

Colour polymorphism and ontogenetic colour changes in *Ischnura rufostigma* (Odonata: Coenagrionidae)

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Abstract. We describe female colour morphs and ontogenetic colour changes of *Ischnura rufostigma* in three populations from China. Females showed two colour morphs, one androchrome, identical to males, and one gynochrome, with orange coloration when immature and green to brown thorax when mature. Population frequencies show that gynochrome females are the most common morph (71-97%). In addition, we found high variability in the extent of the blue coloration on the tip of the abdomen of males and androchrome females. We discuss the possible causes of this colour variation and propose that previously described intraspecific forms of *I. rufostigma annandalei*, solely based on the variation of this blue coloration, have no taxonomic relevance.

Further key words. Dragonfly, damselfly, Zygoptera, *Ischnura rufostigma* group, melanism, China.

Introduction

Female colour polymorphism is a widespread phenomenon among odonates, particularly in the family Coenagrionidae (CORDERO & ANDRÉS 1996; FINCKE et al. 2005). This polymorphism includes females coloured and sometimes patterned like males, considered male-mimics (androchromes) and non-male-like females (gynochromes). This phenomenon has attracted attention from several research groups in recent years, but there is still not a general explanation for its evolution and maintenance (reviewed in VAN GOSSUM et al. 2008). In this context, the genus *Ischnura* is of particular interest, because it embraces some of the most perfect mimics of male coloration, which are thought to have evolved as a response to male harassment

(VAN GOSSUM et al. 2008). The fact that this polymorphism appears in several distinct families of odonates suggests that it has evolved independently more than once. Furthermore, different species show contrasting patterns of colorations, maturation, and number of morphs, and there are cases where the polymorphism is found in wing coloration (for instance *Polythore*; BECCALONI 1997) rather than body coloration, indicating that this is a complex phenomenon.

A common character of many *Ischnura* species is the presence of a blue spot in the dorso-lateral part of the final abdominal segments, hence the common name “bluetails”. In the Coenagrionidae, the blue colour is produced by the distribution of two kinds of spheres within the endoplasmic reticulum of the pigment cells (VERON et al. 1974; PRUM et al. 2004). The location of these spheres changes within the cell during the maturation process of teneral and with the change of temperature in those species where the body coloration is temperature dependent (VERON et al. 1974). Perched individuals harassed by others in flight, raise and curve the abdomen against the opponent to reject the interaction (UTZERI 1988), showing the “blue tail” towards the ventral ocular region of the harasser, where the blue wave-length-sensitive opsin genes are expressed more fully (at least in *Ischnura heterosticta*; HUANG 2014). This character seems therefore very important in agonistic interactions. On the other hand, in some species this blue spot presents variability in shape and size, at least in *I. graellsii* (CORDERO 1992) and in *I. genei* (ISV & ACR unpubl.) and is present in gynochrome females only when they are immature, e.g., *I. elegans*, *I. genei*, and *I. graellsii* (ACR unpubl.). This suggests that it is – in females – a signal to indicate their non-readiness to mate (HAMMERS et al. 2009).

Ischnura rufostigma is a common species in South and Southeast Asia, spread from India to Vietnam (WILSON & REELS 2003). In the past two centuries, subspecies and varieties have been described according to differences in anal appendages and in the coloration of S8 of the abdomen (see ASAHINA 1991). Nevertheless, this categorisation is confusing and there is no information about the ontogenetic change of colour and about female morphology and polymorphism of most of them. Our aim is, therefore, to fill this gap by describing female colour morphs in Chinese populations and to analyse their variability and the process of colour changes during maturation.

Material and methods

Three populations of *Ischnura rufostigma* were studied in China during 13 days of June and July of 2015, two of them in Yunnan province (Maandi: 22°45'35.87"N, 103°31'20.01"E, 1 179 m; Longling: 24°33'36.51"N, 98°48'57.81"E, 1 829 m) and one in Guizhou province (Longjiao: 24°33'41.49"N, 98°49'23.13"E, 1 171 m). The first population was observed inside paddy fields. There, the density of *I. rufostigma* was low and the study was performed over a period of 6 hours and 19 min to obtain a large sample size (n = 132). The density of the second population was high, and we collected 78 individuals in 10 minutes. This population inhabited a small and deep pond at the edge of a road. The last population was analysed in a set of connected ponds where *I. rufostigma* had low density and shared the space with two other *Ischnura* species – in order of density: *Ischnura asiatica* > *I. rufostigma* > *I. senegalensis*. There, the population proportions were studied for three days (n = 185) and the maturation of the colour was studied for eight days.

Damselflies were captured with an entomological hand net and individually marked with an indelible pen (Staedtler permanent Lumocolor). Body length and hind wing length were measured in 63 individuals of nine populations, including the three mentioned above, and contrasted by linear mixed models with sex and morph (androchrome and gynochrome) as fixed factors and population as random factor. Statistics were computed employing the software R (R CORE TEAM 2015).

Results

Teneral males of *Ischnura rufostigma* (Fig. 1a) showed the same pattern of coloration as mature individuals but with pale colours. The maturation of their thoracic colour consisted in a darkening process, turning from an initial yellowish-green colour to deep blue at an older age (Fig. 1c). Two different colour phenotypes were found in females: androchrome and gynochrome. Androchrome females showed the same colour changes as males (Figs 1b, d, e). Teneral gynochromes (Fig. 1g) showed a homogeneous pale pink colour covering all the body except in the dorsal region. Immature gynochrome females did not have black ante-humeral stripes (Fig. 1h). The dorso-lateral part of the thorax was light orange until the second lateral su-

ture and light green since this suture to the basal part. The edges of the ante-humeral stripe became brownish with the maturity of the female (Fig. 1i). Around six days later, the ante-humeral stripe turned to dark brown and the dorso-lateral region changed to greenish (Fig. 1j).

The orange colour of the abdomen from S2 to S6 of males and androchromes became dark with age. A black spot was present in the dorsal region of S2 and S6. Gynochromes always had the superior part of the abdomen dark and the ventral part developed a little darkening with age. The posterior surface of the legs and the eyes turned dark in both sexes and morphs. Old individuals presented pruinescence on the thorax.

High variability was found in the coloration of the dorsal part of S8 in males and androchromes, with continuous variation. In one extreme, the blue spot covered the dorsal region of this segment and part of other adjacent segments (Figs 2a, b). Some individuals presented black invaginations in the blue spot in the longitudinal and transversal plane (Figs 2e–g). In the other extreme, the blue colour was completely absent from S8 (Figs 1f, 2h).

The sex ratio of the three populations was biased towards males (males/females = 1.29 ± 0.05). Gynochrome females constituted the most common morph in all populations, ranging from 71 to 97% (Table 1). No males and androchrome females with dark S8 were found in the Longjiao population, the only one where androchromes were commonly found. In Maandi, androchromes with dark S8 were found but all collected males had blue colour on this segment.

Males were smaller than females (body length including anal appendages: $t = 2.31$, $p = 0.04$; hind wing length: $t = 7.09$, $p < 0.001$; Table 2). No size differences were found between female morphs.

Males with different colour on S8 had very similar anal appendages, suggesting they belong to the same species (Fig. 3).

Discussion

Our results indicate that *Ischnura rufostigma* females are dimorphic. The androchrome morph is an almost perfect mimic of males, and also shows similar colour changes over maturation. Gynochromes have orange coloration when immature and change to brown and greenish, and only show one mid-dorsal black stripe on the thorax.

The classification of the *I. rufostigma* group has been based on morphological traits and coloration. VICK (1986) summarised the taxa that had been described, illustrating the taxa and finding some differences in the shape

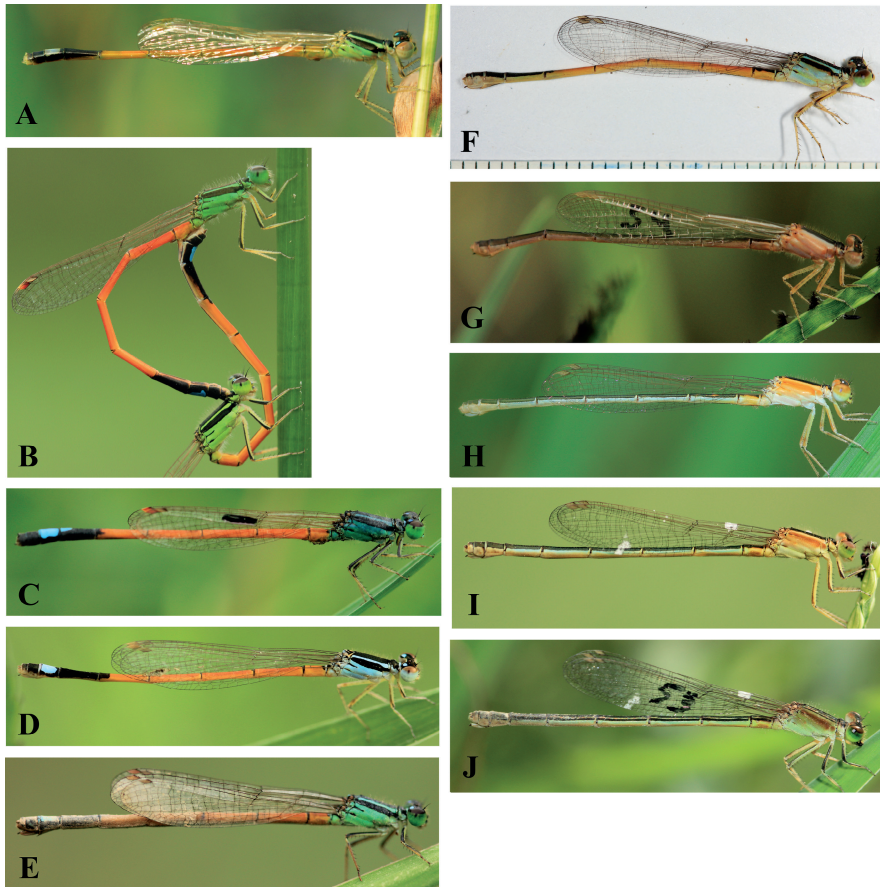


Figure 1. Polymorphism and colour maturation of *Ischnura rufostigma* studied 2015 in a population near Longjiao, Guizhou province, P. R. of China. a – teneral male; b – mating of a mature male with a mature androchrome; c – old male with blue coloration in S7 and S8 and marked with a black stripe on the wing; d – immature androchrome female; e – old androchrome with mud on abdomen and wings after laying eggs; f – immature androchrome without blue on S8; g – marked teneral gynochrome; h – young gynochrome; i – mature gynochrome of intermediate age; j – the same individual six days later, marked and with mud on the abdomen after laying eggs.

and juxtaposition of cerci and in the angle and shape of male S10 dorsal tubercle, suggesting five species for the group (*I. annandalei*, *I. carpentieri*, *I. inarmata*, *I. mildredae*, and *I. rufostigma*).

ASAHINA (1991) followed up with a detailed review, illustrating all known taxa, and even attempting a distribution map. He agreed with the differentiation of *I. inarmata* from Kashmir, as this is a very distinct taxon. He also introduced a new name *montana* for the material from Kathmandu Valley which VICK (1986) had allocated to *carpentieri*. He agreed that the male ap-

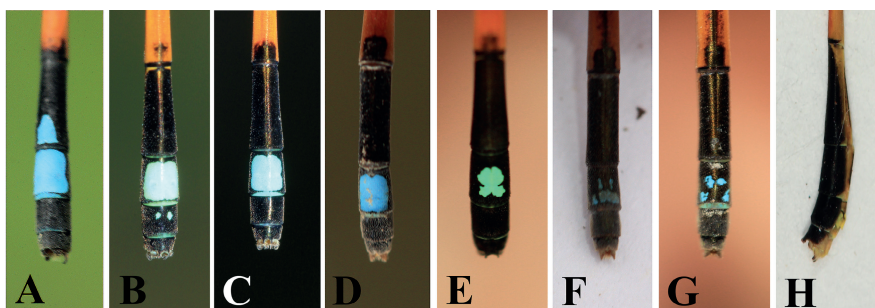


Figure 2. Variation of blue spot on distal abdominal segments in males and androchromes of *Ischnura rufostigma* from a population near Longjiao, Guizhou province, P. R. of China. a, b – spot on S8 extended to adjacent segment; c, d – spot complete only on S8; e–g – spot on S8 shrunk; h – spot absent; a–h – males; d, f, g – androchromes.

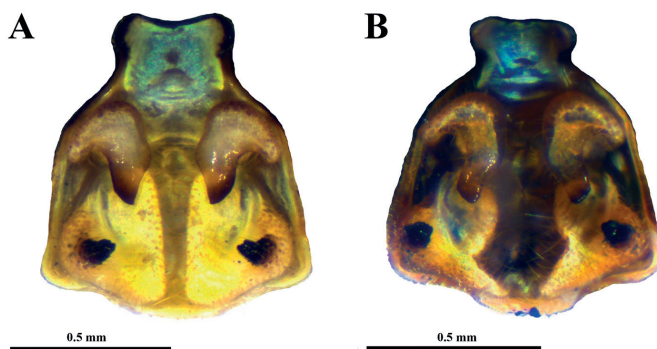


Figure 3. Male anal appendages of *Ischnura rufostigma* in posterior view. a – Male with blue colour on S8; b – male without blue spot on S8.

Table 1. Proportion [%] of colour morphs of *Ischnura rufostigma* in each of three populations studied 2015 in P. R. of China. Mb – male with blue S8; Md – male with dark S8; G – gynochrome; Ab – androchrome with blue in S8; Ad – androchrome with dark S8. Sample size in brackets.

Population	Mb	Md	G	Ab	Ad
Maandi, Yunnan prov.	100 (69)	0 (0)	94.7 (54)	3.5 (2)	1.7 (1)
Longling, Yunnan prov.	79.1 (34)	20.9 (9)	97.1 (34)	0 (0)	2.8 (1)
Longjiao, Guizhou prov.	100 (101)	0 (0)	71.2 (57)	28.8 (23)	0 (0)

Table 2. Mean body size [mm] \pm S.E. of each sex and morph of *Ischnura rufostigma*, in 63 individuals of nine populations from P. R. of China. Mb – male with blue S8; Md – male with dark S8; G – gynochrome; Ab – androchrome with blue in S8; Ad – androchrome with dark S8. Sample size in brackets.

	Males (14)	Mb (12)	Md (2)	Females (49)	G (36)	Ab (11)	Ad (2)
Body	29.86 \pm 0.43	30.27 \pm 0.37	27.4 \pm 0.8	31.08 \pm 0.24	30.91 \pm 0.28	31.8 \pm 0.47	30.2 \pm 1.4
Hind wing	14.62 \pm 0.19	14.78 \pm 0.17	13.65 \pm 0.55	16.87 \pm 0.14	16.81 \pm 0.16	17.2 \pm 0.34	16.15 \pm 0.65

pendages and female prothoracic morphology of this taxon appeared to be appreciably more distinct from those of other taxa to be discussed below, but he still ranked it at the level of subspecies (of either *annandalei* or *rufostigma*).

As far as the other taxa of the *rufostigma* group were concerned, the only difference that ASAHINA (1991) was able to show between them consisted in colour variations and geographical distribution. He pointed out the different extent of blue colour on S8 within the members of the group and the presence of orange S2 and S7 in *I. rufostigma*, in contrast with the black colour of the others.

ASAHINA (1991) remarked that *I. carpentieri* had been synonymised with *I. mildredae* (SCHMIDT 1964) as well as *I. mildredae* with *I. annandalei* (ASAHINA 1970) and suggested the recognition of two subspecies of *I. rufostigma*: *Ischnura r. rufostigma* from Central and East India and Nepal, with males without black dorsally on S2 and S7, and *I. r. annandalei* from East India, Myanmar, Thailand, Laos, South China, and Vietnam, with black dorsally

on S2 and S7. In the latter subspecies, he recognised two forms: forma *annandalei* (males with infrequent and irregular blue spot on S8, from India, Myanmar, Thailand, and Laos) and forma *carpentieri* (males with completely blue-coloured S8, from China and Vietnam). Our studied populations were placed in the middle of these two forms. In our populations, males and androchromes presented a high variability in the coloration of S8 (Fig. 2). Individuals without blue on the abdominal tip were found in two of the three populations examined, and none was found in the only population with high androchrome frequency. In spite of these differences (coloration and population), males presented the same anal appendage configuration. The angle of S10 dorsal tubercle of our specimens presented considerable variation and made VICK's (1986) classification impossible to apply. Our results suggest that the S8 coloration is a continuously variable character of a single species and that the S10 dorsal tubercle is not a reliable trait for identification. Our findings support the fact that *carpentieri* and *annandalei* forms correspond to phenotypic variability of the same species and have no taxonomic relevance. In some European species (*I. elegans*, *I. genei*, and *I. graellsii*) mature males and androchromes might present dark marks in the blue spot (CORDERO 1992 and pers. obs.), which appear since the emergence or develop with age, but no case is known in these European *Ischnura* species of a male or androchrome with the tip of the abdomen completely black. The fact that some immature *I. rufostigma* individuals present a completely dark S8 suggests that the variability of the colour of this region is genetically dependent and not due to age.

The existence of males and androchromes with dark colour instead of a blue tail could be a case of melanism. CORDERO-RIVERA (1988) found a seasonal effect related to melanism in individuals of the same population, in which the first generation of a bivoltine species (*I. graellsii*) presented darker bodies than the second. Melanism was assumed to be due to a lack of UV radiation (BARNARD et al. 2015) in Coenagrionidae reared in captivity, viz. *Coenagrion scitulum* (CORDERO et al. 1995); *Enallagma anna*, *E. annexum*, *E. civile*, *E. carunculatum*, and *E. hageni* (BARNARD et al. 2015); *I. genei* (ISV & ACR unpubl.); *Enallagma cyathigerum* and *Pyrrhosoma nymphula* (ACR unpubl.). The only difference between *I. r. rufostigma* and *I. r. annandalei* is based on the coloration of S2 and S7. Future studies must analyse if indi-

viduals of the same population shown this kind of colour variation across seasons as found in *I. graellsii* (CORDERO-RIVERA 1988).

If the genetic mechanism behind female thoracic colour polymorphism is similar to the system described for other species of *Ischnura* (JOHNSON 1964, 1966; CORDERO 1990; SÁNCHEZ-GUILLÉN et al. 2005), we expect two alleles, one producing the androchrome phenotype and the other determining the gynochrome phenotype, but not expressed in males. An alternative hypothesis to explain the black S8 in androchromes and males would be the existence of a third allele in the colour polymorphism locus, which is also expressed in males or a modifier locus that interacts with the colour polymorphism gene.

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