

On the dimorphism shown by the females of *Boyeria irene* (Odonata: Aeshnidae) in a southern Spain population

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Abstract

A special feature of *Boyeria irene* is a dimorphism of the females, which can have long (f. typica) or short (f. brachycerca) cerci, which occur at different frequencies. The aim of this work was to investigate whether there is a seasonal change in the occurrence of the two female forms. The exuviae investigated here belong to a population that lives in a permanent highland stream in the central part of the Sierra Morena in the province of Córdoba (Spain) in the southern part of the Spanish distribution area. Female exuviae of the brachycerca form accounted for 76.4 to 85.2% in the four years examined, while the annual frequency of the typica form varied between 14.8 and 23.6%. In two of the study years, the proportion of the typica form decreased during the emergence period. Obviously, this is not a pattern that always occurs, but it must nevertheless be considered in the further analysis of the numerical relationships between the female forms.

Resumen

Sobre el dimorfismo mostrado por las hembras de *Boyeria irene* (Odonata: Aeshnidae) en una población del sur de España – El dimorfismo existente en las hembras de *Boyeria irene*, hembras con cercos largos (f. typica) o cortos (f. brachycerca) que aparecen con frecuencias muy diferentes, es una característica especial de esta especie. El objetivo de este trabajo fue investigar si hay una tendencia temporal en la proporción en que se encuentran las dos formas de hembras a lo largo del periodo de emergencia de cada año. Las exuvias aquí estudiadas pertenecen a una población que se desarrolla en un arroyo de montaña permanente, situado en la parte central de Sierra Morena, en la provincia de Córdoba (España), hacia la parte sureña del área de distribución española de esta especie. Las exuvias hembra de la forma brachycerca supusieron del 76,4 al 85,2% en los cuatro años estudiados, mientras que la frecuencia anual de la forma typica osciló entre 14,8 y 23,6%. En dos de los años del estudio, la proporción de la forma typica fue disminuyendo a

lo largo del periodo de emergencia. Aunque no fue un patrón que haya ocurrido siempre, debería ser considerado en posteriores análisis de las proporciones numéricas entre las dos formas de hembras.

Zusammenfassung

Zum Dimorphismus der Weibchen von *Boyeria irene* (Odonata: Aeshnidae) in einer südspanischen Population – Ein spezielles Merkmal von *Boyeria irene* ist ein Dimorphismus der Weibchen, die lange (f. *typica*) oder kurze (f. *brachycerca*) Cerci haben können, die in unterschiedlichen Häufigkeiten auftreten. Ziel dieser Arbeit war es, zu untersuchen, ob es eine saisonale Veränderung des Auftretens der beiden Weibchenformen gibt. Die hier untersuchten Exuvien gehören zu einer Population eines permanenten Hochlandbaches im zentralen Teil der Sierra Morena in der Provinz Córdoba im südlichen Teil des spanischen Verbreitungsgebiets. Exuvien der *brachycerca*-Form machten in den vier untersuchten Jahren 76,4 bis 85,2 % aus, während die jährliche Häufigkeit der *typica*-Form zwischen 14,8 und 23,6 % variierte. In zwei der Untersuchungsjahre nahm der Anteil der *typica*-Form während der Emergenzperiode ab. Dies ist offenbar kein immer auftretendes Muster, muss aber trotzdem in der weiteren Analyse der Zahlenverhältnisse zwischen den Weibchenformen beachtet werden.

Introduction

Boyeria irene (Fonscolombe, 1838) ranges from Central Europe to northern Africa, and some western Mediterranean islands (ASKEW 2004; BOUDOT et al. 2015: 181–182). It persists only in one or two populations in Germany (CLAUSNITZER et al. 2010), and the number of populations in the Maghreb, where it is considered a “Near threatened” species, seems very diminished (SAMRAOUI et al. 2010; KORBAA et al. 2014). It belongs, *sensu lato*, to a chorological type restricted to the western Mediterranean (FERRERAS-ROMERO 1999; BOUDOT et al. 2009: 107), except in Morocco, where the southernmost population known worldwide has been found on the southern slopes of the Anti-Atlas (BOUDOT & DE KNIJF 2012; BOUDOT et al. 2015: 181–182). The species develops in lotic waters, in headwater courses of middle mountains, where the larvae live as claspers hidden among stones, in coarse detritus (twigs) or among tree roots (LEIPELT & SUHLING 2001; WILDERMUTH 2005). In southern Spain *B. irene* is mainly a semivoltine “summer species” (*sensu* CORBET 1954, 1964), the larvae spend the winter before emergence in the last four stadia (mostly in F-1 and F-2), emergence is protracted and asynchronous, and eggs overwinter (FERRERAS-ROMERO 1997). There is also interesting information about egg development, foraging and reproductive behaviour in Spain, France, and Germany (WENGER 1955, 1963; JURZITZA 1967; MILLER & MILLER 1985; WILDERMUTH 2000, 2005; SUHLING & SUHLING 2018).

A well-known but yet unexplained feature of *B. irene* is the existence of female dimorphism regarding the length of the cerci in adults and in the last stadium larvae (WENGER 1959; PETERS 1987), which is also well visible in the exuviae. Females with long cerci are termed *forma typica* and those with short cerci *forma brachycerca* (NAVÁS 1919; WENGER 1959). Some publications on exuviae collections, carried out from southern France to Morocco and Tunisia, have provided very disparate data on the proportions in which both forms appear (VICK 1984; JACQUEMIN 1985; MIKOLAJEWSKI et al. 2000; KUNZ 2005). The females of the other European species of this genus, *B. cretensis* Peters, 1991, possess uniformly short cerci (MÜLLER et al. 2012). Likewise, the American *B. vinosa* has two female forms while the second American species *B. grafiana* has only short appendices. By contrast, the females of the Japanese *B. maclachlani* have only long appendices (WENGER 1959). From the other East Asian *B. sinensis* and *B. karubei* it is not known whether they have female dimorphism; figures of female *B. karubei* show short cerci (ZHANG & TONG 2011; CHO 2020).

The aim of this study was to ascertain whether there is important interannual variation in the proportions of the two female forms of *B. irene* at emergence, and also if some trend is shown along the emergence season. The exuviae here studied belong to a population developing in a permanent upland stream, situated in the Sierra Morena Mountains, southern Spain, towards the southern part of the species' geographical range, which ends in the Maghreb. Proportions of both forms here recorded were compared to those found in populations developing at higher and lower latitudes.

Methods

Study area

The present study was carried out using exuviae of the personal collection of MFR, sampled at the Bejarano Stream (37.9431° N, 4.8907° W), a watercourse in the Sierra Morena Mountains, close to Santa María de Trassierra, Córdoba, Andalusia, Spain. The study site was a small, permanent, mountain stream, about 400 m a.s.l., with a closed canopy of trees along most of its length. Alder *Alnus lusitanica*, elm *Ulmus minor*, chestnut *Castanea sativa* and hazel *Corylus avellana* grew along the banks. In the watercourse here studied, three other species of Odonata existed as stable populations: *Calopteryx haemorrhoidalis*, *Onychogomphus uncatus*, and *Cordulegaster boltonii* (FERRERAS-ROMERO & CORBET 1999; FERRERAS-ROMERO et al. 1999, 2000). At the sampling site the stream's width was 2–5 m and its mean water depth was 35–50 cm. Additional data relating to abiotic attributes of this watercourse were recorded by FERRERAS-ROMERO (1997). At each visit in 1992, water temperature in the stream was recorded to the nearest 0.5°C, most of the time at 09:00 G.M.T., from 10 May (before the first exuviae were collected, 15.5°C) to 2 October (16.0°C). The highest water temperatures recorded were 19°C (3 and 18 August) and 18.5°C (19 and 29 July, and 23 August), and the lowest

Table 1. Annual sums of exuviae of *Boyeria irene* recorded in southern Spain. – **Tabla 1.** Comparación de las cantidades anuales de exuvias de *B. irene* recolectadas en el sur de España. – **Tabelle 1:** Jährliche Exuvienzahlen von *B. irene* mit Anteilen von Weibchen und f. typica-Exuvien in Südspanien.

Exuviae	1989	1990	1991	1992	Σ
Total numbers	338	190	670	1,054	2,252
% female	54.1	53.2	56.9	53.1	54.4
% f. typica	20.8	14.8	23.6	19.1	20.4

were 15.0°C (4 June) and 14.5°C (27 September). When the first exuviae were found (15 May) water temperature was 17.5°C.

Exuviae collection

During late spring and summer of the years 1989 to 1991, exuviae (of final-instar larvae) were collected fortnightly. In 1992, from 25 April to 12 October, sampling effort was tripled to five-day intervals. The temporal pattern of emergence is described in an earlier publication (FERRERAS-ROMERO & CORBET 1995).

Identification of the female forms

WENGER (1959) showed that female larvae of *B. irene* can be readily separated into the two forms by examination of the lengths of the cerci, i.e. the form with very short anal appendages (f. brachycerca) and the type form (f. typica) with long appendages, which is usually considered to be rare (AGUESSE 1968). The two forms are very distinct: appendage lengths are about 2 and 6 mm respectively for the two forms (VICK 1984). ROBERT (1958), in his description of the larva, says »les app sup. (cerci) atteignent le tiers ou les trois-cinquièmes des cerques...«. VICK (1984) describes as a method for separation that »if the cerci were appreciably less than half the length of the paraprocts the specimen was f. brachycerca, if they were appreciably more than half the length of the paraprocts the specimen was the “long appendage” form«. As in Vick’s work cited, in the present study no doubtful intermediate specimens were found.

A set of 2,252 F-0 exuviae collected from 1989 to 1992 (Table 1) was inspected. In the laboratory, the sex of each exuvia was determined according to the presence (female) or absence (male) of gonapophyses on the ventral surface of the eighth and ninth abdominal segments. 1,225 exuviae were from female larvae. In the female exuviae, the anal pyramid (abdominal end) was inspected, looking for the relative length of cerci with respect to the paraprocts: $\frac{1}{3}$ (f. brachycerca) versus $\frac{3}{5}$ (f. typica) (ASKEW 2004), approximately, using a Nikon binocular microscope; 10× magnification is sufficient.

To compare records from different years, only samples with more than 15 female exuviae in the single samples or fortnightly records were considered, because at lower numbers of exuviae the samples were not representative and the proportions of the two female forms were very heterogeneous (Fig. 1). At both ends of the emergence period, lower frequencies were obtained, which were very heterogeneous and therefore considered not very consistent.

We plotted the proportions of forma typica females over time (fortnights). To analyse for trends of increase or decrease of forma typica females in each of the sample years we used Spearman rank correlations using PAST 3.22 software; significance was tested with an exact test (because $n < 9$) (HAMMER et al. 2001).

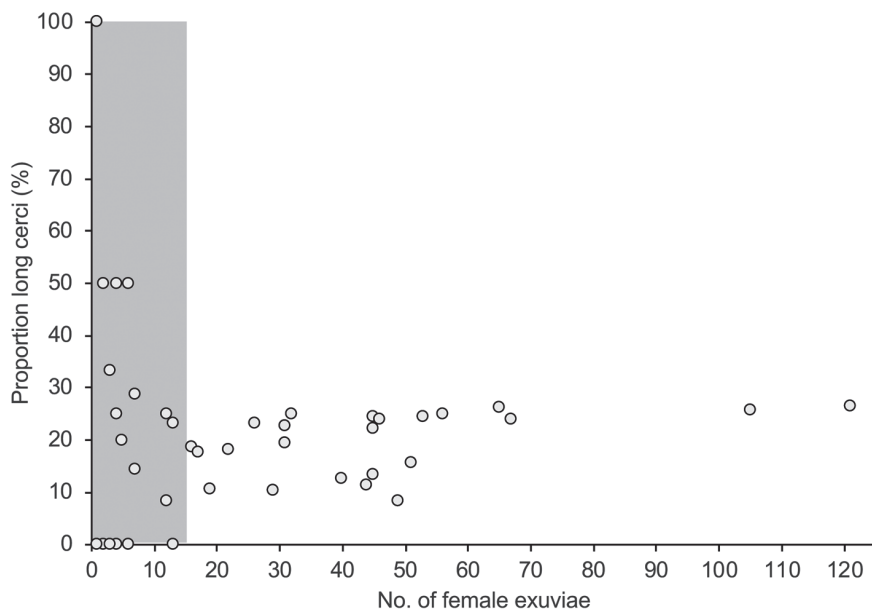


Figure 1. Proportion of exuviae of *Boyeria irene* with long cerci (f. typica) in relation to the total numbers of female exuviae found per sample occasion at Bejarano Stream in the Sierra Morena Mountains, Spain. Note that below 15 exuviae the proportions were highly heterogeneous varying from 0 to 100% (marked with hatched field). – **Figura 1.** Proporción encontrada de exuvias de *B. irene* con cercos largos (forma typica) en cada uno de los muestreos realizados en el arroyo del Bejarano, en la cordillera de Sierra Morena, España, respecto a la cantidad total de exuvias de larvas hembras. Se destaca el hecho de que por debajo de 15 exuvias las proporciones obtenidas fueron muy heterogéneas, variando desde 0 a 100% (área sombreada). – **Abbildung 1:** Anteil der Exuvien von *B. irene* mit langen Cerci (f. typica) in Relation zur Anzahl von geschlüpften Weibchen pro Sammeltermin am Bejarano, einem Bach in den Sierra Morena Bergen, Spanien. Bei weniger als 15 Exuvien wurden die Anteile sehr heterogen und schwankten von 0–100% (grau eingefärbte Fläche).

Results

Each year female exuviae were more numerous than male exuviae (Table 1). The emergence of both sexes was protracted, there being a “tail” along the second half of summer, resulting in a long flying season from mid-May to early October. The earliest exuviae were found on 15-v, in the years 1991 (2 females) and 1992 (4 males and 7 females). On 02-x-1992, the latest two exuviae were collected, both females. The number of female exuviae with long cerci (f. typica) was in total 250 (20.4%). Annual frequency of this form varied between 14.8 and 23.6%. In the year with the highest number of records (1992) the frequency was 19.1% (Table 1).

The frequency in the fortnightly samples with more than 15 female exuviae fluctuated between 10.5 (July first fortnight, 1990) and 26.4% (June first fortnight, 1991) (Appendix 1). In the single samples with more than 15 female exuviae, the highest and lowest percentages of f. typica varied between around 25% (maximum 26.4, 12-vi-1991, n (females) = 121) and 9% (minimum 8.2, 04-vii-1992, n = 49), respectively.

Over the seasons the proportions of f. typica varied (Fig. 2). In 1990 and 1992, the proportion of f. typica decreased throughout the central period of the emergence (late May to late July), (1990: $\rho = -1$, $p = 0.333$; 1992: $\rho = -0.975$, $p = 0.033$), and in 1992 this trend was also significant when for the analysis the second fortnight record of August was excluded (although $n = 16$, but outside central emergence period) (Fig. 2). However, in 1991 there was no trend in any direction ($\rho = 0.1$, $p = 0.333$); the frequencies were similar during the main emergence period. In 1989, there was an increase of f. typica with season, but only three fortnight-periods could be used in the analysis ($\rho = 1$, $p = 0.333$), thus the trend was not significant.

Discussion

It has long been recognized that in *B. irene* females the short cerci (brachycerca) form is more frequent than the long cerci (typica) form (AGUESSE 1968). However, some studies based on the collection of a low number of exuviae and reduced to a small part of the emergence period have shown opposite data. In late July 1979 (days 25 to 30), VICK (1984) found a significantly higher proportion (40.9%) of female exuviae with long cerci at River Hérault and its tributary, River Vis in southern France, but with a sample size of 22 female exuviae only. Likewise, on 23-vi-1983 JACQUEMIN (1985) collected 13 female exuviae along a small river on the Mediterranean coast of the Rif (North Morocco), eight of which (61.5%) were of the long cerci form. It could happen, circumstantially (or haphazardly), that in collections of small sample size the proportion of typica form has sometimes turned out to be particularly high. However, analysing a larger collection as of

mid of July 1999 at the Gardon river system in southern France the proportion of females with long cerci was about 47% ($n = 160$), while at the same time in the Aveyron catchment the proportion was only 12% ($n = 149$) (MIKOLAJESKI et al. 2000). Also, in Ribeira de Seixe, Algarve region (southern Portugal), 322 exuviae were collected on 4 and 20 August and 12 September (probably second half of the emergence period), and only 10 (7.1%) of the 140 studied female exuviae had long cerci (LOUREIRO 2014). KUNZ (2005) found only f. brachycerca in Tunisia ($n =$

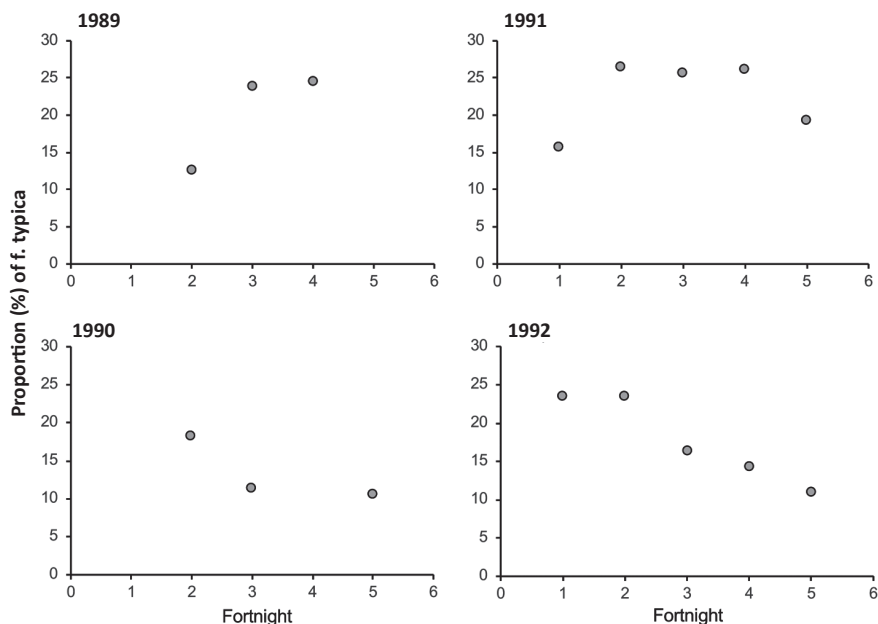


Figure 2. Proportions of f. typica exuviae of *Boyeria irene* over the seasons at Bejarano Stream in the Sierra Morena Mountains, Spain. The fortnight codes are as in Appendix 1. As a general rule, fortnights with less than 15 exuviae were not used for analyses, the 1992 August second fortnight record was excluded too ($n = 16$). – **Figura 2.** Proporción de exuvias de la forma típica de *B. irene* a lo largo de la época de emergencia en el arroyo del Bejarano, en la cordillera de Sierra Morena, España. Los códigos quincenales son los de la Tabla 2. Como regla general, las quincenas con menos de 15 exuvias recolectadas no fueron usadas para estos análisis, también fue excluido el registro de la segunda de agosto de 1992 ($n = 16$). – **Abbildung 2:** Anteile von f. typica-Exuvien von *B. irene* während der Schlupfsaison in den Jahren 1989–1992 am Bejarano, einem Bach in den Sierra Morena Bergen, Spanien. Die Codes der 15-Tages-Perioden sind in Appendix 1 angegeben. Periode mit weniger als 15 Exuvien wurden in der Darstellung nicht berücksichtigt; die zweite August-Periode 1992 ($n = 16$) wurde ebenfalls nicht ausgewertet, da außerhalb der Hauptschlupfzeit.

31). There may be local or regional differences in the occurrence of the two female forms. But, investigating this would require larger datasets from throughout the range of *B. irene* and needs to be discussed considering the biogeographical background, which has not yet been fully resolved, and dispersal routes of *B. irene* (cf. KOHLI et al. 2014).

The population in the Sierra Morena Mountains had an average frequency of ca 20% f. *typica*, hence somewhat in between the extremes. The difference to other datasets was that collection occurred throughout the emergence periods of four consecutive years (1989 to 1992), from first fortnight of May to first fortnight September, which permits analysing temporal variation among and within years. Both forms were present during all the emergence period, even if, on a few dates, only one form was found (e.g. 100% f. *brachycerca* at 29-vii-1992, n = 13). The maximum proportion of f. *typica* was 26.4% (12-vi-1991, n = 121).

In the data of 1992 a decrease of proportion of the long cerci form with ongoing emergence season from May to late July was recorded. But, this did not happen every year. However, since this trend occurred when many more exuviae were collected than in the other years, one cannot exclude that such a trend is more commonly occurring.

In conclusion, there may be a decrease in f. *typica* during the season, as in 1990 and 1992, which may alter the resulting proportions when not collecting exuviae during a whole emergence season. Moreover, generally small numbers of exuviae may result in very high or very low proportions of f. *typica* and therefore have to be interpreted carefully. Both facts have to be considered in further studies.

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Appendix 1. Percentage of f. *typica* exuviae of *Boyeria irene* at Bejarano Stream in the Sierra Morena Mountains, Spain. The fortnight code stands for the successive 15-days periods in which the sampling date is included; from the second half of May (code 1) to the first half of October (code 10). The 1992 records are grouped fortnightly; the greater part are results of three samples/visits with five days intervals between them. – **Apéndice 1.** Porcentaje de exuvias de la forma *typica* de *B. irene* en el arroyo del Bejarano, en la cordillera de Sierra Morena, España. El código quincenal corresponde a los sucesivos periodos de quince días en que está comprendida la fecha de toma de la muestra, desde la segunda mitad de Mayo (código 1) a la primera mitad de octubre (código 10). Los registros de 1992 están agrupados quincenalmente, la mayor parte de ellos son resultado de tres muestreos realizados con intervalo de cinco días entre ellos. – **Anhang 1:** Prozentuale Anteile der f. *typica*-Exuvien von *B. irene*. Der „Fortnight code“ steht für die 15-Tages-Perioden, an deren Ende jeweils Exuvien gesammelt wurden; von der zweiten Maihälfte (Code 1) bis zur ersten Oktoberhälfte (Code 10).

Date	Fortnight code	No. of female exuviae	% f. <i>typica</i>
24-v-1989	1	12	8.3
09-vi-1989	2	40	12.5
21-vi-1989	3	67	23.9
10-vii-1989	4	53	24.5
01-viii-1989	6	7	14.3
14-ix-1989	8	4	50.0
18-v-1990	1	4	25.0
04-vi-1990	2	22	18.2
27-vi-1990	3	44	11.4
17-vii-1990	5	19	10.5
01-viii-1990	6	12	25.0
15-v-1991	(1)	2	0.0
28-v-1991	1	51	15.7
12-vi-1991	2	121	26.4
26-vi-1991	3	105	25.7
12-vii-1991	4	65	26.1
25-vii-1991	5	31	19.3
08-viii-1991	6	6	0.0

Date	Fortnight code	No. of female exuviae	% f. <i>typica</i>
15-v-1992	(1)	7	28.6
30-v-1992	1	98	23.5
14-vi-1992	2	136	23.5
29-vi-1992	3	92	16.3
14-vii-1992	4	126	14.3
29-vii-1992	5	55	10.9
13-viii-1992	6	14	35.7
28-viii-1992	7	16	18.7
12-ix-1992	8	9	11.1
27-ix-1992	9	5	20.0
02-x-1992	10	2	50.0