

Original Article

Evolution in Islands: contrasting morph frequencies in damselfly populations of the Balearic Islands

Adolfo Cordero-Rivera^{1,*}, Anais Rivas-Torres¹, Rosa Ana Sánchez-Guillén^{1,2}

¹Universidade de Vigo, ECOEVO Lab, E.E. Forestal, Campus Universitario, 36005 Pontevedra, Spain

²Instituto de Ecología A.C., Xalapa 91073, Veracruz, México

*Corresponding author. Universidade de Vigo, ECOEVO lab, E.E. Forestal, Campus Universitario, 36005 Pontevedra, Spain. E-mail: adolfo.cordero@uvigo.gal

ABSTRACT

Colour polymorphism is an example of visible phenotypic variability that is often associated with ecological factors and may produce local adaptations. Small populations, particularly in islands, offer opportunities for evolutionary novelties, and are therefore of particular interest to the study of polymorphisms. Here we study the dynamics of female colour morphs in the damselfly *Ischnura elegans* in the Balearic Islands. We found that insular populations are small, show low density, low mating activity, and low androchrome frequency. Our surveys suggest that male harassment is a powerful force in the dynamics of this female-limited polymorphism, because high male densities result in lower presence of mature females around the water, where copulation takes place. Non-male-like (infuscans) females have higher mating frequency. Androchromes were rare (15%) in all populations, but the frequency of the two non-male like females (infuscans and aurantiaca) was reversed between islands, despite their geographical proximity. We found a possible novel morph, suggesting that insular conditions allow innovations. Fitness differences between the two non-male-like females of *I. elegans* are still understudied, because most previous research has concentrated on the maintenance of androchromes, and are therefore a priority for future research.

Keywords: Odonata; *Ischnura elegans*; female colour polymorphism; islands; sexual conflict

INTRODUCTION

The existence of discrete phenotypic variation, particularly linked to body coloration, is a clear example of evolution in action, and this explains why colour polymorphisms are one of the favourite topics of field biologists. If the relative frequencies of morphs remain constant over large geographical areas, this can be interpreted as the consequence of stabilizing selection (e.g. Andrés *et al.* 2000). On the other hand, if morph frequency shows geographical clines, this is evidence for selective forces linked to local habitat characteristics and frequency dependence (Takahashi *et al.* 2011).

The relative weight of stochastic and selective forces is always difficult to establish in field surveys, but in general the smaller the population, the larger the expected effect of stochasticity (Takahashi *et al.* 2010). For this reason, islands, where isolation and small population size are combined, offer exceptional opportunities to study local adaptation and the evolution of polymorphisms. Islands are laboratories of evolution, and their biota examples of unique adaptations, that may modify species ecology, producing very large (island gigantism) or very small

(island dwarfism) populations (Baeckens and Van Damme 2020), and, islands situated far from the continents in particular, are home of unique species not found anywhere else (Losos and Ricklefs 2009).

Odonates are a small order of insects, whose ecology and behaviour is so diverse that they are used by many scientists in their research, making them model organisms (Córdoba-Aguilar *et al.* 2022). In oceanic islands, odonates tend to speciate (e.g. Jordan *et al.* 2003, Beatty *et al.* 2017), and novel adaptations may occur. For instance, the only case of parthenogenetic reproduction known in the order Odonata, is found in the Azores archipelago, and is likely the result of a single colonization by an American damselfly (Lorenzo-Carballa and Cordero-Rivera 2009).

Colour polymorphism is widespread in several families of odonates, and the maintenance of several morphs has been the subject of intense research over the past decades (reviewed by Van Gossum *et al.* 2008). This polymorphism includes one male-like female morph (androchrome), which is similar to the conspecific male in body coloration and sometimes also in behaviour, and one or several 'female-like' morphs, known

Received 3 October 2023; revised 13 November 2023; accepted 12 December 2023

© 2024 The Linnean Society of London.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

as gynochrome females, whose coloration is usually darker and less conspicuous than the male and androchromes (Van Gossum *et al.* 2008). Research on the evolutionary dynamics of the frequency of female colour morphs in odonates has demonstrated that this colour polymorphism is likely a consequence of sexual conflicts over mating frequency, particularly in the genus *Ischnura*, where mating system and polymorphism are evolving in a correlated way (Sánchez-Guillén *et al.* 2020).

Several hypotheses have been proposed to explain the adaptive significance of female colour polymorphism in odonates. Although there are many variants, the main ideas can be grouped around the ‘male-mimicry’ hypothesis, which proposes that androchromes are functional male mimics, and therefore are favoured by high population density (Robertson 1985, Hinnekint 1987, Cordero 1992), or around the ‘learned mate recognition’ hypothesis, which postulates that males form a search image and are more attracted to the most common morph in the population (Fincke 2004). Both mechanisms are based in density and/or frequency dependence and are not mutually exclusive. The puzzle around female colour polymorphism in damselflies remains unsolved despite years of research, probably because the variability in morph frequency is idiosyncratic and dependent on several local conditions of the studied populations (Sánchez-Guillén *et al.* 2017).

Ischnura elegans is a widespread Euro-Asiatic species, which has three female colour morphs, with the androchromes being a perfect mimic of the male in coloration and behaviour (e. g. Sánchez-Guillén *et al.* 2005, 2017). Its polymorphism has been intensively studied in several European regions (e.g. Cordero

et al. 1998, Van Gossum *et al.* 2005, Abbott and Gosden 2009, Sánchez-Guillén *et al.* 2011, Reborá *et al.* 2018, Piersanti *et al.* 2021), and the picture that emerges from these studies is that, although in general androchromes are male mimics, local population factors, like density, morph frequencies, and sex-ratio, modulate the interaction between males and females, and affect the relative fitness of female morphs.

Ischnura elegans is among the commonest European damselflies, but is substituted by other related species in most of the Mediterranean islands (e. g. Sanmartín-Villar and Cordero-Rivera 2016). However, this species is found in the Balearic Islands, although nothing is known about the dynamics of its colour polymorphism in the archipelago. We studied populations of *I. elegans* from Mallorca and Menorca, the largest islands of the Balears, to estimate morph frequencies over a period of 4 years (at least eight generations). Our aim was to determine if colour morph frequencies are similar between islands, which is the expectation if comparable selective factors are acting in the region. Furthermore, given the isolation of these populations, we aimed at detecting possible evolutionary novelties, which are more likely in islands (Baeckens and Van Damme 2020).

MATERIALS AND METHODS

Populations of *I. elegans* were sampled on 56 days between 2020 and 2023 on the islands of Menorca (three localities) and Mallorca (two localities), by collecting adult damselflies along the shore of ponds. We measured, once per season, pH, conductivity, and water temperature on the surface of the pond, using a

Table 1. Populations of *I. elegans* sampled in the Balearic Islands, with the frequency of female colour morphs per season and sample size (*N*, only mature females found alone). Conductivity is presented in mS/cm and Temp refers to water temperature (°C) on the surface of the shoreline of the pond.

Population	Island	Latitude (N)	Longitude (E)	pH	Conduct.	Temp.	Date	androchrome	infuscans	aurantiaca	<i>N</i>
La Vall	Menorca	40.0482	3.92541	n.a.	n.a.	n.a.	Sep 2020	0.158	0.632	0.211	19
				6.89	11.98	28.8	Jun 2021	0.091	0.818	0.091	11
				6.69	14.04	27.2	Sep 2021	0.077	0.615	0.308	13
				6.60	3.54	24.7	May-Jun 2022	0.286	0.714	0.000	7
				6.93	1.80	28.5	Sep 2022	0.100	0.650	0.250	20
Cala Tirant	Menorca	40.0434	4.10329	8.36	10.07	25.3	Jun 2023	0.385	0.308	0.308	13
				6.87	5.91	27.4	Jun 2021	0.000	0.839	0.161	31
				7.19	6.84	25.8	Sep 2021	0.200	0.600	0.200	30
				6.78	3.98	26.1	May 2022	0.056	0.833	0.111	18
				6.61	4.82	27.7	Sep 2022	0.259	0.667	0.074	27
Son Bou	Menorca	39.9013	4.07022	8.06	8.90	21.6	May-Jun 2023	0.133	0.600	0.267	15
				7.69	3.09	24.3	Sep 2023	0.222	0.722	0.056	18
				8.05	1.49	24.4	Jun 2021	0.067	0.600	0.333	30
				7.20	9.11	26.8	Sep 2021	0.225	0.650	0.125	40
				n.a.	10.67	27.1	May 2022	0.286	0.543	0.171	35
S'Albufera	Mallorca	39.7956	3.10438	8.27	6.76	26.5	May-Jun 2023	0.067	0.800	0.133	15
				8.47	>20.00 ^a	31.3	Sep 2023	0.083	0.917	0.000	12
				6.41	5.12	27.5	Jun 2022	0.161	0.355	0.484	31
				7.33	8.39	23.3	Aug-Sep 2023	0.140	0.512	0.349	43
Canyamel	Mallorca	39.6602	3.43798	7.20	5.37	27.1	Jun 2022	0.214	0.321	0.464	28

^aConductivity was above the range of the tester.

Hanna HI98130 tester. Details of the sampled sites are given in Table 1. All populations were found at the end of streams, where they become retrodunar ponds in the immediate vicinity of the sea, at the same altitude and partially exposed to marine winds. Vegetation on the shoreline was dominated by *Juncus acutus* (and other *Juncus*), *Typha dominguensis*, *Phragmites australis*, and shrubs (*Tamarix africana*, *Juniperus phoenicea*, and some *Pistacia lentiscus*).

We used insect nets to collect all the damselflies seen in sampling sessions of 5–30 min, between 8:00 and 13:00, with a mean time of effective sampling per day and locality of 42.3 ± 2.5 min (mean \pm SE). After collecting, each individual was examined to determine sex, age (by thorax colour and wing flexibility), female colour morph (as described in Sánchez-Guillén *et al.* 2005 see Figure 5 for colour pictures), and the presence of parasites (mites and the ceratopogonid midge *Forcipomyia paludis*). Juvenile females of the violacea morph were assigned to the androchrome morph if their eighth abdominal segment lacked black spots, and to the infuscans morph when had black spots. This is in general the case, but a small proportion of infuscans females do not show any black mark on the eighth abdominal segment (Sánchez-Guillén *et al.* 2005), so our estimates of juvenile infuscans females may be an infra-estimation the true frequency of this morph. Each specimen was released after being marked with a small, coloured dot on the wings to avoid counting the same specimen more than once.

Water is a scarce resource in the Balearic Islands, which means that the number of adult damselflies found in each population was generally very low. To estimate population morph frequencies, we sampled each population for 2–4 days, when possible both in spring (May–June) and summer (August–September). Population frequencies were estimated from the sample of mature females found alone (excluding those found mating). Our goal was to examine at least 30 mature females per population and season, because frequencies estimated from small samples have a large intrinsic variability, and therefore can induce erroneous conclusions (Cordero-Rivera and Andrés 2001). However, in many cases the density of the population was so low that only a few individuals were found after several hours searching. The detailed data for each day of sampling are presented at Supporting Information.

Females observed in copula were counted separately, to compare the frequencies of morphs between lone females and mating females (Cordero-Rivera and Sánchez-Guillén 2007). Nevertheless, the density of populations was usually very low and only a few mating pairs were observed on most sampling days. Means are presented with their standard error and (sample size).

To test statistical hypotheses, we used Generalized Linear Mixed Models (GLMMs), with the appropriate error structure for the response variate, and including as random terms the population, island, and season (early/late) to control for non-independence of data. Statistical analyses were done with xlStat 2022 and GenStat 23rd edition.

RESULTS

Population density and age structure

Mature males outnumbered mature females in 48 of the 54 sampling dates. On average, $70.8 \pm 2.1\%$ ($N = 54$) of mature

individuals were males. However, among young individuals, most were females, males representing only $32.7 \pm 3.7\%$ ($N = 44$). No young specimens were found in 10 out of the 54 sampling days. Mature males were always more abundant than young males representing $91.8 \pm 1.8\%$ in the early season (May–June) and $94.6 \pm 1.3\%$ in the late season (Aug–Sept). Mature females were less common in the early season ($48.3 \pm 4.8\%$) compared to the late season ($79.5 \pm 4.5\%$).

The density of mature males varied between 0.01 and 5.3 males/minute, with an average of 1.3 ± 0.1 (54) males/min. We tested the hypothesis that male density affects female presence along the shoreline (Hinneking 1987). We found that the proportion of mature females in the population was negatively related to mature male density (number of mature females as response variate, GLMM with binomial errors using the total number of females as binomial totals, and including population, island, and season as random terms; $F_{1,37.9} = 7.13$, $P = 0.011$; Fig. 1A). However, the proportion of young males in the population was not affected by mature male density (number of young males as a response variate; GLMM with binomial errors, and the same random terms as above; $F_{1,11.7} = 2.17$, $P = 0.167$; Fig. 1B).

We did not find a single case of parasitism by water mites among the over 3600 damselflies examined during the field-work. Parasitism by *Forcipomyia paludis* (Fig. 5F) was only detected in the early season (May–June), in the populations of Tirant and Son Bou in Menorca, and S'Albufera in Mallorca, but was extremely rare (see Cordero-Rivera 2021).

Frequency of female morphs

Sample size per day varied between one and 28 mature females per population. Overall, the frequency of androchrome females was very similar between islands, with $15.0 \pm 0.04\%$ (13) in Mallorca and $15.8 \pm 0.03\%$ (41) in Menorca. However, the proportion of infuscans was $45.9 \pm 0.10\%$ (13) in Mallorca vs. $65.3 \pm 0.04\%$ (41) in Menorca, and aurantiaca was common in Mallorca [$39.1 \pm 0.10\%$ (13)] but rare in Menorca [$18.9 \pm 0.03\%$ (41)].

Table 1 presents a summary of morph frequencies per population and season. To test for temporal effects on morph frequencies, we analysed the effect of Population and Date on mature Morph frequencies, using a GLMM with binomial errors, including the total number of mature females as the binomial total. Results indicate no significant effects of Date or Population on androchrome frequencies (deviance ratio_{5,53} = 0.42, $P = 0.833$). The frequency of infuscans was significantly lower in Mallorca compared to Menorca (Canyamel: $t_{48} = -3.25$, $P = 0.002$; S'Albufera: $t_{48} = -3.17$, $P = 0.003$), but Date had no effect ($t_{48} = 0.21$, $P = 0.831$). In the case of aurantiaca, both populations from Mallorca had a significantly higher frequency (Canyamel, $t_{48} = 3.47$, $P = 0.001$; S'Albufera, $t_{48} = 4.04$, $P < 0.001$) and again Date had no effect ($t_{48} = -1.30$, $P = 0.199$).

Given the above results, we investigated the effect of Male density, Mature sex-ratio, and Island on Morph frequency, using a GLMM with binomial errors, with the number of Mature females as the binomial total, including Population as a random term. Results indicate that Male density had no significant effect on Androchrome frequency, but the tendency is to a negative

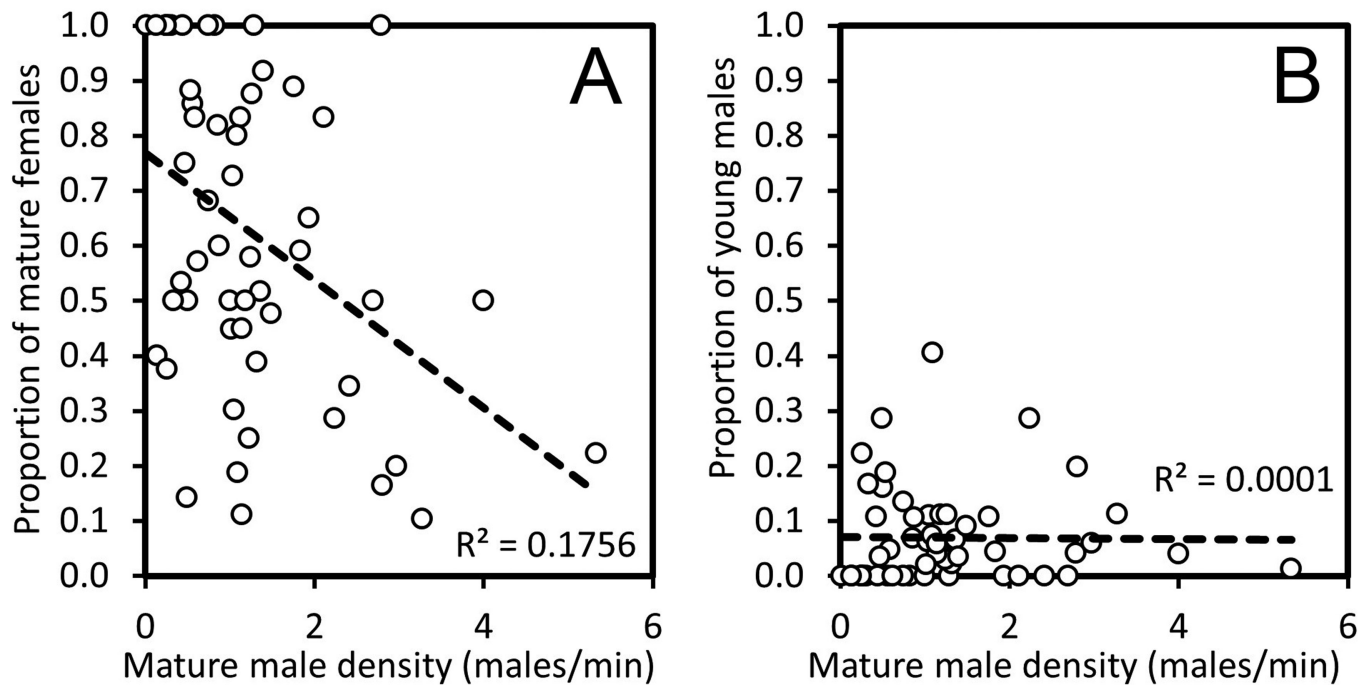


Figure 1. The relationship between mature male density (number of mature males/min) and (A) the proportion of mature females found alone (mature females/total alone females) and (B) the proportion of young males in the population. The effect of male density is significant in (A), but not in (B).

effect ($F_{1,50} = 3.41$, $P = 0.071$), a significant negative effect on infuscans frequency ($F_{1,50} = 11.71$, $P = 0.001$), and also a negative effect on aurantiaca frequency ($F_{1,47.6} = 4.29$, $P = 0.044$). Mature sex-ratio, on the contrary, had a positive effect on androchrome frequency ($F_{1,50} = 8.64$, $P = 0.005$), but no significant effect on infuscans ($F_{1,50} = 3.21$, $P = 0.085$) and aurantiaca ($F_{1,50} = 2.06$, $P = 0.158$). Finally, the effect of Island was only significant for infuscans frequency, with an increase in Menorca compared to Mallorca ($F_{1,35.5} = 4.33$, $P = 0.045$).

Figure 2 shows the relationship between the frequency of female colour morphs in juvenile specimens and in the mature population, grouped per season. If mature frequencies were similar to juvenile frequencies, the points should be around the diagonal in the figure. We compared the observed mature frequency with the expected given morph frequencies in juveniles, using a goodness of fit χ^2 test, for the 11 seasons where at least 10 juvenile and 10 mature females were found. There were significant differences in six out of 10 seasons (in the 11th season the expected mature frequency for androchromes was zero, and the test could not be calculated). In the case of androchromes, mature frequency was higher than expected on three occasions and lower in one case (indicated by the large symbols in **Fig. 2**). For infuscans females, mature frequencies were higher than expected on two occasions and lower in one. Finally, aurantiaca females were on three occasions found in a higher proportion in the mature sample compared to the juvenile sample.

Mating activity

On average, 8.9 ± 3.2 ($N = 27$) mating pairs were found on days with reproductive activity. On most sampling days (29 days out of 56), no mating pairs were found at all. However, on a few days, mating activity was intense. For instance, on 28 May 2023, a total

of 84 mating pairs were found at Cala Tirant. Two days later, only 10 were found, and on 3 June, only four were observed. We analysed whether the number of mating pairs observed per day was related to Male density, Mature sex-ratio, and the number of Mature females found alone, using a GLMM with Poisson errors and log link, with Population as a random term. Results indicated that Male density ($F_{1,16.7} = 11.65$, $P = 0.003$) and the number of Mature females ($F_{1,20.3} = 3.34$, $P = 0.020$) had a positive effect on Mating activity (**Fig. 3**), but the effect of Mature sex-ratio was not significant ($F_{1,13.2} = 0.03$, $P = 0.860$). However, these relations were dependent on the extraordinary data of 28 May 2023 (closed symbol in **Fig. 3**), and if this data point is excluded, results become not significant.

The relationship between population morph frequency and mating frequency is presented in **Figure 4**, aggregating data per season and population. The number of matings observed per season and population varied from 1 to 98. In most days, infuscans females were the majority morph among mating females, even in the S'Albufera population, where aurantiaca represented 48% of mature females, but only four mating pairs were found there (all infuscans). We compared population and mating frequencies using a goodness of fit χ^2 test, for the two seasons with the largest sample size of mating pairs (it could not be calculated when the observed frequency for one morph was zero). Only in one case was there a significant difference between both frequencies (large symbol in **Fig. 4**).

A possible novel female morph of *I. elegans*

In all sampled populations we found the previously described three female morphs of *I. elegans* (see **Sánchez-Guillén et al. 2005**), i.e. androchromes (male-like; **Fig. 5A**), and the gynochromes infuscans (**Fig. 5B**) and aurantiaca (**Fig. 5C**).

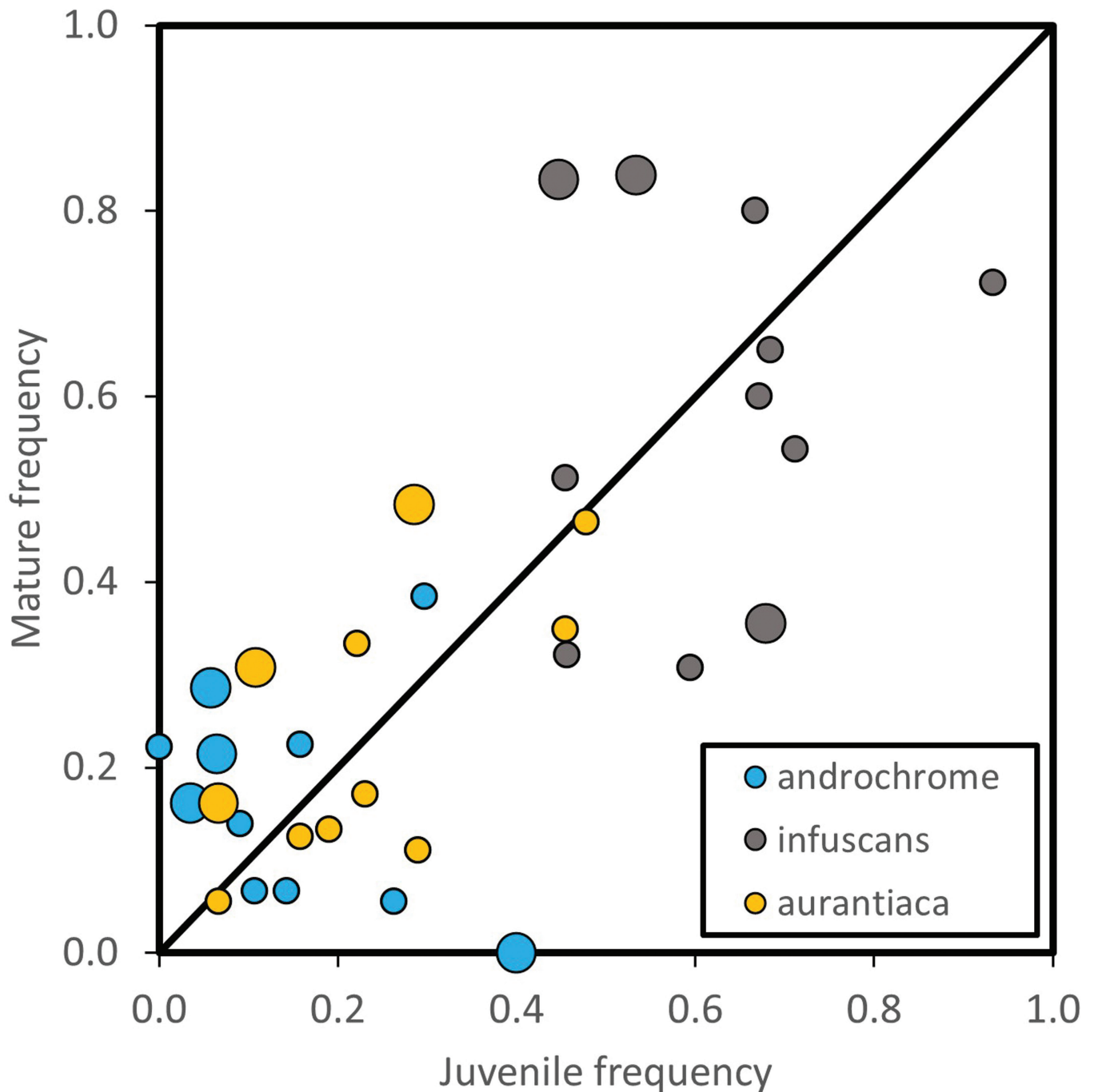


Figure 2. The relationship between the frequency of female colour morphs in juvenile specimens, and their frequency in the sample of mature females for all seasons where at least 10 individuals of each age were found. The diagonal indicates the expectation if frequencies were identical. Large symbols indicate significant deviations.

However, in June 2021 we found two juvenile females (Fig. SH-I) that could not be assigned to any of the known female morphs of *I. elegans*. Both specimens were found in the population of Son Bou (Menorca), and were characterized by the presence of only one medio-dorsal black line on the thorax, as is typical of the aurantiaca morph (Fig. 5G) (Cordero-Rivera 2015), but had a light violet thorax instead of pink-orange, which is the colour expected for the aurantiaca morph. This violet coloration is shown by the juvenile infuscans and some androchrome females of *I. elegans* (Fig. SD-F). Furthermore, these females showed

orange antehumeral lines, with some black over them, producing an intermediate coloration between the morphs aurantiaca and infuscans (Fig. SH-I). The specimens were not collected, and this morph was not detected in the following years in any population.

DISCUSSION

Our results confirm some previous findings on the same and similar species, but also reveal novel facts, which may be related

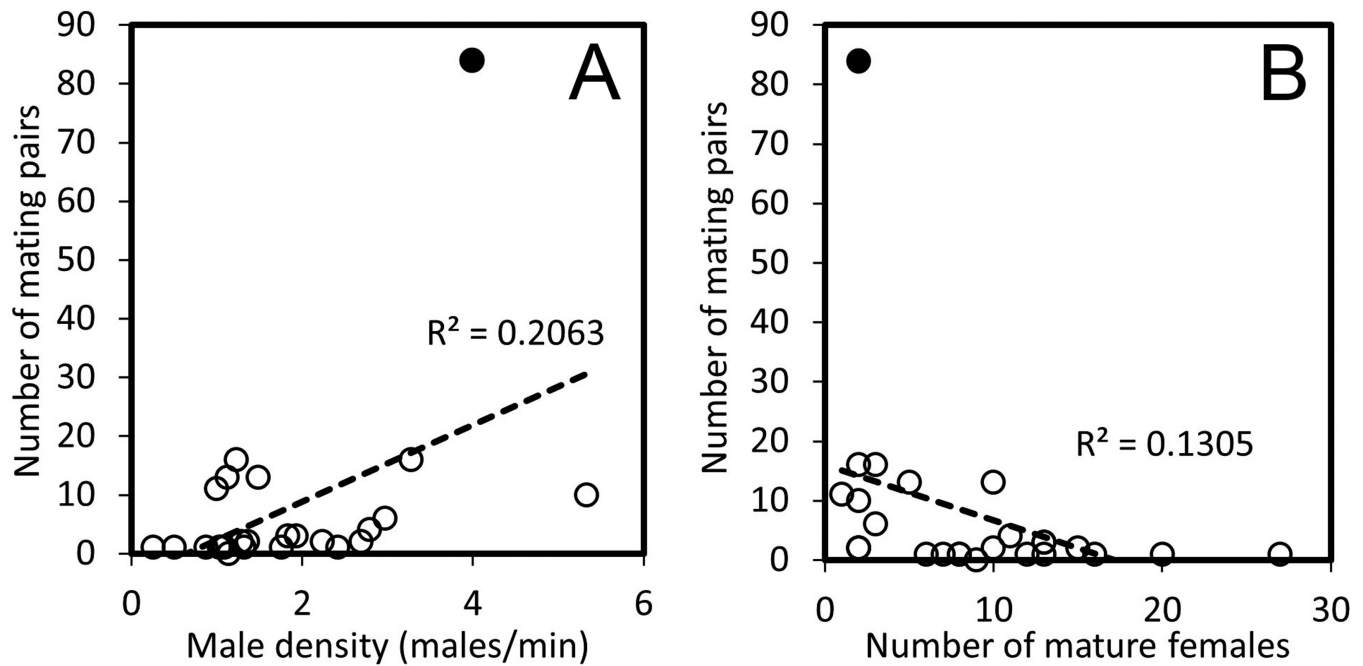


Figure 3. The effect of mature male density (A) and the number of mature females found alone (B) on the number of mating pairs observed each day. In both cases the effect is significant, but if the data point from 28 May 2023 (closed symbol) is excluded, the effect becomes not significant.

to the particularities of these insular populations. First, mature males were much more abundant than mature females, making the operational sex-ratio highly male biased, which is typical of odonates and damselflies in particular (Cordero-Rivera and Stoks 2008). Second, all populations were polymorphic, as has been reported for *I. elegans* in previous studies (e.g. Cordero *et al.* 1998, Sánchez-Guillén *et al.* 2005). Third, the frequency of infuscans females tended to be higher among mating females than in the mature population (Cordero-Rivera and Sánchez-Guillén 2007, Hammers and Hans Van Gossum 2008, Gosden and Svensson 2009), although given the small sample size, significant differences were detected only in one season in this study. Among the novel findings, we found that male density affected the presence of mature females in the shore of the ponds in a negative way (Fig. 1A), that juvenile and mature morph frequencies are sometimes significantly different, and we found a previously unknown colour morph, which could represent a novel morph (Fig. SH-I).

The populations of *I. elegans* in the Balearic Islands are characterized by their low density and isolation. Male density averaged 1.3 males/min, and in 76% of the sampling days male density was below 1.5 males/min, much lower than the maximum we have found in other regions (14.7 males/min; unpublished data). Furthermore, on some occasions the number of individuals found was so low that sampling had to be aborted. Hinnekint (1987) hypothesized that the concentration of males around the water would produce intense harassment on mature females, so that they would be obliged to move far from the pond. Until now there has been little evidence for this, but we have found a negative effect of male density on the proportion of mature females found around the water (Fig. 1A). This result suggests that males are pulling away mature females due to their insistent mating attempts. Male density has been considered one of the drivers of

the maintenance of female colour polymorphism in *Ischnura*, because androchrome females would be favoured at high densities, given that their mimicry allows them to avoid unnecessary matings (Hinnekint 1987, Cordero 1992). Observations of mature females in the field indicate that androchromes use more open habitats and confront approaching males, whereas gynochrome females hide and fly away when a male approaches (Van Gossum *et al.* 2001, Sánchez-Guillén *et al.* 2017). Males produce more harassment (clasp attempts) on gynochrome females compared to androchromes (e.g. Piersanti *et al.* 2021), and this effect is more intense when female density increases in the case of gynochromes, but not in androchromes (Gosden and Svensson 2009).

The low density of *I. elegans* in our populations may explain why androchrome females were consistently rare in both islands (around 15%; in June 2023 they were the majority morph in La Vall, but sample size was only 13 females). Androchrome frequency varies considerably between regions in *I. elegans*. For instance, a review of studies in the UK, France, Italy and Spain, indicated that androchromes were between 8% and 90% of mature females, and in 12 out of 23 cases this morph was the commonest (Cordero-Rivera and Sánchez-Guillén 2007). In the Netherlands and Belgium, 30–80% of females were androchromes (Hammers and Hans Van Gossum 2008). In Ukraine and Poland, 22–33% were androchromes, and in Spain between 3 and 75% were androchromes (Sánchez-Guillén *et al.* 2011). In general, androchromes increase to the North of the distribution of *I. elegans* in Europe, being the commonest morph in Sweden (Gosden *et al.* 2011). In Belgium and the Netherlands, androchrome frequency is negatively related to the mean temperature of June and the distance to the sea (Hammers and Van Gossum 2012). Summer temperatures in the islands of Mallorca and Menorca are usually well

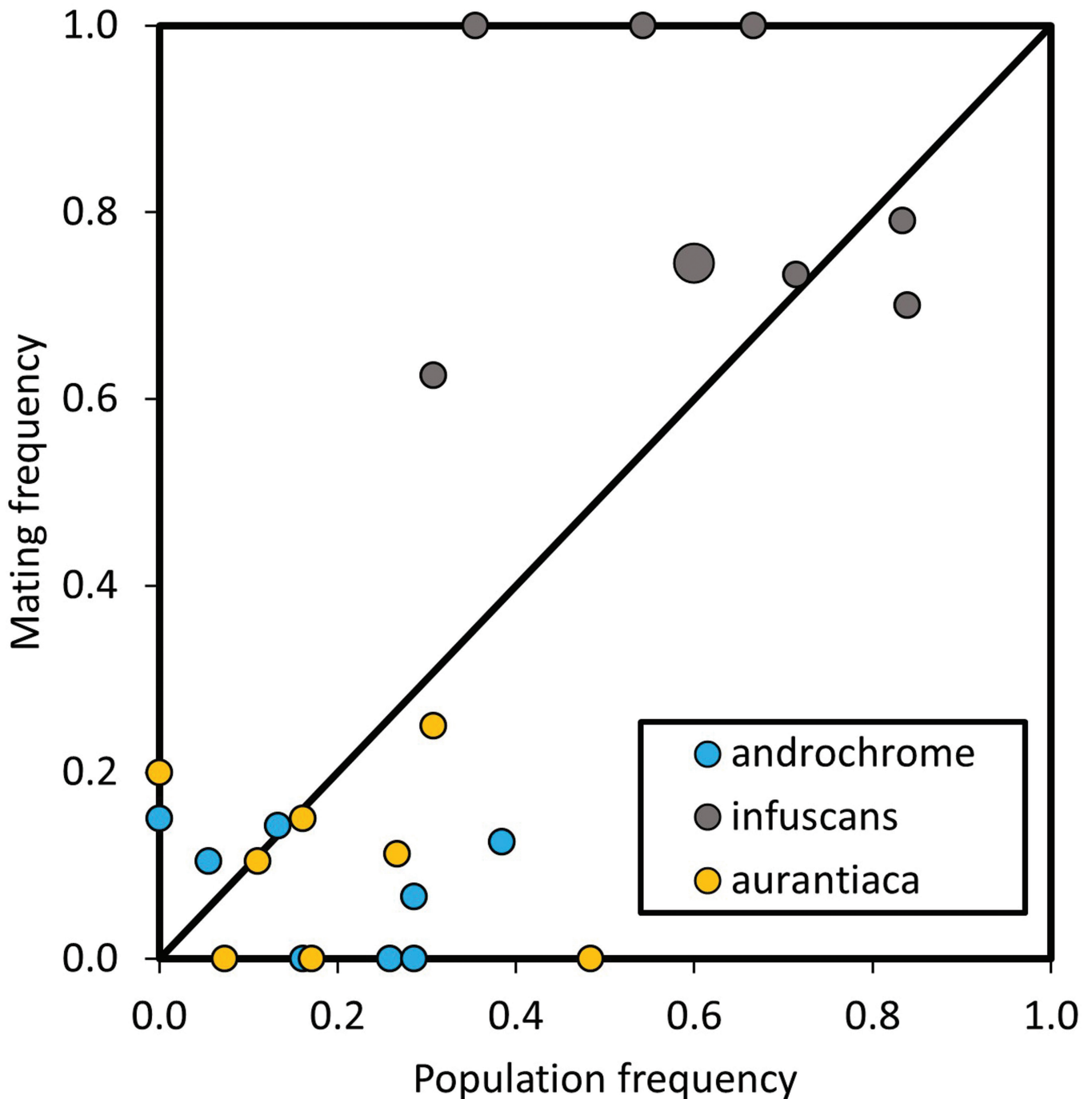


Figure 4. The relationship between the frequency of each morph in the population (estimated from mature females found alone) and their frequency among mating pairs, for all seasons where at least three mating pairs were found. In general, infuscans females were over-represented among mating females, but, given the sample size, the difference is only significant in the case indicated by a larger dot.

above 30 °C, and water temperatures between 22 and 29 °C (Table 1). If androchromes are more sensitive to higher temperatures, this might be one of the factors that explain their low frequency in our populations. In fact, in other Mediterranean localities, androchromes were also rare (3.3–36.0%; Sánchez-Guillén *et al.* 2011). However, in another polymorphic damselfly, androchrome females were more common in more exposed habitats, suggesting that higher temperatures favour androchromes (Cooper *et al.* 2016). Furthermore, in *Ischnura denticollis*, where temperature has been invoked as a relevant

factor in the maintenance of female colour polymorphism, higher temperatures are also more favourable for androchromes (Castillo-Pérez *et al.* 2021). In our study species, *I. elegans*, egg survival and hatching time does not differ among female morphs maintained at different temperatures (Bouton *et al.* 2011), but androchromes were found to have better condition than gynochromes in favourable weather conditions (Bots *et al.* 2009). These contrasting results indicate that the possible effects of temperature must be studied in detail in the future, because they are not generalizable.

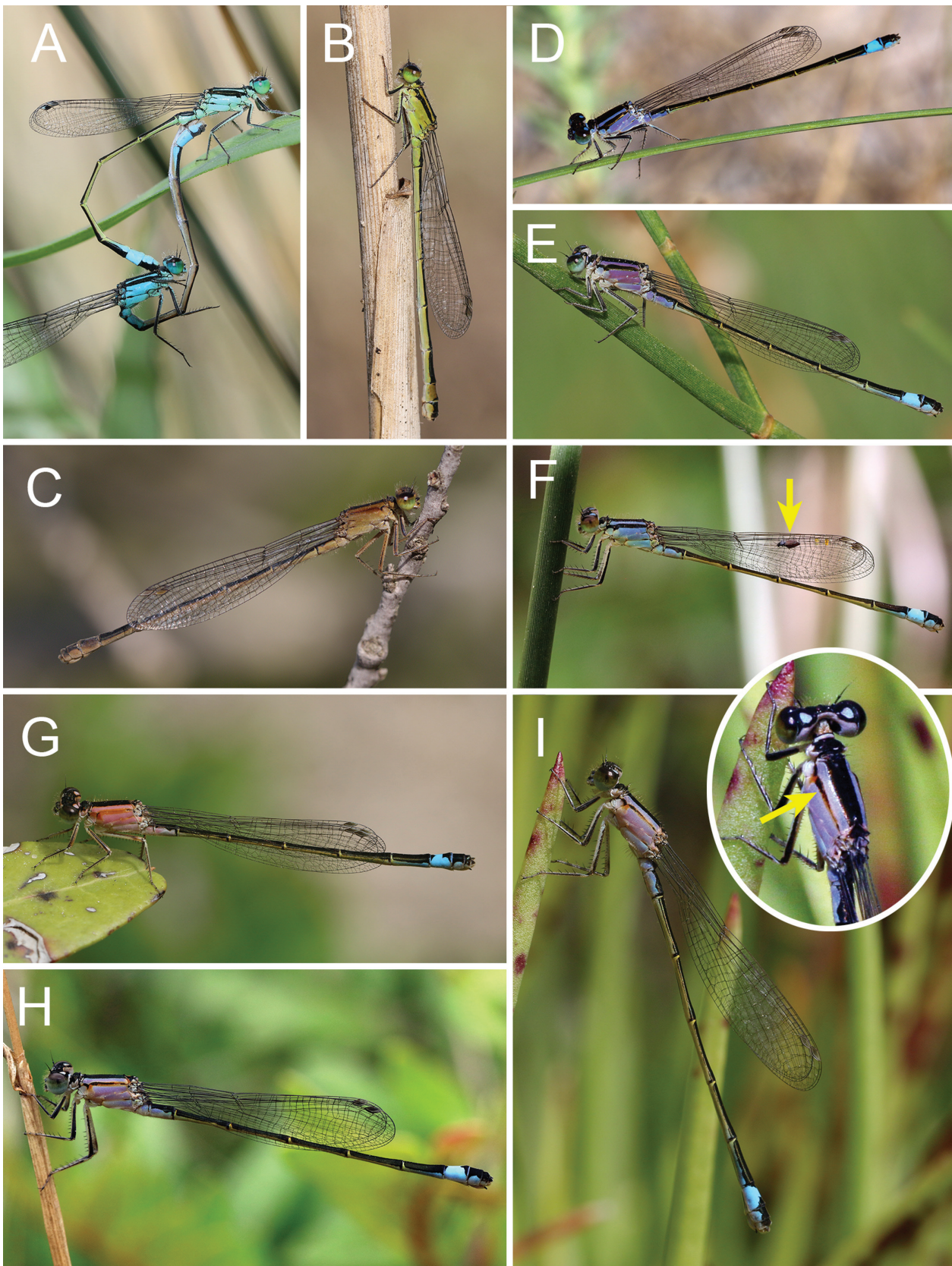


Figure 5. Colour variation of *I. elegans* from the Balearic Islands. (A) Androchrome (male-like) female mating. (B) Mature female infuscans in the olive-green phase (it becomes brown with age). (C) Mature female aurantiaca, in the brown phase. (D-F) Three examples of the juvenile violet coloration of infuscans females. Note the parasitic midge on (F) (arrow). (G) Juvenile female of the aurantiaca morph in the pink-orange phase, with only one medio-dorsal black line, and orange antehumeral lines. (H-I) The two juvenile females of the new morph, showing a violet thorax but orange antehumeral lines, with some black over the orange, as is indicated with the arrow on the insert. Pictures taken at Son Bou and Tirant in June 2021 by A.C.-R.

Contrary to the density-dependent hypothesis, which suggests that androchromes are favoured at higher densities, we found no significant effect of male density on androchrome frequency, but we did find a negative effect on gynochrome frequency, which indirectly points to the same conclusion. The lack of effect of male density on androchrome frequency might be explained by the fact that the variation of androchrome frequency was very limited in our populations. On the other hand, we found that sex-ratio was positively related to androchrome frequency, which is another prediction of the density-dependent hypothesis (Hinneking 1987, Cordero 1992). It is possible that androchromes were less prone to abandon the shore of the ponds at increasing male density, compared to gynochromes, as some behavioural observations in the field suggest that androchromes are more likely to face-off approaching males, i.e. they are more aggressive (Sánchez-Guillén *et al.* 2017). Several studies of polymorphic damselflies have found differences between androchrome and gynochrome females in fitness, including fecundity (Abbott and Gosden 2009, Takahashi and Watanabe 2010a, b, Galicia-Mendoza *et al.* 2017, Sánchez-Guillén *et al.* 2017), male harassment (Van Gossum *et al.* 2005, Gosden and Svensson 2007, 2009, Takahashi and Watanabe 2010b, Gering 2017, Piersanti *et al.* 2021), and mating frequency (Cordero *et al.* 1998, Svensson *et al.* 2005, Hammers and Hans Van Gossum 2008, Sánchez-Guillén *et al.* 2017). These differences are usually not generalizable between species (and even sometimes are opposite) nor between populations of the same species, which suggests that the maintenance of this polymorphism is not explained by a single hypothesis.

We found that the frequency of female colour morphs in juvenile females sometimes does not match their frequency among mature females (Fig. 2). The deviations were nevertheless inconsistent, suggesting that this fact is affected by several uncontrolled factors. If survivorship during the sexual maturation period, which in this species takes about 5–6 days since emergence (Parr 1973), was similar among morphs, then mature frequency should be similar to juvenile frequency. There is some evidence that points out that survivorship of female colour morphs is similar, and their recapture rate also (Sanmartín-Villar and Cordero-Rivera 2022), but this is mainly based on animals marked when already mature. The daily survival rates of juvenile odonates average 0.887, significantly lower than the survival rate of mature females, which was estimated as 0.898, but their recapture rates did not differ (Sanmartín-Villar and Cordero-Rivera 2022). Even if survival during sexual maturation were the same for all morphs, differences could arise if the spatial distribution of morphs differ, for instance, if androchromes were more likely to remain close to the water than the other morphs. Furthermore, morph-biased dispersal could also bias the estimation of morph frequencies, making juvenile frequencies different to mature frequencies. The possibility that juvenile females show different behaviour in function of their colour morph is still understudied. For instance, there is evidence for differences in mating propensity between aurantiaca and violacea (the juvenile colour of infuscans and androchromes) females (Hammers *et al.* 2009).

Two studies have linked the maintenance of female colour polymorphism with parasite tolerance, specifically with the effect of water mites (Sánchez-Guillén *et al.* 2013, Willink and Svensson 2017). Although water mites can be a relevant factor

in some damselfly populations (e.g. Andrés and Cordero 1998), their prevalence is in general too low in many regions, and they are absent (or almost absent) in some islands (Lorenzo-Carballa *et al.* 2011, Cordero-Rivera *et al.* 2018), as we have also found in the Balears. The only parasites found were blood-sucking midges, but again, their prevalence was too low to be significant (Cordero-Rivera 2021).

One of the most surprising results of our fieldwork in the Balears is the low mating activity detected on most days. *Ischnura elegans* is one of the species of damselflies more easily observed mating, because it is normally very abundant and copulations in this species last for up to 7 h (Miller 1987), and start very early in the morning in the Mediterranean region (e. g. Piersanti *et al.* 2021). In our populations mating pairs were rarely observed (with a few exceptional days), a fact that might be related to the low density of these populations (Fig. 3). Seasonal variability in the reproductive activity is expected, given the fluctuations of rain and temperature, as has been reported for *Ischnura graellsii* (Cordero Rivera 2002). In our populations, high temperatures (and in late summer, also strong winds) are clearly a limiting factor for the activity of *I. elegans*, and we discovered that after noon, finding these damselflies becomes almost impossible, because they look for shelter inside shrubs. Low mating frequency might also contribute to the low frequency of androchromes observed in these islands.

The finding of a possible novel female colour morph (Fig. 5H-I) was a surprise, an *insular surprise*. In the genus *Ischnura*, polymorphic species show two or three female morphs (Sánchez-Guillén *et al.* 2020), which are under the control of two to three alleles, the androchrome allele being dominant in *I. elegans* (Sánchez-Guillén *et al.* 2005). The novel morph shows an intermediate phenotype between infuscans and aurantiaca, being detectable when the individuals are juvenile (Fig. 5H-I). It is likely that, upon maturation, these females become brown, and therefore cannot be distinguished from aurantiaca females. This coloration might be produced by a novel allele, whose prevalence is very low, or could be also the result of a modifier gene, which alters the expression of the aurantiaca and infuscans alleles. Unfortunately, this morph is very rare, and we could detect it in only one population and season. It would be interesting to rear the offspring of these females and study the inheritance mechanisms.

In conclusion, we have found that Balearic populations of *I. elegans* are characterized by low density, low mating activity, and low androchrome frequencies. In both Mallorca and Menorca, androchromes are the minority morph, but the frequency of infuscans and aurantiaca is very different between islands. This fascinating phenomenon is much more complex and needs more experimental work to disentangle the differences between the two gynochrome morphs, which is a priority for future research.

SUPPORTING INFORMATION

Supplementary data is available at the *Biological Journal of the Linnean Society* online. Table S1. Excel spreadsheet with all the field data compiled during this study.

ACKNOWLEDGEMENTS

We thank Esther Soler for help in Menorca and the staff of S'Albufera of Mallorca Natural Park for their help with the organization of

fieldwork. Collection permits were provided by the Regional Government of Illes Balears to A.C.-R. This work was funded by grant PGC2018-096656-B-I00 to A.C.-R. from Ministerio de Ciencia, Innovación y Universidades MCIN/AEI/10.13039/501100011033 and from 'European Regional Development Fund: A way of making Europe', by the 'European Union'. Funding for open access charge: Universidade de Vigo/CISUG.

DATA AVAILABILITY

All data are included in the Supporting Information.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

- Abbott JK, Gosden TP. Correlated morphological and colour differences among females of the damselfly *Ischnura elegans*. *Ecological Entomology* 2009;**34**:378–86. <https://doi.org/10.1111/j.1365-2311.2009.01087.x>
- Andrés JA, Cordero A. Effects of water mites on the damselfly *Ceriagrion tenellum*. *Ecological Entomology* 1998;**23**:103–9. <https://doi.org/10.1046/j.1365-2311.1998.00125.x>
- Andrés JA, Sánchez-Guillén RA, Cordero-Rivera A. Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. *Evolution* 2000;**54**:2156–61.
- Baeckens S, Van Damme R. The island syndrome. *Current Biology* 2020;**30**:R338–9. <https://doi.org/10.1016/j.cub.2020.03.029>
- Beatty CD, Sánchez Herrera M, Skevington JH *et al.* Biogeography and systematics of endemic island damselflies: the *Nesobasis* and *Melanesobasis* (Odonata: Zygoptera) of Fiji. *Ecology and Evolution* 2017;**7**:7117–29. <https://doi.org/10.1002/ece3.3175>
- Bots J, De Bruyn L, van Dongen S *et al.* Female polymorphism, condition differences, and variation in male harassment and ambient temperature. *Biological Journal of the Linnean Society* 2009;**97**:545–54. <https://doi.org/10.1111/j.1095-8312.2009.01238.x>
- Bouton N, Iserbyt A, Van Gossom H. Thermal plasticity in life-history traits in the polymorphic blue-tailed damselfly, *Ischnura elegans*: no differences between female morphs. *Journal of Insect Science (Online)* 2011;**11**:112. [insectscience.org/11.112. 10.1111/j.1095-8312.2011.01619.x](https://doi.org/10.1111/j.1095-8312.2011.01619.x)
- Castillo-Pérez EU, Nava-Bolaños A, Rocha-Ortega M *et al.* Does heat tolerance explain female polymorphism in damselflies? *Journal of Insect Behavior* 2021;**34**:41–8. <https://doi.org/10.1007/s10905-021-09767-z>
- Cooper IA, Brown JM, Getty T. A role for ecology in the evolution of colour variation and sexual dimorphism in Hawaiian damselflies. *Journal of Evolutionary Biology* 2016;**29**:418–27. <https://doi.org/10.1111/jeb.12796>
- Cordero A. Density-dependent mating success and colour polymorphism in females of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). *Journal of Animal Ecology* 1992;**61**:769–80. <https://doi.org/10.2307/5630>
- Cordero A, Santolamazza-Carbone S, Utzeri C. Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). *Animal Behaviour* 1998;**55**:185–97.
- Cordero-Rivera A. Influencia de la selección sexual sobre el comportamiento reproductor de los odonatos. In: Soler M (ed.), *Evolución: la Base de la Biología*. Granada: Proyecto Sur, 2002, 497–507.
- Cordero-Rivera A. El género *Ischnura* en España y Portugal: guía de identificación a partir de fotografías. *Boletín Rola* 2015;**6**:37–58.
- Cordero-Rivera A. *Forcipomyia paludis* (Diptera: Ceratopogonidae) on the wings of *Ischnura elegans* (Odonata: Coenagrionidae) in Minorca (Balearic Islands). *Boletín de la Sociedad Entomológica Aragonesa* 2021;**69**:212–3.
- Cordero-Rivera A, Andrés JA. Estimating female morph frequencies and male mate preferences of polychromatic damselflies: a cautionary note. *Animal Behaviour* 2001;**61**:F1–6.
- Cordero-Rivera A, Sánchez-Guillén RA. Male-like females of a damselfly are not preferred by males even if they are the majority morph. *Animal Behaviour* 2007;**74**:247–52.
- Cordero-Rivera A, Stoks R. Mark-recapture studies and demography. In: Córdoba-Aguilar A (ed.), *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research*. Oxford: Oxford University Press, 2008, 7–20.
- Cordero-Rivera A, Vieira V, Utzeri C. Clonal damselflies (*Ischnura hastata*) are not significantly affected by mite parasitism. *Entomologia Experimentalis et Applicata* 2018;**166**:583–91.
- Córdoba-Aguilar A, Beatty CD, Bried JT (eds). *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research*. Oxford: Oxford University Press, 2022.
- Fincke OM. Polymorphic signals of harassed female odonates and the males that learn them support a novel frequency-dependent model. *Animal Behaviour* 2004;**67**:833–45. <https://doi.org/10.1016/j.anbehav.2003.04.017>
- Galicia-Mendoza DI, Sanmartín-Villar I, Espinosa-Soto C *et al.* Male biased sex ratio reduces the fecundity of one of three female morphs in a polymorphic damselfly. *Behavioral Ecology* 2017;**28**:1183–94.
- Gering EJ. Male-mimicking females increase male-male interactions, and decrease male survival and condition in a female-polymorphic damselfly. *Evolution* 2017;**71**:1390–6. <https://doi.org/10.1111/evo.13221>
- Gosden TP, Svensson EI. Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. *PLoS One* 2007;**2**:e580. <https://doi.org/10.1371/journal.pone.0000580>
- Gosden TP, Svensson EI. Density-dependent male mating harassment, female resistance, and male mimicry. *The American Naturalist* 2009;**173**:709–21. <https://doi.org/10.1086/598491>
- Gosden TP, Stoks R, Svensson EI. Range limits, large-scale biogeographic variation, and localized evolutionary dynamics in a polymorphic damselfly. *Biological Journal of the Linnean Society* 2011;**102**:775–85. <https://doi.org/10.1111/j.1095-8312.2011.01619.x>
- Hammers M, Van Gossom H. Variation in female morph frequencies and mating frequencies: random, frequency-dependent harassment or male mimicry? *Animal Behaviour* 2008;**76**:1403–10.
- Hammers M, Van Gossom H. Helpende en misleidende signalen in vrouwelijke lichaamskleur bij het Lantaarntje (*Ischnura elegans*). *Brachytron* 2012;**15**:16–24.
- Hammers M, Sánchez-Guillén RA, Van Gossom H. Differences in mating propensity between immature female color morphs in the damselfly *Ischnura elegans* (Insecta: Odonata). *Journal of Insect Behavior* 2009;**22**:324–37. <https://doi.org/10.1007/s10905-009-9175-2>
- Hinnekin B. Population dynamics of *Ischnura e. elegans* (Vander Linden) (Insecta: Odonata) with special reference to morphological colour changes, female polymorphism, multiannual cycles and their influence on behaviour. *Hydrobiologia* 1987;**146**:3–31. <https://doi.org/10.1007/bf00007574>
- Jordan S, Simon C, Polhemus D. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata: Coenagrionidae). *Systematic Biology* 2003;**52**:89–109. <https://doi.org/10.1080/10635150390132803>
- Lorenzo-Carballa MO, Cordero-Rivera A. Thelytokous parthenogenesis in the damselfly *Ischnura hastata* (Odonata, Coenagrionidae): genetic mechanisms and lack of bacterial infection. *Heredity* 2009;**103**:377–84. <https://doi.org/10.1038/hdy.2009.65>
- Lorenzo-Carballa MO, Beatty CD, Haitlinger R *et al.* Larval aquatic and terrestrial mites infesting parthenogenetic *Ischnura hastata* (Odonata: Coenagrionidae) from the Azores Islands. *Experimental and Applied Acarology* 2011;**54**:225–41. <https://doi.org/10.1007/s10493-011-9437-5>
- Losos JB, Ricklefs RE. Adaptation and diversification on islands. *Nature* 2009;**457**:830–6. <https://doi.org/10.1038/nature07893>

- Miller PL. An examination of the prolonged copulations of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 1987;**16**:37–56.
- Parr MJ. Ecological studies of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae) I age groups, emergence patterns and numbers. *Odonatologica* 1973;**2**:139–57.
- Piersanti S, Salerno G, Di Pietro V *et al.* Tests of search image and learning in the wild: insights from sexual conflict in damselflies. *Ecology and Evolution* 2021;**11**:4399–412. <https://doi.org/10.1002/ece3.7335>
- Rebora M, Frati F, Piersanti S *et al.* Field tests of multiple sensory cues in sex recognition and harassment of a colour polymorphic damselfly. *Animal Behaviour* 2018;**136**:127–36. <https://doi.org/10.1016/j.anbehav.2017.12.015>
- Robertson HM. Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. *Animal Behaviour* 1985;**33**:805–9. [https://doi.org/10.1016/s0003-3472\(85\)80013-0](https://doi.org/10.1016/s0003-3472(85)80013-0)
- Sánchez-Guillén RA, Van Gossum H, Cordero-Rivera A. Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biological Journal of the Linnean Society* 2005;**85**:471–81.
- Sánchez-Guillén RA, Hansson B, Wellenreuther M *et al.* The influence of stochastic and selective forces in the population divergence of female colour polymorphism in damselflies of the genus *Ischnura*. *Heredity* 2011;**107**:513–22. <https://doi.org/10.1038/hdy.2011.36>
- Sánchez-Guillén RA, Martínez-Zamila SMJ, Jiménez-Cortés JG *et al.* Maintenance of polymorphic females: do parasites play a role? *Oecologia* 2013;**171**:105–13. <https://doi.org/10.1007/s00442-012-2388-7>
- Sánchez-Guillén RA, Wellenreuther M, Chávez-Ríos JR *et al.* Alternative reproductive strategies and the maintenance of female color polymorphism in damselflies. *Ecology and Evolution* 2017;**7**:5592–602. <https://doi.org/10.1002/ece3.3083>
- Sánchez-Guillén RA, Ceccarelli S, Villalobos F *et al.* The evolutionary history of colour polymorphism in *Ischnura* damselflies (Odonata: Coenagrionidae). *Odonatologica* 2020;**49**:333–70.
- Sanmartín-Villar I, Cordero-Rivera A. The inheritance of female colour polymorphism in *Ischnura genei* (Zygoptera: Coenagrionidae), with observations on melanism under laboratory conditions. *PeerJ* 2016;**4**:e2380. <https://doi.org/10.7717/peerj.2380>
- Sanmartín-Villar I, Cordero-Rivera A. Odonata survival: insights from mark-recapture experiments. In: Córdoba-Aguilar A, Beatty CD, Bried JT (eds), *Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research*. Oxford: Oxford University Press, 2022, 129–40.
- Svensson EI, Abbott J, Härdling R. Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *The American Naturalist* 2005;**165**:567–76. <https://doi.org/10.1086/429278>
- Takahashi Y, Watanabe M. Morph-specific fecundity and egg size in the female-dimorphic damselfly *Ischnura senegalensis*. *Zoological Science* 2010a;**27**:325–9. <https://doi.org/10.2108/zsj.27.325>
- Takahashi Y, Watanabe M. Female reproductive success is affected by selective male harassment in the damselfly *Ischnura senegalensis*. *Animal Behaviour* 2010b;**79**:211–6. <https://doi.org/10.1016/j.anbehav.2009.10.032>
- Takahashi Y, Yoshimura J, Morita S *et al.* Negative frequency-dependent selection in female color polymorphism of a damselfly. *Evolution* 2010;**64**:3620–8. <https://doi.org/10.1111/j.1558-5646.2010.01083.x>
- Takahashi Y, Morita S, Yoshimura J *et al.* A geographic cline induced by negative frequency-dependent selection. *BMC Evolutionary Biology* 2011;**11**:256. <https://doi.org/10.1186/1471-2148-11-256>
- Van Gossum H, Stoks R, De Bruyn L. Frequency-dependent male mate harassment and intra-specific variation in its avoidance by females of the damselfly *Ischnura elegans*. *Behavioral Ecology and Sociobiology* 2001;**51**:69–75.
- Van Gossum H, De Bruyn L, Stoks R. Male harassment on female colour morphs in *Ischnura elegans* (Vander Linden): Testing two frequency-dependent hypotheses (Zygoptera: Coenagrionidae). *Odonatologica* 2005;**34**:407–14.
- Van Gossum H, Sherratt TN, Cordero-Rivera A. The evolution of sex-limited colour polymorphisms. In: Córdoba-Aguilar A (ed.), *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research*. Oxford: Oxford University Press, 2008, 219–29.
- Willink B, Svensson EI. Intra- and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society B: Biological Sciences* 2017;**284**:20162407.