A new host plant for *Danaus plexippus* (LINNAEUS, 1758) in Europe. A study of cryptic preimaginal polymorphism study within *Danaus chrysippus* (LINNAEUS, 1758) in southern Spain (Andalusia)

(Lepidoptera, Nymphalidae, Danainae)

by

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Abstract: During the 1980's the migrant species *Danaus plexippus* (L.) and *Danaus chrysippus* (L.) established a number of colonies in the south of the Iberian Peninsula (Andalucia). During the early 90's these disappeared, but over the last few years (2001-2005) both species have re-established themselves (GIL-T. & GIL-UCEDA, 2006), with a complete biological cycle, in coastal areas of the provinces of Granada and Almeria (SE. Spain). As a result of field observations carried out on these colonies, *Cynanchum acutum* L. is here registered as a new larval host plant for *Danaus plexippus* (L.). It is the first native plant to be accepted by larvae of this species on the European continent, where the butterfly has successfully developed its full biological cycle; furthermore, larvae frequently share a leaf with those of *D.chrysippus* (L.) without rivalry or rejection of either species.

Different pupal and larval forms of *Danaus chrysippus* (L.) from the south of the Iberi-an Peninsula have been studied and illustrated. According to published references the pupal colour forms are not yet well-known. However, while a green form is predominant in nature, creamy-yellowish-white and amber colour forms were also obtained when the species was reared in captivity, this being clearly dictated by the ground colour of the surrounding environment (cryptic polymorphism) prior to pupation.

Resumen: Las especies migradoras *Danaus plexippus* (L.) y *Danaus chrysippus* (L.) establecieron varias colonias (ciclo biológico completo) en el sur de la Península Ibérica (Andalucía) en la década de los años 80. Dichas colonias desaparecieron a principios de los 90. En los últimos años (periodo 2001-2005) se han establecido nuevas colonias (GIL-T. & GIL-UCEDA, 2006) de las dos especies, con ciclo biológico completo, en zonas costeras de las provincias de Granada y Almería (SE. Spain).

Como resultado de las observaciones realizadas en las anteriores colonias, se registra una nueva planta nutricia larval, *Cynanchum acutum* L., en condiciones naturales, para *Danaus plexippus* (L.). Esta es la primera planta autóctona aceptada por este lepidóptero en el continente europeo. La mariposa desarrolla su ciclo biológico completo sobre esta planta, sus orugas comparten con frecuencia los mismos pies de la planta nutricia con orugas de *D. chrysippus* (L.), no existiendo ningún rechazo o competencia entre las dos especies.

Se estudian e ilustran las distintas formas pupales y larvales obtenidas de *Danaus* chrysippus (L.) en el sur de la Península Ibérica. El color de las formas pupales (aparentemente no bien conocidas, según algunas referencias publicadas), tanto en la forma

verde (forma pre-dominante en la naturaleza) como en las formas de color crema-blanco. amarillento y de color ámbar (las dos últimas obtenidas principalmente en cría en cautividad), está claramente influido por el color de fondo del entorno (polimorfismo críptico) antes de la fase de crisalida.

Zusammenfassung: Die beiden Wanderfalterarten *Danaus plexippus* (L.) and *Danaus chrysippus* (L.) besiedelten in den 80er Jahren des letzten Jahrhunderts durch mehrere Kolonien (kompletter Entwicklungszyklus) den Süden der Iberischen halbinsel (Andalusien). Diese verschwanden wieder Anfang der 90er Jahre, bildeten jedoch in den letzten Jahren (2001-2005) neue Kolonien mit einem kompletten Entwicklungszyklus in der Küstenregion von Granada und Almeria in SE-Spanien (GIL-T. & GIL-UCEDA, 2006).

Als Untersuchungsergebnis an den Koloniene im Freiland konnte *Cynanchum* acutum L. als neue Raupenfutterpflanze von *Danaus plexippus* (L.) festgestellt werden. Dies ist die erste bodenständige Art, die von dieser Art in Europa akzeptiert wird und an der diese den vollständigen Entwicklungszyklus durchlaufen kann. Darüberhinaus teilen die Raupen dieser Art häufig diese Futterpflanze mit denen von *D.chrysippus* (L.) ohne daß Rivalitäten zwischen diesen feststellbar sind.

Verschiedene Farbmorphen der Raupen und Puppen von *Danaus chrysippus* (L.). die im Süden der Iberischen Halbinsel gefunden werden konnten, wurden untersucht und abgebildet. Die Farbmorphen der Puppe (in Anlehnung an die zitierte Literaur ungenügend bekannt), eine grüne Form (in der Natur vorherrschend), eine cremig-gelb-weiße und eine bernsteinfarbige Form (die letzten beiden, vor allem unter Laborbedingungen erzielt) werden eindeutig durch die Grundfarbe der Umgebung (Kryptischer Polymorphismus) vor der Verpuppung induziert.

Introduction: Danaus chrysippus (L.) established its first colony (Fig.1) in the Iberian Peninsula (Spain), in the province of Almeria during 1979. Subsequently, in 1980 it was observed in the province of Malaga (Torrox) where it established several colonies in diverse localities along the coast line. During the 80's colonies were extended to other areas of the southeast and northeast coast of the peninsula.

In 1980 it was verified that *Danaus plexippus* (L.) had become successfully established (Fig. 2) in the province of Malaga, in various colonies sympatric with *D. chrysippus* (L.); Torrox, Nerja ect., (Fig 3 arrow M). These colonies reached maximum growth during the period 1983-1987. It was suggested that the original pioneering imagos of *D. plexippus* (L.) which had settled in the southern Iberian Peninsula might possibly have originated from the Canary islands.

After 1988 both species began to disappear from localities near Torrox (Malaga) ie: Nerja. After 1991 *D. chrysippus* (L.) disappeared from the province of Malaga and, according to TARRIER (1994), *D. plexippus* (L.) numbers were also reduced by 90-95%. Within a year or two both species were considered extinct in the Mediterranean coastal districts of the Iberian Peninsula. The disappearance of both species from their original sites in Malaga coincided during a period of intensive urbanization in the area of Torrox, where they had formerly settled and flourished. At that time, it was considered that the destruction of their breeding area, together with the loss of the known host plant *Asclepias curassavica*, had

heen the main factors that had caused their extinction (examples: CITORES, 1993, TARRIER, 1993 ect).

In GIL-T. & GIL-UCEDA (2006) the establishment of new colonies of both species in the south of the Iberian Peninsula was reported. These settlements were thoroughly studied during the period 1998-2005 for *D. chrysippus* (L.), and during 2001-2005 for *D. plexippus* (L.) in Granada province (coastal areas near Torrenueva, Motril, Salobreña and Almuñecar) and in the province of Almeria (El Alquian, Viator, Rioja and in the neighbourhood of the capital Almeria) see Fig. 3: arrow G= Granada province area; arrow A=Almeria province area. Consequently, we disagree with the hypothesis drawn up with regard to the reasons for its disappearance during the late 80's, and are of the opinion that other factors are more relevant.

Meteorological influences - cyclic drought periods and/or high temperature periods were highly important and had much to do with the appearance and establishment of these colonies and their subsequent extinction in the Iberian Peninsula. Climate has never been taken into account in any of the numerous publications related to the Iberian danainids. The optimum temperatures for the survival of larvae of both species were analyzed and their annual life cycle estimated (details to follow herein). Cannibalism took place, which was previously unknown (pupae were devoured by larvae of the same kind or even by its sister species), and sporadic attempts at copulation between different species [$\sigma\sigma$ of D. chrysippus (L.) attempting to copulate with freshly emerged (prior to wing development) \$9 of D, plexippus (L.)] was also observed. Furthermore, a new host plant was discovered for D *plexippus* (L.) on the European continent. This is a native asclepiadaceous plant, which proves that D. plexippus (L.) can adapt to nutritional resources different than those presently accepted and which are of alien origin and/ or have been recently introduced in Europe. Analytical summaries are supplied, together with illustrations of the larval and pupal forms of D. chrysippus (L.), of which very little is known aside from disparate published opinions. Moreover, a study of cryptic polymorphism has been carried out, as well as the that into normal variation.

A new host plant for Danaus plexippus (L.)

As a result of searching out the larvae of *D. chrysippus* (L.) on shrubs of the native asclepiadaceous (Milkweed) *Cynanchum acutum*, amongst the abundant larvae found of *D. chrysippus* (L.) we also found a fair number of *D. plexippus* (L.) caterpillars. These successfully completed their life cycle exclusively on this plant (Fig. 4). C. acutum is endemic in Mediterranean countries and has a vast geographic distribution (Portugal, Spain, France, Italy, Romania, Albania, Bulgaria, ex Yugoslavia, Greece, ect.). *Asclepias curassavica* and *Gomphocarpus fruticosus* were the recorded host plants for *D. plexippus* (L.), both of which are of alien origin. *C. acutum* should therefore now be considered a new native host plant for this species in Europe.

C. acutum is a climbing shrub, which can be found abundantly in shady humid areas, it being preferably situated between patches of cane and reed bed. These occupy fairly large areas along the coast line and near rivers in the area under consideration. The seeds are covered with cotton-like hairs and are therefore easily dispersed by the wind;

quite often they germinate in public parks and gardens. It is a recognised foodplant for *D. chrysippus* (L.) but, as previously explained, it has never been recorded for *D. plexippus* (L.). In North America more than 27 different host plants have been listed for *D. plexippus* (L.) (MALCOM & BROWER, 1989). In the Canary Islands, Gossypium arboreum (Malvaceae, Cotton plant) has been recorded along with the already known A. curassavica and *G. fruticosus*.

There is no doubt that in spite of the European-wide scarcity of its classic host plants, *D. plexippus* (L.) took advantage and adapted its feeding habits to the resources native to the Mediterranean area (*C. acutum*). The exploitation of this new and widespread plant disproves the theory expressed in various references (ex.: TARRIER, 1993) that the reason for the disappearance of *A. curassavica* from the south of Spain was due to urbanization. It is quite obvious that as there is an abundance of the alternative foodplant (*C. acutum*), which exists over hundreds of square kilometres of the coast, the destruction of the butterfly's habitat cannot be responsible for its disappearance on the coast line of eastern Andalucia.

It is well known that the chemical defences held by the larvae and adults of *Danaus* originate from the Asclepidadaceae. These plants generate toxic cardenolid components (specifically cardio-actives glucosids or glycosids) which are ingested by the larvae. Subsequently, these toxins are transferred to the adults (MALCOM & BROWER, 1989). The concentration of cardiac glucosids decreases in direct proportion to their age (ALONSO-MEJIA & BROWER, 1994). The majority of predators (birds, mammals, etc) avoid consumption on account of their toxicity.

Notes on climate and annual life cycle of *Danaus* species in Southern Spain

South Andalucia is the only area of the Iberian Peninsula where *D. plexippus* (L.) has succeeded in establishing itself. It is located under the influence of the subtropical western coastal or Mediterranean climates, a transition fringe between medium latitudes and tropical climates. This means that autumns and winters are mild (especially along the coast), alternating with dry and markedly hot summers. Summer droughts are more acute here than in any other Spanish region and sunshine in some areas of the coast of Almeria exceeds 3000 hours annually, this being one of the hottest parts of Spain. The Mediterranean coast is frost-free for more than 350 days a year; in other words, frost is almost completely absent here. If we take into consideration the whole Andalucian coast line, the annual number of frosts is less than 5 days, and in areas of Almeria, Granada, Malaga and Cadiz the period drops down to only one day per year. These are relevant characteristics which exactly explain the marked preference of these Danaid species for the localities within the Mediterranean subtropical and Mediterranean subdesert climates, whose main features are:

Subtropical Mediterranean climate: Affects the greater part of the Andalucian coastal areas, and is characterized by mild winter temperatures which are influenced by the sea and the southerly orientation of the coast. The monthly average temperature during the winter season ranges between 12∞ C and 15∞ C. Furthermore, the high minimum temperature prevents frosts.

Mediterranean sub-desert climate: Mainly affects the southeast sector of the province of Almeria, the driest area of Europe, with very high temperatures and extreme isolation. In Table 1 (' \underline{X} , X, x, x/' = Major to minor record scale; '-' = no records), in accordance with the recording carried out (larvae and imagoes, during climatologically favourable years), the approximate annual life cycle can be determined for both species in southern Spain (Andalusia).

$\begin{array}{l} X = very \ abundant \\ X = abundant \end{array}$		Jan		Feb		Mar-July	A	ug	Se	pt	0	ct	N	ov 1	D	ec 1
x = very s	carce ificant	1q	2q	1q	2q		Iq	2q	lq	2q	1q	2q	1q	2q	1q	2q
Danaus	Imago	-	-	-	-		-	x/	x	X	X	X	X	x	-	-
chryssipus	Larva	-	-	-	-	no r	-	-	x	X	X	X	X	x/	x/	-
Danaus	Imago	x/	<i>x/</i>	x/	-	ecor	-	-	x/	x	X	X	x	<i>x/</i>	x/	<i>x/</i>
plexippus	Larva	-	-	-	-	.ds	-	-	-	x	X	X	x	<i>x/</i>	x/	

Table 1. Records (q=two weeks) of imagos/ larvae Danaidae in S. Spain

Larval and pupal cryptic polymorphism in D. chrysippus (L.)

Some published references show disparities when describing the normal colouration of pupae. For example: WIEMERS (1995) describes the "usual" colour of the pupa as a "turquoise" colour (bluish-green?); in VERDUGO (1984), his description of *D. chrysippus* (L.) pupae is "yellowish"; in"LEIGHEB & CAMERON-CURRY (1999:63) it is considered that this butterfly only shows two pupal forms; one is light green and the other a "milkish-white" colour, "it being adding that the "milkish-white" is "more common" than the green form. Our findings do not agree with any of the these conclusions. Throughout our breeding experience of *D. chrisippus* (L.) in captivity (Table 2) we obtained the following larval-pupal forms from a sample of 64 larvae collected from coastal areas of both Granada and Almeria.

Pupae: 42 (Fig 8) were predominantly green coloured, of a rather lighter tone than those of *D. plexippus* (L.); 12 (Fig 10) were of a creamy-white colour, and partially yellow; 7 (Fig 9) were amber coloured (orange-yellow), this being a form of which we were unable to trace any records; and 3 exhibited a brown-rose abdomen and green thorax, this being the rarest form.

Larvae: 53 caterpillars (see Fig. 5) were of the predominant typical form; 9 (Fig. 6) were of a greenish-yellow colour, with a yellow head and lacking the typical black transversal stripes; and 2 (Fig 7) were yellow coloured and thinly striped in black with a yellowish-green head.

Larval fo	rms	Pupal forms					
Fig. 5 (predominant)	82,81%	Fig. 8 (predominant)	65,63%				
Fig. 6	14,06%	Fig. 10	18,75%				
Fig. 7	3,13%	Fig. 9	10,94%				
		Otra	4,69%				

Table 2.-Synopsis larval-pupal forms of D. chrysippus (L.) (S. Spain)

We found all of these larval forms in nature. However, we did not find any other nupal forms apart from the usual green. The markings on the larvae show clearly that it is a cryptic camouflage dictated by its host plant. The colour of the chrysalis bears no relationship to that shown by the caterpillars (Fig. 5, 6 or 7), whatever their colour and markings. The colour of the pupa is the result of an imitation or crypsis (note: except for green, the imitated colour is not always perfect) of the prevailing colour of its environment whether it belongs to the existing vegetation, or to the object or support (natural or artificial where the larvae attaches its cremaster for pupation. Our results support this statement and also by that made by ROTHSCHILD et al. (1978), in which it was pointed out that the colour of pupae is closely related to their ability to merge into the background where they develop. When rearing high numbers in captivity, green was still the predominant colour (Table 2). Amber and creamy-white pupae were obtained in variable numbers, these depending on the different colour of the walls of the breeding box, as they adapted their colouring to that of the box. After several experiments we noticed that while the final colour tone of the pupae was similar to that of the interior of the cage, the match was not precise Neither form (Figs. 9 and 10) exceeded the indicated percentage for the creamy-white forms shown in Table 2.

Note: The unusual amber colour was obtained as a result of having reared larvae du-ring their final stage inside a container with a caramel-coloured interior, although the majority of the resultant pupae were green (the same colour of the host plant leaves C. acutum). This confirms our previous considerations related to their ability to merge with the background colour of their surrounding environment. The results obtained in some of the previously mentioned references [WIEMERS (1995), VERDUGO (1984), LEIGHEB & CAMERON-CURRY (1999: 63), and others] with respect to pupal colouration were probably also influenced by the cryptic coloration adopted by the pupae, and also by a smaller sample used during captive breeding.

Acknowledgements: I am very grateful to RAFAEL ESTEVEZ RODRIGUEZ (E-Vigo, Pontevedra) for his essential help in the translation of the text from Spanish into English, and to COLIN PRATT for the final English version.

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Fig. 3: New danainid colonies located in the South of the Iberian Peninsula ('G' & 'A' arrows).

Colour plate 1/ Farbtafel 1, p. 279

Fig. 1: Imago of Danaus chrysippus (L.). Fig. 2: Imago of Danaus plexippus (L.).

Fig. 4: *D. plexippus* (L.) larvae on *Cynanchum acutum*, new host plant. Fig. 5: *D. chrysippus* (L.) larva, predominant form. Fig. 6: *D. chrysippus* (L.) larvae, green form.

Fig. 7: *D. chrysippus* (L.) larva, yellow form. Fig. 8: Pupae: on the left *D. plexippus* (L.); on the right *D. chrysippus* (L.). Normal forms. Fig. 9: *D. chrysippus* (L.) pupa, amber colour form. Fig. 10: *D. chrysippus* (L.) pupae, pearl white colour form.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Atalanta

Jahr/Year: 2006

Band/Volume: 37

Autor(en)/Author(s): Gil-T. Felipe

Artikel/Article: <u>New host plant for Danaus plexippus (Linnaeus, 1758) in</u> <u>Europe. \ study of cryptic preimaginal polymorphism study within Danaus</u> <u>chvysippus (Linnaeus, 1758) in southern Spain (Andalusia) 143-149</u>