of agriculture, invasive species, or global warming, and therefore we suggest to monitor populations of *P. c. nufrellensis* as well as *P. c. gennargenti* and implement conservation measures to protect these endemic taxa.

Taxonomic conclusions

Both island endemic taxa, *nufrellensis* as well as *gennargenti*, were originally described at the rank of subspecies of *Polyommatus (Lysandra) coridon* PODA, 1761 (SCHURIAN 1977, LEIGHEB 1987). Later authors usually followed this concept (e.g. MARCHI et al. 1996, KLEINE-KUHLE 1999, CASULA et al. 2004, SALA et al. 2005, DIRINGER 2009). In 2003, however, *P. (L.) c. gennargenti* was raised to species status by JUTZELER et al. (2003), based on various morphological and ethological differences. Similar arguments could also be raised for *P. (L.) coridon nufrellensis*. Apart from morphological characteristics which are mentioned earlier in this paper, this taxon also differs in its life history. Larval developmental time differs considerably between both taxa. Whereas larvae of *P. c. gennargenti* only hatch after a long period in the egg at the end of September or the beginning of October (D. JUTZELER in litt. 23. v. 2001), and are thus similar to Central European populations of *P. coridon*, the larvae of *P. c. nufrellensis* already hatch after a few days, as is the case in other bivoltine taxa of the *coridon*-group. Feeding behaviour in egg-laying ♀♀ in captivity also differs from the nominotypical subspecies. Whereas ♀♀ of Central European *coridon* can be left unattended for extended periods of time, those of *nufrellensis* die quickly if they do not regularly come into contact with a feeding source. Despite these characteristics which prove the differen-

tiation of *P. c. nufrellensis* from other subspecies of *P. coridon*, we suggest to continue considering *nufrellensis* as a subspecies of *P. coridon*, in accordance with TALAVERA et al. (2013). Our results indicate that isolation of *nufrellensis* is of relatively recent origin, and time was insufficient to develop reproductive isolation mechanisms, as evidenced by the successful cross-breeding until the F₂ generation.

Addendum to Part II (SCHURIAN et al. 2011)

The hybrid experiments between *P. coridon nufrellensis* and *P. coridon gennargenti* resulted in a fertile F₁ generation (SCHURIAN et al. 2011). Experimental hybridisation was continued and resulted in 5 matings of the F₁ generation at the end of 2007:

F₁-Hybr. (*nufrellensis* × *gennargenti*) ♂ × F₁-Hybr. (*nufrellensis* × *gennargenti*) ♀.

One of the ♀♀ died without laying an egg and three ♀♀ died after laying only few eggs which did not produce any larvae. The remaining two ♀♀ laid a total of 262 eggs (♀ I: 146 eggs, ♀ II: 116 eggs). The eggs of ♀ I only produced 3 caterpillars, whereas 66 caterpillars hatched from those of ♀ II. The breeding in December and January turned out to be difficult, partly due to suboptimal weather and food conditions. The caterpillars only grew very slowly and most of them died due to diseases. Only four caterpillars survived until the end of January 2008, and of these, only one pupated on 29. I. 2008, which led to a ♀ of the F₂ generation, emerging on 10. II. 2008.

Morphology of the F₂ generation

(Figs. 10–12.)

The single emerged ♀ is very similar to *nufrellensis*. It is blue on the upperside and lacks the brown colouration along the veins which is so typical for ♀♀ of *gennargenti*. The reddish caps above the marginal spots in the submarginal region, which are hardly visible or completely lacking in ♀♀ of *gennargenti*, are well developed in our specimen, as is the case in *nufrellensis*. The underside also does not differ from *nufrellensis*, neither in ground colour, nor in the arrangement and size of the ocelli. Its forewing length is 12.5 mm and therefore it is smaller than wild-caught specimens.

In conclusion, the results provide evidence that *P. (L.) coridon gennargenti* und *P. (L.) coridon nufrellensis* can experimentally be cross-bred to the F₂ generation.

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Literature

- CASULA, P., SCANU, D., CRNJAR, R., GRILL, A., & MARCHI, A. (2004): The fragmented population structure of the Sardinian chalk hill blue butterfly (Lepidoptera, Lycaenidae). — *Journal for Nature Conservation*, Jena, **12**: 77–83.
- CLEMENT, M., POSADA, D., & CRANDALL, K. A. (2000): TCS: a computer program to estimate gene genealogies. — *Molecular Ecology*, Hoboken, **9**: 1657–1660.
- DINCA, V., DAPPORTO, L., & VILA, R. (2011): A combined genetic-morphometric analysis unravels the complex biogeographical history of *Polyommatus icarus* and *Polyommatus celina* Common Blue butterflies. — *Molecular Ecology*, Hoboken, **20** (18): 3921–3935.
- DIRINGER, Y. (2009): Chronique d'élevage 1: Historique de la découverte et élevage du *coridon* corse, *Polyommatus (Lysandra) coridon nufrellensis* SCHURIAN (1977) (Lepidoptera: Lycaenidae). — *Lépidoptères, Revue des Lépidoptéristes de France*, Paris, **18** (44): 92–98.
- GAMISANS, J., HUGOT, L., & JUTZELER, D. (2011): *Hippocrepis conradiae* GAMISANS & HUGOT (Fabaceae), une nouvelle espèce de Corse, liée à un papillon endémique. — *Candollea*, Genève, **66** (2): 273–280.
- HAUSMANN, A., HASZPRUNAR, G., SEGERER, A. H., SPEIDEL, W., BEHOUNEK, G., & HEBERT, P. D. N. (2011): Now DNA-barcoded: the butterflies and larger moths of Germany. — *Spixiana*, München, **34**: 47–58.
- JUTZELER, D., CASULA, P., GASCOIGNE-PEES, M., LEIGHEB, G., & GRILL, A. (2003): Confirmation du statut spécifique de *Polyommatus gennargenti* (LEIGHEB, 1987) de Sardaigne comparé à *Polyommatus coridon* (PODA, 1761) de la région de Schaffhouse (CH) par élevage parallèle (Lepidoptera: Lycaenidae), 2 parts. — *Linneana Belgica*, Wetteren, **19** (3): 109–118; (4): 149–160.
- KANDUL, N. P., LUKHTANOV, V. A., DANTCHENKO, A. V., COLEMAN, J. W. S., SEKERCIOGLU, C. H., HAIG, D., & PIERCE, N. E. (2004): Phylogeny of *Agrodiaetus* HÜBNER 1822 (Lepidoptera: Lycaenidae) inferred from mtDNA sequences of COI and COII and nuclear sequences of EF1-α: karyotype diversification and species radiation. — *Systematic Biology*, Oxford, **53** (2): 278–298.
- KLEINEKUHLE, J. (1999): Die Tagfalter (Rhopalocera) Sardiniens aus biogeographischer Sicht. — *Oedipus*, Schweinfurt, **16**: 1–60.
- KUDRNA, O., HARPKE, A., LUX, K., PENNERSTORFER, J., SCHWEIGER, O., SETTELE, J., & WIEMERS, M. (2011): Distribution atlas of butterflies in Europe. — Halle (Gesellschaft für Schmetterlingsschutz), 576 pp.
- LEIGHEB, G. (1987): *Lysandra coridon* ssp. *gennargenti* nova (Lepidoptera, Lycaenidae), nuovo licenide della Sardegna. — *Bollettino del Museo Regionale di Scienze Naturali*, Torino, **5** (2): 447–454.
- (1991): La femmina di *Polyommatus (Lysandra) coridon gennargenti* (LEIGHEB 1987) (Lepidoptera, Lycaenidae). — *Bollettino del Museo Regionale di Scienze Naturali*, Torino, **9** (2): 369–373.
- LUKHTANOV, V., SOURAKOV, A., ZAKHAROV, E. V., & HEBERT, P. D. N. (2009): DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. — *Molecular Ecology Resources*, Hoboken, **9** (5): 1302–1310.
- MARCHI, A., ADDIS, V., EXPOSITO HERMOSA, V., & CRNJAR, R. (1996): Genetic divergence and evolution of *Polyommatus coridon gennargenti* (Lepidoptera: Lycaenidae) in Sardinia. — *Hereditas*, London, **77**: 16–22.

- PARMENTIER, L., & ZINSZNER, E. (2013): Contribution to the knowledge of two endemic Corsican butterflies, *Polyommatus coridon nufrellensis* and *Plebejus bellieri* (Lepidoptera: Lycaenidae) in relation to *Hippocrepis conradiae*: first evidence as hostplant, discovery of a new locality and update on distribution, biology and conservation. — *Phegea*, Merksem, **41** (2): 26–41.
- POSADA, D., & CRANDALL, K. A. (2001): Intraspecific gene genealogies: trees grafting into networks. — *Trends in Ecology & Evolution*, London, **16** (1): 37–45.
- SALA, G., CRNJAR, R., & GUIDI, M. (2005): *Polyommatus coridon nufrellensis* SCHURIAN, 1977 (Lepidoptera: Lycaenidae) rediscovered. — *Linneana Belgica*, Wetteren, **20** (4): 121–122.
- SANUDO-RESTREPO, C. P., DINCA, V., TALAVERA, G., & VILA, R. (2013): Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). — *Molecular Phylogenetics and Evolution*, Amsterdam, **66** (1): 369–379.
- SCHURIAN, K. [G.] (1976): Zur Biologie von *Lysandra coridon manleyi* (Lep., Lycaenidae). — *Entomologische Zeitschrift*, Essen, **86** (6): 49–53.
- (1977): Eine neue Unterart von *Lysandra coridon* PODA (Lep., Lycaenidae). — *Entomologische Zeitschrift*, Essen, **87** (3): 13–18.
- (1989): Revision der *Lysandra*-Gruppe des Genus *Polyommatus* LATR. (Lepidoptera: Lycaenidae) [Dissertation Frankfurt am Main]. — *Neue Entomologische Nachrichten*, Markt-leuthen, **24**: 7–181.
- (1990): Hybridisierungsversuche mit Lycaeniden (Lepidoptera). — *Verhandlungen des Westdeutschen Entomologentags*, Düsseldorf, **1989**: 257–264.
- , GASCOIGNE-PEES, M., & DIRINGER, Y. (2006): Contribution to the life-cycle, ecology and taxonomy of *Polyommatus (Lysandra) coridon nufrellensis* SCHURIAN (1977) (Lepidoptera: Lycaenidae). — *Linneana Belgica*, Wetteren, **20** (5): 180–192.
- , WESTENBERGER A., DIRINGER, Y., & WIEMERS, M. (2011): Contribution to the biology and taxonomy of *Polyommatus (Lysandra) coridon nufrellensis* (SCHURIAN, 1977) (Lepidoptera: Lycaenidae), Part II: An experimental hybridization of *P. (L.) c. gennargenti* × *P. (L.) c. nufrellensis*. — *Nachrichten des Entomologischen Vereins Apollo*, Frankfurt am Main, N.F. **31** (4): 177–186.
- TALAVERA, G., LUKHTANOV, V. A., PIERCE, N. E., & VILA, R. (2012): Establishing criteria for higher-level classification using molecular data: the systematics of *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae). — *Cladistics*, New York, **29** (2): 166–192.
- , —, RIEPPEL, L., PIERCE, N. E., & VILA, R. (2013): In the shadow of phylogenetic uncertainty: The recent diversification of *Lysandra* butterflies through chromosomal change. — *Molecular Phylogenetics and Evolution*, Amsterdam, **69** (3): 469–478.
- VILA, R., BELL, C. D., MACNIVEN, R., GOLDMAN-HUERTAS, B., REE, R. H., MARSHALL, C. R., BÁLINT, Z., JOHNSON, K., BENYAMINI, D., & PIERCE, N. E. (2011): Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. — *Proceedings of the Royal Society*, London, **B 278** (1719): 2737–2744.
- VODOLAZHISKY, D. I., & STRADOMSKY, B. V. (2008): Исследование филогенеза подрода *Polyommatus* (s. str.) LATREILLE, 1804 (Lepidoptera: Lycaenidae) с использованием маркеров мтДНК, Часть II [Phylogenetic analysis of subgenus *Polyommatus* (s. str.) LATREILLE, 1804 (Lepidoptera: Lycaenidae) based on mtDNA markers, part II]. — *Caucasian Entomological Bulletin*, Rostov-on-Don, **4** (2): 237–242.
- WIEMERS, M. (2003): Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*) — a molecular phylogenetic approach. — PhD dissertation, University of Bonn, 198 pp. + 2 col. pls. [URL: nbn-resolving.de/urn:nbn:de:hbz:5n-02787].
- , & FIEDLER, K. (2007): Does the DNA barcoding gap exist? — a case study in blue butterflies (Lepidoptera: Lycaenidae). — *Frontiers in Zoology*, **4**: 8.
- , KELLER, A., & WOLF, M. (2009): ITS2 secondary structure improves phylogeny estimation in a radiation of blue butterflies of the subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*). — *BMC Evolutionary Biology*, **9**: 300.

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