

**Interpopulation variation in
female color-type frequency of
Ischnura senegalensis in Okinawa Island, Japan
(Odonata: Coenagrionidae)**

Kouji Sawada, Kazunori Yamahira¹ & Takashi Kuriwada²

Fukuoka High School, 1-29-1, Katakasu, Hakata-ku, Fukuoka, Japan;
<kouji.senegalensis@gmail.com>

¹ Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan;
<yamahira@lab.u-ryukyu.ac.jp>

² Faculty of Education, Kagoshima University, Kagoshima, Japan;
<kuriwada@edu.kagoshima-u.ac.jp>

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Abstract. *Ischnura senegalensis* (Rambur, 1842) has two female color-types: gynochromes, which are brown, and androchromes, which are green and similar in appearance to conspecific males. *Ischnura senegalensis* females from Okinawa Island, a small island in the Ryukyu Archipelago, Japan, show color-type frequencies that vary greatly, even among adjacent local populations. For example, androchrome frequencies were very low (0–4.3%) in northern populations of the island. However, in the southern populations androchrome frequencies were high and ranged widely (0–67.2%). Periodical surveys from 2011 to 2013 of two adjacent southern populations revealed that the frequency of color-type remained constant over time. To our knowledge, this is the first study of temporally stable and extremely high interpopulation variation in female color-type frequency in Odonata. We discuss possible reasons for the evolution of such high interpopulation variation in color-type frequency.

Key words. Dragonfly, damselfly, Zygoptera, female-limited polychromatism, local population, Ryukyu Archipelago

Introduction

Within the Odonata, female polychromatism (color polymorphism) occurs in seven families of the Zygoptera and three of the Anisoptera (CORBET 1999: 278). More than 130 species have female-limited color polymorphism among the seven families of Holarctic Odonata (FINCKE et al. 2005). In many cases, adult females have two color-types (dichromatism): gynochrome females have different coloration than conspecific males, whereas

androchrome females show coloration similar to conspecific males (ROBERTSON 1985). The inheritance of female limited polychromatism appears to be controlled by a single gene locus with two or three alleles (JOHNSON 1964, 1966; CORDERO 1990; ANDRÉS & CORDERO 1999; SÁNCHEZ-GUILLÉN et al. 2005). However, despite its prevalence, the evolutionary factors that maintain color polymorphism are still controversial.

Interpopulation variation in the frequency of color types within a species may provide some insight into the mechanisms of color polymorphism, because this variation may be related to environmental differences between populations. Variation in color-type frequencies at regional scales has been documented in some damselfly species. For example, ISEBYT et al. (2010) reported that female color-type frequencies of *Nehalennia irene* (Hagen, 1861) geographically vary across Canada, from the Atlantic to Pacific coasts (~7,000 km); and *Ischnura elegans* (Vander Linden, 1820) exhibits a longitudinal gradient in color-type frequency in Europe, from Spain to Ukraine (~4,500 km; WELLENREUTHER et al. 2011).

Ischnura senegalensis (Rambur, 1842) is a common damselfly, widely distributed throughout Africa and Asia, which shows also female dichromatism with two color-types in mature females: gynochrome females are brownish and are well camouflaged, whereas androchrome females are greenish and similar in appearance to conspecific males (SAWADA 1998). Females and monomorphic, non-territorial males inhabit freshwater environments, such as ponds, slow streams, and paddy fields (SAWADA 1995). In the Japanese Archipelago, the frequency of *I. senegalensis* female color-types varies stepwise across latitudes of 31–38°N, from Honshu Island to Kyushu Island (~2,000 km; TAKAHASHI et al. 2011). However, no one has studied the frequency of variation in *I. senegalensis* at smaller, local scales. Such small-scale variation may provide a new perspective on the factors maintaining female color polymorphism in this species.

In this study, we report interpopulation variation at a local scale in the frequency of female color types in *I. senegalensis* on Okinawa Island (Okinawa-jima), in the Ryukyu Archipelago, Japan, situated approximately 600 km from the Japanese main islands. Although many small islands between the main islands and Okinawa Island harbor *I. senegalensis*, the long distance from the main islands enables us to eliminate the effect of constant gene

flow on interpopulation variation within Okinawa Island. Moreover, the island contains diverse habitats within its relatively small land area (approximately 1,200 km²), which makes it a good model system for studying local adaptation.

Materials and methods

To evaluate interpopulation variation in the frequency of female color-type of *Ischnura senegalensis*, field surveys were conducted in each of 18 populations on Okinawa Island, Okinawa Prefecture, Japan (Tab. 1). We sampled each population once in 2011 or 2012 with the exception of Oyama, which was sampled twice. Females were collected using butterfly nets and their color-type was recorded. To avoid double counts, females were marked on their hind wing before release.

To evaluate a possible temporal fluctuation in the female color-type frequencies, this mark-release census was repeated at two localities: five times at Makabe (29-iv- and 29-x-2011, 28-iv- and 11-xi-2012, 20-xi-2013), and four times at Kakinohana (12-xi-2011, 27-iv- and 12-xi-2012, 20-xi-2013).

To examine differences in the color-type frequency among the 18 populations sampled across the island, a Fisher's exact probability test by Monte Carlo simulation based on 10,000 replications was conducted using the 2×18 contingency table. To examine temporal fluctuations in the color-type frequency at Makabe and Kakinohana, Fisher's exact probability tests were conducted using the 2×5 and 2×4 contingency tables, respectively. All tests were performed using R (ver. 2.15.2).

Results

Between two and 64 females were collected from each of the 18 populations on Okinawa Island (Tab. 1). The frequency of color-types differed among the populations (Fig. 1) and the interpopulation difference was statistically significant (Fisher's exact probability test by Monte Carlo simulation based on 10,000 replications, $P < 0.001$). In general, the frequency of androchrome females was low in northern populations. Androchrome females were found only at Sesoko, and their frequency was only 4.3%. In contrast, southern populations varied in the frequency of androchromes, which ranged from 0% at Yamada, Yonashiroteruma, Hiyagon, and Senbaru to 67.2% at Ka-

Table 1. Localities and years of field collections of *Ischnura senegalensis* in Okinawa Island, Ryukyu Archipelago, Japan. N indicates the total sample size and numerals in parentheses indicate the frequency of androchromes.

| Populations (abbreviation) | Date | Latitude | Longitude | Habitat | N |
|----------------------------|----------------------|------------|-------------|-------------------|------------|
| Tobaru (Tob) | 28-iv-2012 | 26°44'29"N | 128°09'45"E | slow stream | 4 (0%) |
| Hanji (Han) | 10-xi-2012 | 26°43'49"N | 128°09'55"E | paddy fields | 2 (0%) |
| Kijoka (Kij) | 11-xi-2012 | 26°42'23"N | 128°09'03"E | paddy fields | 57 (0%) |
| Taiho (Tai) | 28-iv-2012 | 26°39'23"N | 128°07'50"E | paddy fields | 4 (0%) |
| Kori (Kor) | 10-xi-2012 | 26°42'06"N | 128°01'22"E | pond | 2 (0%) |
| Sesoko (Ses) | 11-xi-2012 | 26°38'44"N | 127°51'43"E | pond | 46 (4.3%) |
| Nago (Nag) | 28-iv-2012 | 26°36'37"N | 127°59'32"E | pond | 7 (0%) |
| Abu (Abu) | 10-xi-2012 | 26°32'43"N | 128°05'00"E | pond | 3 (0%) |
| Kanna (Kan) | 29-iv-2012 | 26°29'27"N | 127°57'19"E | pond | 3 (33.3%) |
| Yamada (Yam) | 10-xi-2012 | 26°25'29"N | 127°46'41"E | pond | 10 (0%) |
| Ishikawa (Ish) | 30-iv-2011 | 26°25'40"N | 127°48'55"E | slow stream | 15 (6.7%) |
| Yonashirote-ruma (Yon) | 29-iv-2012 | 26°21'02"N | 127°52'46"E | paddy fields | 27 (0%) |
| Hiyagon (Hiy) | 28-iv-2012 | 26°18'32"N | 127°49'27"E | pond | 4 (0%) |
| Oyama (Oya) | 29-iv- & 12-xi- 2011 | 26°16'39"N | 127°44'25"E | paddy fields | 2 (50.0%) |
| Senbaru (Sen) | 09-iv-2011 | 26°14'57"N | 127°45'48"E | pond | 9 (0%) |
| Chinenshikiya (Chi) | 28-iv-2012 | 26°09'09"N | 127°47'54"E | watercress fields | 21 (38.1%) |
| Kakinohana (Kak) | 11-xi-2012 | 26°08'51"N | 127°48'04"E | watercress fields | 64 (67.2%) |
| Makabe (Mak) | 11-xi-2012 | 26°06'27"N | 127°41'05"E | pond | 54 (3.7%) |

kinohana (Fig. 1). Androchrome frequency differed even among adjacent populations. For example, Makabe and Kakinohana were situated approximately 13 km apart from each other, but the androchrome frequencies were 3.7 and 67.2 %, respectively.

In both Makabe and Kakinohana, the frequencies of androchrome females were constant across years. The androchrome frequency was 0–3.9 % from 2011 to 2013 (mean 1.5 %, N = 5) in Makabe, while it was 57.7–75.4 % from 2011 to 2013 (mean 66.7 %, N = 4) in Kakinohana (Fig. 2). The androchrome frequencies were not significantly different among the sampling occasions (Fisher’s exact probability test, Makabe: $P = 0.729$; Kakinohana: $P = 0.266$).

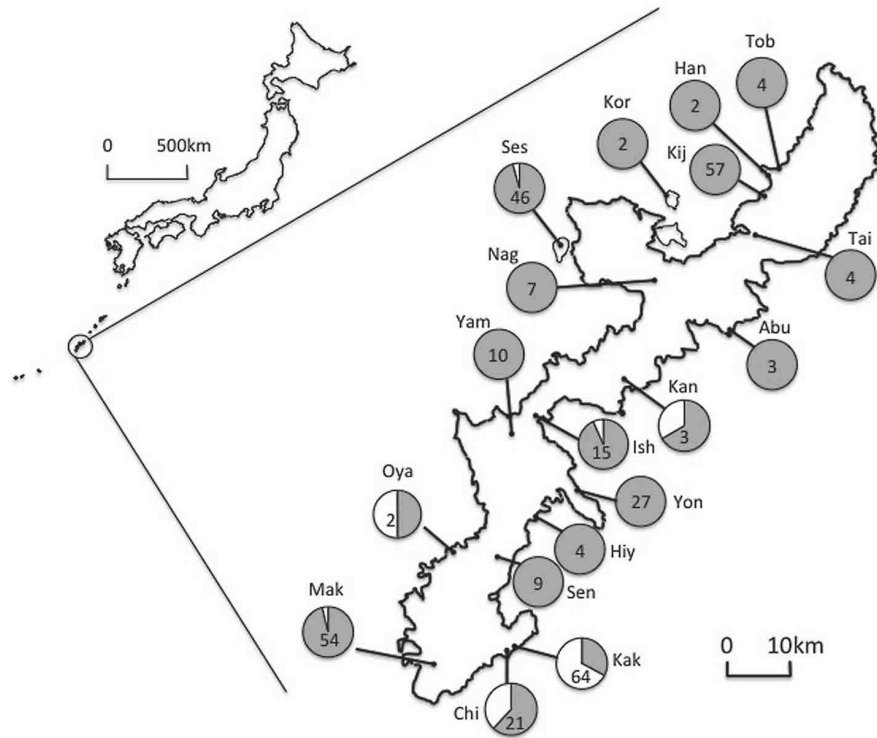


Figure 1. Female color-type frequencies of 18 local populations of *Ischnura senegalensis* in Okinawa Island, Ryukyu Archipelago, Japan (gray – gynochrome; white – androchrome). Numerals within circles indicate the total sample size. Abbreviations for population names are indicated around circles.

Discussion

The frequency of female color-types in *Ischnura senegalensis* varied among local populations on Okinawa Island, a small island with a size of only 1,200 km². The variation was greater in the southern part of the island where the androchrome frequency varied from 0 to more than 60%, even among adjacent populations. Similar interpopulation variation in color-type frequency at local scales has been reported for *Ischnura elegans* where the androchrome frequency varies from approximately 55 to 90% among local populations within an area of less than 1,000 km² in southern Sweden (SVENSSON & ABBOTT 2005). In *I. elegans*, however, the local color-type frequencies changed from year to year, and the interpopulation variation gradually disappeared within three years (SVENSSON & ABBOTT 2005). The local color-type frequencies of *I. senegalensis* were stable and the large variation in color-type frequencies between the two adjacent southern populations (Makabe and Kakinohana) persisted during three years. To our knowledge, this is the first study of temporally stable, extremely high interpopulation variation in female color-type frequency in Odonata.

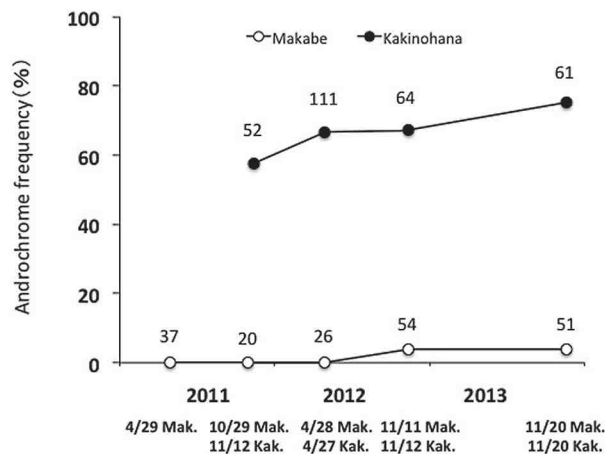


Figure 2. Temporal changes in androchrome frequency in two southern populations of *Ischnura senegalensis* in Okinawa Island, Ryukyu Archipelago, Japan: Makabe and Kakinohana. Numerals above the dots indicate the total sample size.

It is not clear why such high interpopulation variation in color-type frequencies evolved. One possibility is local adaptation. TAKAHASHI et al. (2011) concluded that the latitudinal cline in the color-type frequency of *Ischnura senegalensis* is adaptive because clutch size (number of ovarioles) reverses between the two color-types across latitudes, i.e., adult gynochrome females are more fecund in the south, while androchrome females are more fecund in the north. TAKAHASHI et al. (2011) suggested that the two color types may differ in their response to environmental factors that differ across latitudes, such as temperature. In our study, populations varied in color-type frequencies even within a very small latitudinal range (26°06'N and 26°49'N), on Okinawa Island. All the populations that were examined were located at approximately the same altitude (less than 70 m above sea level) where water temperatures are likely similar. It remains unclear which factors determine local variation in color-type frequency of *I. senegalensis* on Okinawa Island.

Local variation in color-type frequency may be explained not only by local adaptation but also by neutral processes, such as genetic drift, founder effect, and/or bottleneck effect. One way to distinguish local adaptation versus neutral processes is to compare the degree of population differentiation between the locus for color-type and neutral loci, such as some mitochondrial genes or nuclear microsatellites. For example, ISEBYT et al. (2010) reported that genetic drift and historical events are important in explaining the geographical variation in female color-type frequencies of *Nehalennia irene* in Canada by analyzing variation in mitochondrial DNA (mtDNA) gene fragments. However, ABBOTT et al. (2008) reported that the observed temporal change of color-type frequencies toward equilibrium in *Ischnura elegans* in southern Sweden reflects local adaptations driven by stabilizing selection by analyzing neutral amplified fragment length polymorphism (AFLP) loci. Furthermore, based on analysis of microsatellite loci, SÁNCHEZ-GUILLÉN et al. (2011) suggested that divergent selection has been operating on the color locus in Spanish populations of *I. elegans*. TAKAHASHI et al. (2014) also suggested that divergent selection has established the latitudinal cline in color-type frequencies of *I. senegalensis* across the Japanese Archipelago. To test whether the interpopulation variation in female color-type frequencies in *I. senegalensis* on Okinawa Island reflects local adaptations or whether it is

due to neutral processes, the degree of population differentiation at neutral loci should be evaluated.

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