

Karyology and distribution as tools in the taxonomy of Iberian *Agrodiaetus* butterflies (Lepidoptera : Lycaenidae)

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Summary

A cytotaxonomical study in the main distribution areas of *Agrodiaetus ripartii*, *A. fabressei*, *A. ainsae* and *A. fulgens* revealed differences between these species. In the species with brown males, *A. ripartii* has $n = 90$ with two macrochromosomes and *A. fabressei* the same chromosome number, but three large chromosomes. In the species with blue males, *A. fulgens* (considered here a true species) and *A. ainsae* have $n = ca. 103$ and $n = 108$ with two and six macrochromosomes respectively. Macrochromosomes proved to be the best genetic marker to identify the species of *Agrodiaetus* in the Iberian Peninsula. By combining karyological and morphological data we were able to construct detailed UTM maps for the four species. *A. ripartii* is found from Catalonia to the Cantabrian Mountains, in the south to the Sistema Ibérico, *A. fabressei* flies in the Sistema Ibérico and Andalusian Sierras, *A. fulgens* is a Catalanian species with a very restricted distribution range and *A. ainsae* lives in the Central and Western Pyrenees and the Cantabrian Mountains. The taxon recently described as *A. violetae* is considered a subspecies of *A. fabressei*, based on the similar chromosome morphology and number. The group seems to have evolved very quickly karyologically, but morphological and ecological differences are not so evident.

Resumen

El estudio citotaxonomico en las principales áreas de distribución de *Agrodiaetus ripartii*, *A. fabressei*, *A. ainsae* y *A. fulgens* reveló diferencias entre las especies. Las especies de machos castaños *A. ripartii* y *A. fabressei* tienen respectivamente $n = 90$ y dos macrocromosomas y el mismo número de cromosomas, pero con tres cromosomas grandes. En el caso de las especies de machos azules *A. fulgens* (elevada aquí a la categoría de especie) y *A. ainsae* tienen $n = ca. 103$ y $n = 108$ con dos y seis macrocromosomas respectivamente. Los macrocromosomas resultaron ser el mejor marcador genético para identificar las especies del género en la Península Ibérica. Mediante el análisis conjunto de datos cariológicos, morfológicos y de distribución (estudiada con mapas

detallados en proyección UTM), obtendremos una idea clara de la taxonomía del grupo. Así *A. ripartii* se encuentra desde Cataluña hasta el Sistema Cantábrico, siempre al norte del Sistema Ibérico, *A. fabressei* vive en el Sistema Ibérico y sierras andaluzas, *A. fulgens* está restringida a unas pocas localidades catalanas y *A. ainsae* vive en los Pirineos Centrales y Occidentales y los Montes Cantábricos. La recientemente descrita *A. violetae* se considera una subespecie de *A. fabressei* por su similar número y morfología cromosómicas. El grupo parece haber evolucionado muy rápidamente desde el punto de vista cariológico, mientras que las diferencias morfológicas y ecológicas no son tan patentes.

Introduction

The taxonomy of the genus *Agrodiaetus* Hübner, [1822] is one of the most complicated among Palaearctic butterflies. The genus lives mainly in the Mediterranean and Middle East, but can also be found in Central Europe and Russia (HIGGINS & HARGREAVES, 1983 ; KUDRNA, 1986). The biology is similar in the different species of the group, the larvae use sainfoin (*Onobrychis*) as foodplants, and overwinter in this stage, feeding during the following spring on the young leaves of the plant (SCHURIAN, 1976 ; Lepidopterologische Arbeitsgruppe der Schweiz, 1987 ; MANINO *et al.*, 1987; MUNGUIRA, unpublished data). Pupation takes place at the base of the plant and adults begin to fly usually after a month. The flight period ranges from July in hotter places to August in populations living at higher altitudes.

Interest in the group lies in the controversial taxonomic position of most species. Before chromosome studies were made, morphological research attempted to provide a clear view of the systematics and taxonomy of the group. Some comprehensive reviews attempted this difficult task (FORSTER, 1961), but the confusion generated by this approach proved the inadequacy of the methods based solely on morphological analysis. Chromosomal studies started by de Lesse in 1952, clearly showed that morphology itself was not enough to understand relationship among the species (DE LESSE, 1960a). Unfortunately these studies are not a panacea either and the species' chromosome numbers also show a complicated pattern, with very different chromosome numbers in otherwise identical taxa. This shows how hard it is to give simple answers to difficult questions in a group that is undergoing a splitting process at the moment we are studying it. For example, in Italy the taxa previously grouped under the name *Agrodiaetus ripartii* (FREYER, 1830) has been split into three different species with different chromosome numbers : *A. humedasmae* Toso & Balletto, 1976

with $n = 38$; *A. galloi* Balletto & Toso, 1979 with $n = 66$; and *A. ripartii* with $n = 90$ (TROIANO *et al.*, 1979).

AGENJO (1947) lists the Spanish species as *Plebejus (Agrodiaetus) damon* (Denis & Schiffermüller, 1775), *P. dolus* (Hübner, [1823]), *P. admetus* (Esper, 1785) and *P. ripartii*. FORSTER (1961) describes *ainsae* as a subspecies of *dolus* and considers *Agrodiaetus* as an independent genus. Following the popular book by GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO (1974) the Iberian species of the genus are *Agrodiaetus damon*, *A. dolus*, *A. fabressei* (Oberthür, 1910), *A. ripartii* and *A. ainsae* Forster, 1961, to which *A. vioetae* Gomez-Bustillo, Expósito & Martínez, 1979 was added later as a new species. *A. fabressei* corresponds to what AGENJO (1947) named *P. admetus*, now known to be restricted to Eastern Europe and Asia Minor. There is a tendency to split the species of the group into new species whose validity has been discussed in several reviews (see for example FERNÁNDEZ-RUBIO, 1992). HIGGINS (1975) also considers within the genus the species *A. amanda* (Schneider, 1792), *A. thersites* (Cantener, 1834) and *A. escheri* (Hübner, [1823]), but although this inclusion has strong arguments in its favour, the resulting genus is less homogeneous. KUDRNA (1986 : 161, 229-231) in the latest European checklist grouped the *Agrodiaetus* within the genus *Polyommatus* and listed the following species as present in Spain : *P. ainsae*, *P. damon*, *P. dolus*, *P. fabressei*, *P. ripartii*, and *P. vioetae*. The first three taxa have blue males and the last three brown males. The inclusion of *Agrodiaetus* in *Polyommatus* is not supported by recent research (LELIEVRE, 1992) and we do not assume it for simplicity.

We centered our study only in the *Agrodiaetus sensu stricto* group (following HIGGINS & RILEY, 1970), but excluded *A. damon* which is a fairly distinct species that has never been mistaken with the others anywhere in its range. The objectives of our study were to identify the specific entities of the *Agrodiaetus* group living in the Iberian Peninsula based on cytotaxonomical and morphological information and to give accurate UTM distributions for every species.

Materials and methods

Chromosome number studies were made on male testes of at least five specimens for each species and/or geographical area. Testes were dissected *in situ* under a stereomicroscope in distilled water and fixed with a solution of ethanol : acetic acid (3:1). They were kept at ca. 5°C until analysis was possible. After staining the samples with lacto-propionic orceine they were observed under a phase contrast microscope

to count chromosomes using the squash technique (LORKOVIC, 1990) with small fragments of the testes. Brown males were studied from Sierra de Cazorla (SW Spain), Sistema Ibérico (Central Spain), Catalonia (NE Spain) and the Cantabrian Mountains (N Spain). Blue males were studied from Catalonia and the Pyrenees. This covered all the taxa previously described and the geographic range of the group in the Iberian Peninsula and was considered enough to give an idea of the chromosome numbers for each area and species.

Distribution data were collected using faunistic records from the literature and from specimens in the collections of the following Spanish institutions: Museo Nacional de Ciencias Naturales, Sociedad de Ciencias Naturales Aranzadi, Museo de Ciencias Naturales de Vitoria, Museo de Zoología de Barcelona and Universidad Autónoma de Madrid. Private collections from Fidel Fernández-Rubio, José Luis Yela, Arcadi Cervelló, José María Font and José Luis Nuñez and personal communications from Albert Masó, José Bellavista and Francisco Abós were used for distribution records. Data were also gathered visiting a total of 36 localities from 12 different Provinces in which specimens were taken for morphological analysis. The specimens used for this study are preserved in the zoological collection of the Universidad Autónoma de Madrid (UAMZ). A database was created to process all the faunistic data with information on localities, provinces, dates, UTM coordinates, altitudes and bibliographic references. This is available upon request for anyone interested, but its volume (more than 700 records) made its inclusion in this paper impossible.

Maps were produced using a commercial program for automated cartography (CYANUS) for the Iberian Peninsula and Balearic Islands. Each record was assigned to a particular species using karyological data for the localities from which chromosome studies were made. For the rest of the localities identification was based on geographical and morphological affinities with the former records. The morphological analysis of the records for which we had specimens, showed that the individuals identified by this method had the typical morphology of the species to which they were assigned.

The biology of the group was studied at the different localities visited, where we gathered data on foodplants, overwintering stages and immature stage morphology.

Table 1

Localities, sample size and chromosome numbers of the studied material and all the literature references for *Agrodiaetus* species in the Iberian Peninsula.

"No. males" refers to the number of males that produced metaphase I plates suitable for chromosome counts and the total sample is given in brackets.

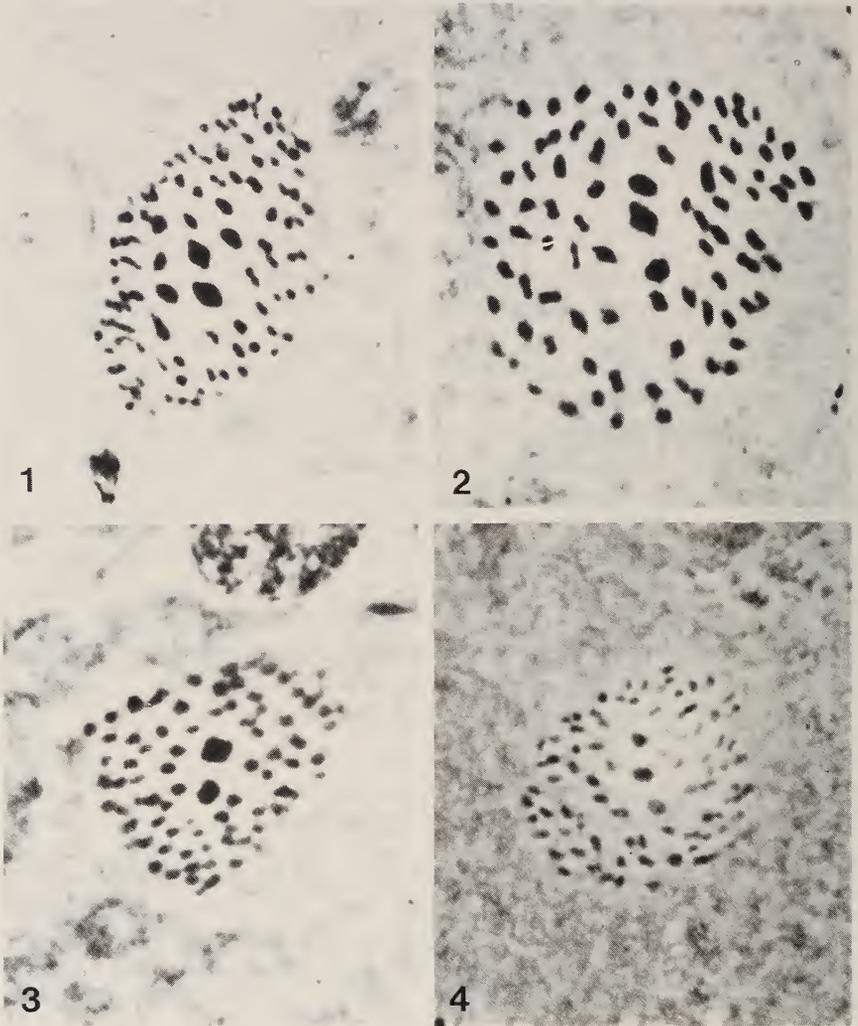
References are as follows : (1) DE LESSE, 1960b (2) DE LESSE, 1961a (3) DE LESSE, 1962 (4) DE LESSE, 1968 and (*) our own observations

| Locality | Province | No. males | Chr. No. | Macrochr. | Species | Ref. |
|--------------|-------------|-----------|-----------|-----------|------------------|------|
| Villanueva | Burgos | 2 | 108 | ? | <i>ainsae</i> | 3 |
| Peñahorada | Burgos | 1 (1) | ? | 2 | <i>ainsae</i> | * |
| Bernués | Huesca | 3 | 108-110 | ? | <i>ainsae</i> | 3 |
| Jaca | Huesca | 1 (2) | — | — | <i>ainsae</i> | * |
| Taradell | Barcelona | 3 (5) | 103 | 6 | <i>fulgens</i> | * |
| Noguera | Teruel | 8 | 90 | 4 | <i>fabressei</i> | 1 |
| Albarracín | Teruel | 1 (9) | — | — | <i>fabressei</i> | * |
| Peñalén | Guadalajara | 1 (2) | 90 | 3 | <i>fabressei</i> | * |
| Cazorla | Jaén | 6 (12) | 90 | 3 | <i>fabressei</i> | * |
| Morella | Castellón | 1 | 90 | 4 | <i>fabressei</i> | 2 |
| Villarroya | Teruel | 1 | 90 | 4 | <i>fabressei</i> | 2 |
| Olocan | Castellón | 2 (15) | 90 | 2 | <i>ripartii</i> | 2 |
| Amorós | Barcelona | 4 | 90 | 2 | <i>ripartii</i> | 4 |
| Collsuspina | Barcelona | 1 (2) | ca. 88 | 2 | <i>ripartii</i> | * |
| Taradell | Barcelona | 3(10) | ca. 88 | 2 | <i>ripartii</i> | * |
| Santa Coloma | Tarragona | 0 (2) | — | — | <i>ripartii</i> | * |
| Peñahorada | Burgos | 1 (6) | 90 | 2 | <i>ripartii</i> | * |
| Gredilla | Burgos | 2 (5) | ca. 85-90 | 2 | <i>ripartii</i> | * |
| Jaca | Huesca | 1 (2) | ca. 88 | 2 | <i>ripartii</i> | * |
| Jaca | Huesca | ? | ? | 2 | <i>ripartii</i> | 2 |

Results

Chromosome numbers in different populations

Table 1 gives a summary of the data from our study pooled with data from DE LESSE's 1960b, 1961a, 1962 and 1968 papers. From these results it is clear that the best genetic marker for the identification of the species of the group in the Iberian Peninsula is the number of macrochromosomes (see WHITE, 1973 for the use of the term, that appears as "gros chromosomes" in DE LESSE, 1960b). These can be defined in Lepidoptera as chromosomes with two to four times the normal size, located in the centre of metaphase I plates and surrounded by normal-sized chromosomes (Figs 1-4). The location of macrochromosomes contrasts with their peripheral location in Orthoptera, amphibians and reptiles (WHITE, 1973). In lycaenids the number of macrochromosomes is always low while in the other groups it usually outnumbers that of microchromosomes (see WHITE, 1973 for compa-



Figs 1-4. Metaphase I plates. 1 — *Agrodiaetus fulgens*. (Taradell, Barcelona Province, type locality for species) $n = 103$, six macrochromosomes ; 2 — *Agrodiaetus fabressei* (Peñalén, Guadalajara Province), $n = 90$, three macrochromosomes ; 3 — *Agrodiaetus ripartii* (Collsuspina, Barcelona Province), $n = \text{ca. } 88$, two macrochromosomes ; 4 — *Agrodiaetus fabressei violetae* (Sierra de Cazorla, Jaén Province), $n = 90$, three macrochromosomes.

ri-son), and it is probable that these configurations depend on mechanical aspects of the cell division process. The number of chromosomes is also distinctive, but the metaphase I plates of *Agrodiaetus* have large chromosome numbers and accurate counts are difficult in most cases. The results from Table 1 support the following species' arrangements: *A. ainsae* with $n = ca. 108$ lives in the Pyrenees and a wide area surrounding the Cantabrian Mountains, whereas in Catalonia the specimens previously assigned to *A. dolus* should now be considered a distinct species. The name *A. fulgens* (Sagarra, 1925) is valid for this species, because it was given to what was thought a subspecies of *A. dolus* in the same localities where the samples for chromosome studies were taken. The reason for considering *fulgens* a distinct species is the different chromosome number ($n = ca. 103$, while *dolus* has $n = 124$ and *ainsae* $n = 108$) and above all, the different number of macrochromosomes of these specimens (six for *fulgens* (Fig. 1) as opposed to four in *dolus*, DE LESSE, 1961b). de Lesse (1962, 1966) only gives the chromosome number for *A. ainsae* and does not provide figures for it, but our specimen from Peñahorada (Burgos Province, northern Spain) had two macrochromosomes although the metaphase I plates we obtained were not suitable for accurate chromosome counts. The number of macrochromosomes is therefore six in *A. fulgens*, four in *A. dolus* and two in *A. ainsae*. Although our sample for *fulgens* is very small (only 3 specimens with metaphase I plates), the separation of *ainsae* from *dolus* was made with similar samples (five specimens, DE LESSE, 1962). Moreover it is not chromosome number, but the number of macrochromosomes that we consider to be a strong argument to split *fulgens* from *ainsae*, because it is highly improbable that specimens with such a different chromosome morphology can belong to the same species.

A. ripartii and *A. fabressei* share the same chromosome number ($n = 90$) but clearly differ in the morphology of chromosomes, *fabressei* having three (Fig. 2) and *ripartii* two (Fig. 3) macrochromosomes. DE LESSE (1960a, Table 1) states that *fabressei* has four macrochromosomes, but after examining our photographs and drawings we have concluded that the species clearly has three large chromosomes constantly seen in all the metaphase I plates. The difference between de Lesse's results and ours is due to the existence of one or two medium sized chromosomes that in some plates resemble macrochromosomes, but are driven out of the centre of the spindle in a majority of our figures from *fabressei*. Large chromosomes are always located in the centre of metaphase I plates, and they are easily spotted when comparing long series of plates. This has been possible with the material from the Sierra

de Cazorla, previously regarded as *A. violetae*, where the presence of three macrochromosomes is clear (Fig. 4). The latter race has therefore a true *fabressei* karyotype, which supports the idea outlined in FERNÁNDEZ-RUBIO (1992) that it is a subspecies of *fabressei* and not of *ripartii*, as has been proposed (BALLETO, pers. comm.). The distribution of both karyotypes seems to be clearcut from the data in Table 1. *A. ripartii* is a species living from the Cantabrian Mountains to the Catalonian Pyrenees whereas *A. fabressei* lives in central Spain and in some southern mountain ranges.

Morphology

There is not a single character clearly separating all *ripartii* specimens from *fabressei*, and *ainsae* from the Catalonian species *fulgens*. In the first case a group of characters can identify most of the specimens, but in the latter this proves to be very difficult. Generally speaking *fabressei* lacks the white band along the v4 on the hind wing's underside, but there is a great variability on this character. Traditionally brown males without the white band have been regarded as *fabressei*, and this has produced records of the species in the Pyrenees (GÓMEZ, 1988) and Catalonia (GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO, 1974). The book by MANLEY & ALLCARD (1970) is unusual because it illustrates an individual with white band taken at Peñahorada (Burgos Province) under the name *fabressei* and a very similar specimen (with white band) from Albarracín (Teruel) as *ripartii*. These identifications are in disagreement with our karyological results. On the other hand a white band is also present in some *fabressei* specimens leading some authors to consider these as *ripartii* (GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO, 1974; MANLEY & ALLCARD, 1970 for the male collected in Albarracín, Teruel). The Catalonian race *agenjoi* Forster, 1965 has been considered a different species (HIGGINS & HARGREAVES, 1983), a subspecies of *fabressei* (MANLEY & ALLCARD, 1970; GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO, 1974) or a subspecies of *ripartii* (AGENJO, 1964; DE LESSE, 1968, PÉREZ, 1979). Black spots on the underside are larger in *fabressei* than in the normal *ripartii*, but again the Catalonian specimens are an exception to this and resemble typical *fabressei*. Another distinctive feature of *fabressei* has traditionally been the enlargement of the black spot between v2 and v3 in the forewing's underside (DE LESSE, 1968), but our material from the whole geographic range of both species does not support the distinctness of this feature. The apical angle of the forewing is smaller in *fabressei* than in *ripartii*. Although this seems to be a constant character, it is hard to measure and by itself is not useful enough to identify all the specimens clearly. The third supposed

species with brown males, *A. violetae*, has a mixture of morphological characters from *ripartii* and *fabressei* with a white band in the underside of the hindwing (absent in six of our sample of 22 butterflies) and a small angle in the apex of the forewing. As a result of its chromosome morphology we consider it a subspecies of *A. fabressei*. Although there are exceptions to all the characters mentioned above, most of the specimens can be identified by a mixture of characters. Therefore specimens without white band in the underside, large spots in the underside and small apical angle can be considered *fabressei*, provided they are in the distribution range of the species. Individuals outside the normal species' range need chromosome study to be sure of their identity.

As far as the species with blue males is concerned identification is also difficult. *A. fulgens* has traditionally been regarded as a race with brighter blue and lack of the white band in the hindwing's underside. These two features are useful for most specimens, but again a substantial morphological variation takes place in *ainsae* for both characters, making individual diagnosis difficult in some cases.

Distribution

Given that it is very difficult to identify the species of the group based on morphological features, we considered the possibility to use both chromosome numbers and distribution data to assign a given individual to a species of the group. In almost all the studied northern localities, three *Agrodiaetus* species live together: *damon*, which is a well characterized distinct species, a species with blue males and a species with brown males. We assumed that each area has a single brown and/or blue species (this was confirmed by the chromosome study) and by mapping the species tried to define areas isolated by barriers separating species. In the localities of the Sistema Ibérico (Central Spain) only a species with brown males flies together with *damon* and the chromosome studies revealed this to be *fabressei* (DE LESSE, 1960b, and our observations, Table 1).

A. fabressei (Fig. 5) is therefore restricted to the Sistema Iberico, a mountain range running from north to south in Central Spain and to the Sierras of Andalusia. The species lives in the Serranías of Cuenca and Albarraçín, the high plains of La Alcarria, some calcareous outcrops in Segovia Province and north of Madrid, and reaches the Province of Soria in the north, being isolated from *ripartii* by the Picos de Urbión, whose northern slopes flow down to the Ebro Valley. *A. fabressei violetae* is found in a total of twelve UTM squares (10×10 km)

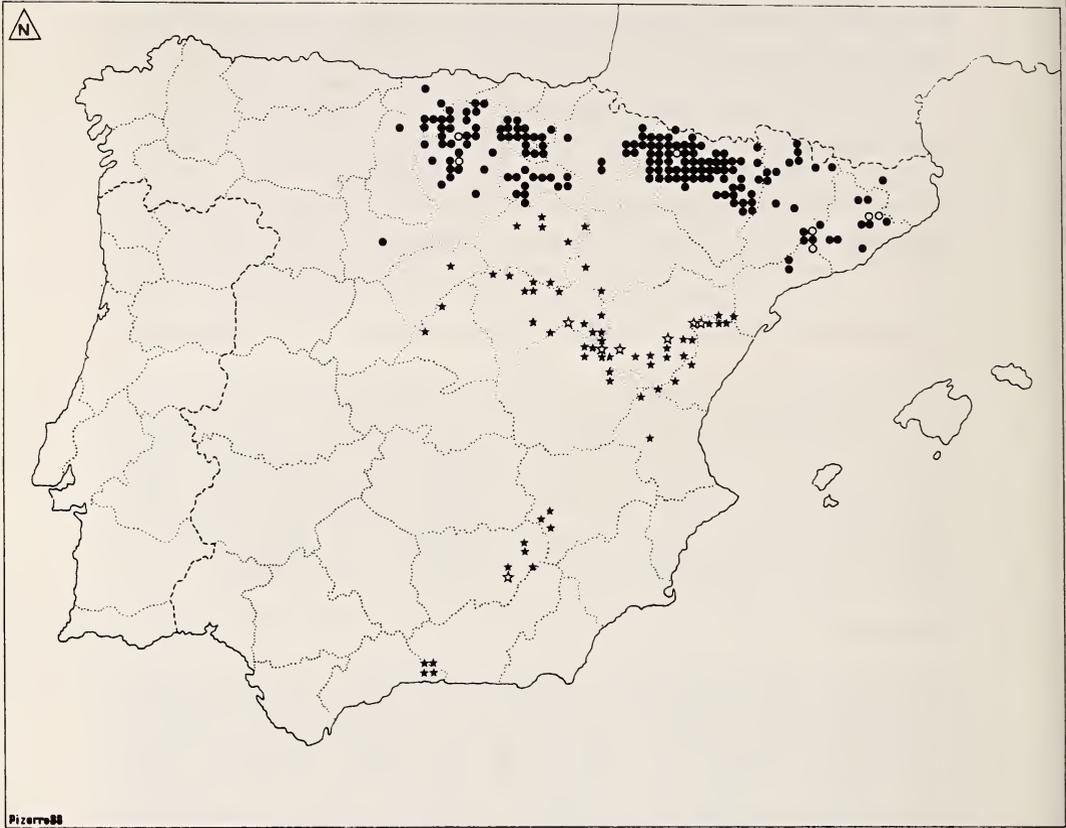


Fig. 5. Distribution of *Agrodiaetus ripartii* (circle) and *A. fabressei* (star) in the Iberian Peninsula. Each symbol represents the presence of the species in a 10×10 km UTM square. Open symbols represent localities where chromosome studies were available.

from four mountain ranges in Southern Spain (Sierra Tejada, Sierra de Almirajara, Sierra de Cazorla and Sierra de Alcaraz).

A. ripartii (Fig. 5) lives over a wide area, from Catalonia through the Pyrenees to the Cantabrian Mountains, where it lives mainly on the southern slopes. Two subspecies can be distinguished: the Catalanian *agenjoi* and the Pyrenean and Cantabrian *ripartii*, separated by an area that more or less matches the boundary between the Pyrenees and a mountain range running from north to south in eastern Catalonia (Serralada Vertical) in which the species becomes scarce (Fig. 5). DE LESSE (1961a) describes the presence of specimens with a typical *ripartii*



Fig. 6. Distribution of *Agrodiaetus ainsae* (circle) and *A. fulgens* (star) in the Iberian Peninsula. Each symbol represents the presence of the species in a 10×10 km UTM square. Open symbols represent localities where chromosome studies were available.

karyotype in Olocau del Rey (Castellón Province), an area where *fabressei* is widespread. If this finding is confirmed it may be possible that *ripartii* has some populations more or less mixed with *fabressei* colonies in this contact zone.

A. ainsae (Fig. 6) is the most widespread species with blue males in this area. Specimens from the western Pyrenees and the Cantabrian mountains belong to this species, but not a single locality has been found south to the Picos de Urbión, suggesting that the southern limit for *ripartii* is also valid for the blue species of the group.

A. fulgens (Fig. 6) has until now been considered a subspecies of *dolus* (GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO, 1974, HIGGINS, 1975). It has only been cited from a handful of localities in Catalonia and between the strongholds of this species and the previous one there is a wide area with scarcity of records that can be seen when the distribution of both species is plotted together (Fig. 6). It should be noted that this lack of records is not a consequence of lack of information, for this is a well explored area.

Biology

All the studied species use *Onobrychis* as larval foodplants. In the Catalanian localities, the Pyrenees, the Sistema Ibérico and the Cantabrian Mountains the foodplant is always *O. viciifolia* Scop. The only population that uses a different foodplant is *A. fabressei violetae* in the Sierra de Cazorla. Its foodplant is *O. argentea* Boiss., but we do not consider this difference to be relevant with respect to its taxonomic status, because the two plants are ecologically similar, and *O. argentea* is the only species of this plant genus living in Sierra de Cazorla. In all other aspects the biology of the studied species is very similar. They all overwinter as third instar larvae (we studied this for *fabressei*, *ripartii* and *ainsae*) and pupate during the spring at the base of the foodplant. The fullgrown larva is very similar in species living in the same area. Thus we were unable to distinguish between *A. fabressei* and the very different *A. damon* in Albarracín (Teruel Province), and between *A. ripartii* and *A. ainsae* in Jaca (Huesca Province). All the larvae have tentacles and dorsal nectary organs (Newcomer's gland), and the coloured band in the lateral zone of the larva can have different colours, being yellow in the specimens from Jaca in the Pyrenees and pink in the larvae from Albarracín. Whether this can be a distinctive feature between *ripartii* and *fabressei* or just a character related to the ecological peculiarities of the site requires a more comprehensive study. Egg morphology is being studied under the SEM microscope by two of us (MUNGUIRA & MARTÍN, in preparation) and all the species exhibit a very similar chorionic pattern, with no distinctive features among them.

Discussion

Previous results have shown how complicated a taxonomic study of this difficult butterfly group can be. Although butterfly taxonomy in Europe is often regarded as being settled, there are some groups in which a great deal of research is still needed. Two of the main

taxonomic groups within which the boundaries between species are not yet well defined are found in *Agrodiaetus*, and *Polyommatus* (*Lysandra*) of the *coridon* (Poda, 1761) group (DE BAST, 1985 ; MENSI *et al.*, 1988 ; LELIEVRE, 1992).

Our approach to the taxonomy of the group was to begin to identify the different karyotypes found in the Iberian Peninsula. Then we tried to study as many areas as possible to assign karyotypes to relevant areas from the biogeographical point of view. Plotting this information with distribution maps, we tried to draw accurate maps of every species. The separation of species in contact areas such as the boundaries between *A. ainsae* and *A. fulgens* is still tentative and a karyological study of almost every locality is needed to be completely sure. The limit between *A. ripartii* and *A. fabressei* also needs some further study, but in this case we think that our proposal is closer to reality because the morphology of specimens from Abejar (Soria) resembles the typical *fabressei*, with most butterflies lacking the white band on the hind-wing's underside (MANLEY & ALLCARD, 1970). On the other hand the specimens from the northern slopes of the Sistema Ibérico (e. g. Castañares de las Cuevas in La Rioja) are morphologically true *ripartii* with the white band present in all the studied material. Some research needs to be done on the identity of some populations close to the town of Morella in central eastern Spain, where DE LESSE (1961a) identified karyotypes belonging to *fabressei* and *ripartii* in nearby populations, but never in the same one. This area may prove to be a contact zone for the two species, and the segregation of populations may not be as clearcut as depicted in the distribution maps. In a locality between the towns of Olocan and Forcall, de Lesse (1961a) found males with a typical *ripartii* karyotype and a single male with *fabressei* morphology, for which a chromosome study was not possible. Speaking of this *fabressei* specimen he writes that "on a vu qu'aucun doute ne peut exister sur son identité". Therefore a contact area between the two species might exist in Morella, but this statement has to be proven through more chromosomic studies. On the other hand, although some authors have reported the two species from the same locality, nobody has yet identified specimens of the two different karyotypes living together.

Populations having different chromosome numbers are usually thought to belong to different species. Although DE LESSE (1960a ; 1966) is very cautious to split species by their distinct chromosome numbers, his data have always been used as a proof to make new species' arrangements (GÓMEZ-BUSTILLO & FERNÁNDEZ-RUBIO, 1974 ; HIGGINS, 1975). Some intraspecific variation in chromosome numbers and chromosome

morphology can not be plainly rejected (WHITE, 1973), particularly in such similarly looking and difficult groups as the *Agrodiaetus*. Nevertheless, before a more thorough study is done on the significance of chromosome number variability in the evolution of lycaenids, we assume it is safer to assign populations with different chromosome numbers to different species. This approach was made in most chromosomal studies in butterflies, as for example to support the separation of *A. humedasa* (TROIANO, *et al.*, 1979), to split a species complex in several species previously regarded as identical (SOUMALANIEN & BROWN, 1984) or as a general rule to explain chromosome number variability (see WHITE, 1973). On the other hand a karyological feature of great taxonomic importance has emerged during our study: the variability of the number of macrochromosomes observed in the centre of metaphase I plates. This character was previously used by DE LESSE (1960b), but our data prove that it is the best genetic marker to identify the species of *Agrodiaetus* in the Iberian Peninsula. We can therefore recognize *fulgens* by its six macrochromosomes and separate it from *ainsae* that has only two, whereas the species with brown males differ in having two (*ripartii*) or three (*fabressei*) of these large chromosomes.

As a conclusion to our study, the Spanish *Agrodiaetus* with their chromosome numbers and distribution are: *A. ripartii* ($n = 90$, two macrochromosomes) living from Catalonia to the Cantabrian Mountains, *A. fabressei* ($n = 90$, three macrochromosomes) living in the Sistema Ibérico (South to the Picos de Urbión), the limestone outcrops of Central Spain and some southern mountain ranges (Sierra Almirajara, Cazorla, Alcaraz and Tejada), *A. ainsae* ($n = 108$, two macrochromosomes) from the Pyrenees and Northern Spain and *A. fulgens* ($n = \text{ca. } 103$, six macrochromosomes) living in Catalonia.

Acknowledgements

We are very grateful to Fidel Fernández-Rubio, Arcadi Cervelló, José María Font, José Luis Nuñez and José Luis Yela for kindly giving access to their private collections. Isabel Izquierdo, Ibón de Olano and Jesús Aldaba gave facilities for the revision of public collections and Albert Masó and Francisco Abós provided unpublished distribution records. José Serrano and Jaime Gosálvez helped us in many ways with cytogenetical analysis, discussion of data and giving access to their laboratories. Enrique García-Barros made valuable suggestions to a previous draft of the manuscript.

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Jahr/Year: 1994

Band/Volume: [17](#)

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Artikel/Article: [Karyology and distribution as tools in the taxonomy of Iberian Agrodiaetus butterflies \(Lepidoptera : Lycaenidae\) 125-140](#)