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Sexual conflict and the evolution of monandry: The case of the damselfly *Ischnura hastata* (Odonata: Coenagrionidae) in the Galápagos Islands

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Abstract

1. Sexual selection favours the evolution and maintenance of polygamy, which is the dominant reproductive strategy in insects. Monogamy can evolve in very short-lived species due to time constraints. Here we study adult activity and mating behaviour of a population of the damselfly *Ischnura hastata*, a species rarely seen mating, and which has been suggested to be monandric, in wetlands of Isabela Island, Galápagos.
2. By means of mark-recapture methods, we estimated that the daily survival rate was low, ranging from 0.385 to 0.876, yielding average life expectancies of mature individuals of only 1.2–3.2 days. Adults showed very low activity before 7:00, indicating that mating does not occur early. The number of male–female interactions and mating attempts was extremely low, with only 44 copulations recorded on over 230 h of observations.
3. Copulations were brief, with a mean duration of 11 min (but only two were observed from the start). Males showed clear preference to attempt to grasp in tandem females of intermediate age (in 94.3% of cases), rather than young (31.3%) or mature females (24.0%). Males were very persistent once a tandem was achieved, retaining females for up to 139 min, but most females resisted and did not copulate.
4. We conclude that females of *I. hastata* show a very short time window to mate, exactly when they change colour from juvenile to mature, and live only enough to mate once. Short lifespan has selected for female monandry in *I. hastata*, creating an intense sexual conflict over mating rates.

KEYWORDS
Ischnura, monandry, monogamy, Odonata, sexual conflict, short-lived species

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INTRODUCTION

Sexual selection is the engine of multiple mating (Parker & Birkhead, 2013), and consequently, polygamy is the most common behavioural strategy in animal populations. Insects are highly polygamous (Thornhill & Alcock, 1983; Choe et al., 1996; Shuker & Simmons, 2014) and both sexes frequently mate more than once. Females might mate with several males before starting to lay eggs, with long-term sperm storage, producing the conditions for sperm competition (Parker, 1970) and cryptic female choice (Eberhard, 1996). Monandry, the reproductive strategy of females mating only once in their life, is very rare in insects (Cordero-Rivera, 2022) and even in strictly monandric species, there are reasons for females to re-mate. For example, monandrous females may mate again if the first male fails to inseminate (García-González, 2004), if there is genetic incompatibility or if the amount of sperm received in one mating is not enough for the lifespan of the female (Cordero-Rivera, 2022).

The evolution of monandry has been linked to two alternative scenarios. First, females might maximise their reproductive success by mating only once, particularly if the cost of mating is very high and males do not provide nutrients or other benefits (Arnqvist & Kirkpatrick, 2005). Also, if females are short-lived and the encounter rate between males and females is very low, females might benefit from monogamy due to time constraints (Kvarnemo, 2018). On the contrary, males usually benefit by inducing female monandry even if this is not the best option for their mates, creating intense sexual conflict over mating frequency. In this case, monandry is beneficial to males, but not necessarily so for females, and therefore experimentally inducing remating by females would result in benefits for them (Arnqvist & Andrés, 2006). Consequently, monandry can be the result of females resisting remating (female control) or of males eliminating female receptivity to further matings (male control), even if this is detrimental to females (Hosken et al., 2009). However, both alternatives are very difficult to disentangle in the field.

As mentioned above, most insect species that have been studied in detail are highly polygamous, but monandry seems to have evolved several times, as this reproductive strategy can be found in distantly related orders like the Orthoptera (Ortíz-Jiménez & del Castillo, 2015), Lepidoptera (Xu & Wang, 2011), Diptera (Arnqvist & Andrés, 2006; South & Arnqvist, 2008), Hymenoptera (Kronauer et al., 2011) and Coleoptera (Reid, 1999). In the Odonata, mating behaviour is highly diverse, and most species are promiscuous, with both sexes mating several times over their lifetime, a fact that has led to this group being proposed as a model system to study postcopulatory sexual selection (Córdoba-Aguilar & Cordero-Rivera, 2008; Cordero-Rivera & Córdoba-Aguilar, 2010). However, some odonates are apparently monogamous, or at least they are rarely seen mating, like some species of the genera *Mesamphiagrion* (Palacino-Rodríguez et al., 2020), *Ischnura* (Fincke, 1987) or *Polythore* (Sanmartín-Villar & Cordero-Rivera, 2016). Among North American species of the genus *Ischnura* there is a correlation between life-history, demographic traits and behaviour, with supposedly monandric species showing smaller size, greater sexual size dimorphism, shorter matings and monochromatic females when compared with polyandric species (Robinson & Allgeyer, 1996).

Ischnura hastata is a damselfly species indigenous to America, where it can be found from Canada to Brazil (Koroiva et al., 2020). Mating appears to be rare in this species: for instance, previous fieldwork in Cuba and Galápagos populations yielded only a single observation of a copulation pair, and 9% of field-collected mature females laid only sterile clutches, suggesting that they were unmated (Lorenzo-Carballa et al., 2017). This species has female-only populations in the Azores, constituting the only known example of parthenogenesis in the Odonata (Cordero-Rivera et al., 2005; Lorenzo-Carballa et al., 2009). Although very little is known about the mating behaviour of *I. hastata*, it has been considered monandric by Robinson & Allgeyer (1996). Our goal here was to estimate the daily activities of adult *I. hastata* in a sexual population, to determine when copulations occur, and to describe the interactions between males and females. Specifically, we designed field surveys to follow the activity of focal males and females, with the aim to detect sexual interactions and copulations, and test the hypothesis that females are monandric. Furthermore, we marked a large number of adults to estimate their survival, because short lifespan is generally linked to monandry (Kronauer et al., 2011).

METHODS

Fieldwork was done between 2018 and 2022 at wetlands in the island Isabela, Galápagos (Ecuador). In 2018 we carried out an exploratory fieldwork, to obtain the general patterns of the behaviour of *Ischnura hastata* needed to focus subsequent fieldwork. To do so, we explored the natural brackish wetland “Las Diablas” (latitude: −0.957, longitude: −90.978) and the artificial ponds found at “El Chapín” (−0.944, −90.973), both at sea level. We sampled and marked specimens in a section of 50 m of the shore of the Las Diablas wetland daily between 30 July and 4 August 2018 (a total of 20 h of field observations) and in two ponds at El Chapín between 10 and 15 August (18 h of observations). Las Diablas is a large wetland with marine influence, having a pH of 6.87 and a conductivity of 5.34 mS/cm in the sampled point. For the fieldwork carried out in 2019 (7–21 January; 80 h of observations) and 2020 (14 January–2 February; 113 h of observations) we selected the ponds at El Chapín, because the size of the population of *I. hastata* was smaller, and the accessibility of the habitat allowed a more efficient sampling. These ponds had a pH of 8.06, conductivity of 2.27 mS/cm in August 2018 and pH 7.79–8.14 and conductivity 2.37–3.72 mS/cm in January 2020 and have no connection with the sea. In 2022, additional observations focusing only on mating behaviour were carried out at both ponds, between 8 October and 13 November (35 h of observation).

General methods

Field observations started at 6:30–8:00 and ended at 14:00–17:00, or earlier if the weather was rainy. One or two observers slowly walked

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around the shoreline of the ponds and nearby grassy areas and captured the damselflies using an entomological net. To obtain estimates of survival and recapture rates, animals were marked by writing a number on their wings, using a permanent marker (Faber-Castell Multimark 1523 S pen). Animals were assigned, based on their thorax colouration (Figure 3) and wing flexibility, to one of the following age categories: teneral (newly emerged adults, with pale colouration and glistening wings, up to one day old), young (orange coloured in females, light yellow in males, very flexible wings; about 2–5 days old) and mature (brownish with bluish pruinescence at older ages in females, bright yellow in males; more than 5 days old). Females showing an intermediate colouration between orange and brown were considered separately (see Results). We generally avoided marking very soft teneral specimens, because handling them unavoidably damages their wings, but some tenerals of about 24 h of age were included in the young age group. After marking, animals were immediately released on the shore of the ponds.

In 2018 we recorded air temperature using a datalogger (Gemini Ltd) situated in the shade of a tree, at about 1.5 m above the soil, which took a measurement every 5 min. In 2019, 2020 and 2022 we recorded temperature at irregular intervals using a thermometer under the shade of a tree.

Focal observations

In 2018, we carried out behavioural observation along the shoreline of the ponds, and the grassy fields. Focal observations were carried out in the 2019 and 2020 fieldwork seasons. In 2019 we observed 203 mature females for 5 min (a total of 16.9 h of observation) to determine the frequency of interactions with males and their general activity from 7:00 to 17:00 h. Sample size was 10 females for every 30 min interval (except 7:30–8:00 when 13 females were observed, 12:30–13:00 with 9 females and 13:00–13:30 with 11). Females were observed near the water because we aimed to determine when they lay eggs and expected higher mating frequency near the shoreline, where matings were observed in 2018. We recorded the following behaviours: foraging activity (prey consumption and number of foraging flights), number of refusal displays to approaching individuals (see below), number of males approaching and trying to grasp the female in tandem, number of flights, frequency of grooming behaviours (cleaning the eyes, wings or the abdomen), whether the female was or not ovipositing, the number of non-sexual interactions with conspecifics (males or females), and whether the female remained perched for the entire focal observation period. If the focal female was unmarked, we captured the female after the observation (when possible) and painted a dot on the wings, to avoid observing the same female twice.

Given that no focal females were observed mating in 2019, in 2020 we observed the behaviour of mature males and young females, and of mature females, at random, that is, we searched for individuals in the grassy fields around the ponds and the shoreline. Each individual was observed for 10 min, recording the same behaviours as in 2019, but now including the number of “threat” displays by young females (and males), divided flights into short and long (more than 1 m), and for males we recorded the number of approaches to females and the number of tandem attempts, and whether these ended in tandem or copulation. In total, we observed 174 mature females (9–12 females in every 30-min interval for a total of 29 h), 83 young females (4–6 females per interval for 13.8 h) and 129 males (6–10 males per interval for 21.5 h), from 8:00 to 16:30 h.

Statistical analyses

The recapture histories of marked animals were analysed using Cormak-Jolly-Seber models by means of the software MARK (White & Burnham, 1999). Our goal was to estimate survival (*Phi*) and recapture (*p*) rates of mature and young males and females, to get an estimate of their life expectancy. We first fitted the time-dependent saturated model by groups (sexes and age groups young (including teneral) and mature), denoted by *Phi*(g*t)*p*(g*t) using the notation of Lebreton et al. (1992), and checked that the model was a good approximation to the variability of the dataset, using Test 2 and Test 3 of the program RELEASE, as described in detail in previous published work (Lorenzo-Carballa et al., 2017). We estimated the variance inflation factor (c-hat) and used this value to correct AIC estimates. We used the model *Phi*(g) *p*(g) to estimate survival rates of young and mature males and females, and estimated life expectancy by means of the formula Lifespan $= -1/\log_e$ (survival) (Cook et al., 1967).

Focal observations were sorted by time, and the mean frequency and standard error of each behaviour were calculated for each 30-min time interval. Mean values are presented with their standard errors. Statistical tests were calculated with XLSTAT 2020 (www.xlstat.com) and Genstat 21st edition (www.vsn.co.uk).

RESULTS

Survival rates

Table 1 shows the estimates of daily survival and recapture rates by sex and age at marking, using model Phi(g) p(g). This model was the most supported in 2019 and the second most supported in 2020 (Table S1), when sampling effort was higher, suggesting that there are true differences between sexes and ages. Daily recapture rate was highly variable between groups and years, with a minimum of 0.032 for young males in 2019 and maximum of 0.663 for young females in Las Diablas in 2018. Daily survival rate was also low (0.385–0.876), with young males having the maximum survival in 2018 at Las Diablas and 2019 at El Chapín. These estimates translate to average life expectancies of only 1.2–3.2 days for females marked with orange (young) coloration and 2.4–3.2 for females marked as mature specimens (Table 1).

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TABLE 1 Estimates of survival rate (phi) and recapture rate (p) and their standard errors using model phi(g) p(g). Life expectancy (days) was calculated from survival rate following Cook et al. (1967).

Population	Age/sex	Phi	SE	p	SE	Expected lifespan
Las Diablas 2018	Mature males	0.716	0.084	0.245	0.062	3.0
	Young males	0.870	0.362	0.072	0.073	7.2
	Mature females	0.678	0.100	0.261	0.076	2.6
	Young females	0.533	0.066	0.663	0.108	1.6
El Chapín 2018	Mature males	0.502	0.193	0.192	0.132	1.4
	Young males	0.385	0.307	0.190	0.241	1.0
	Mature females	0.661	0.084	0.425	0.112	2.4
	Young females	0.683	0.202	0.237	0.164	2.6
El Chapín 2019	Mature males	0.697	0.044	0.134	0.027	2.8
	Young males	0.876	0.107	0.032	0.019	7.6
	Mature females	0.733	0.036	0.220	0.036	3.2
	Young females	0.424	0.147	0.111	0.075	1.2
El Chapín 2020	Mature males	0.650	0.034	0.189	0.028	2.3
	Young males	0.594	0.054	0.155	0.037	1.9
	Mature females	0.741	0.028	0.214	0.028	3.3
	Young females	0.733	0.036	0.113	0.021	3.2

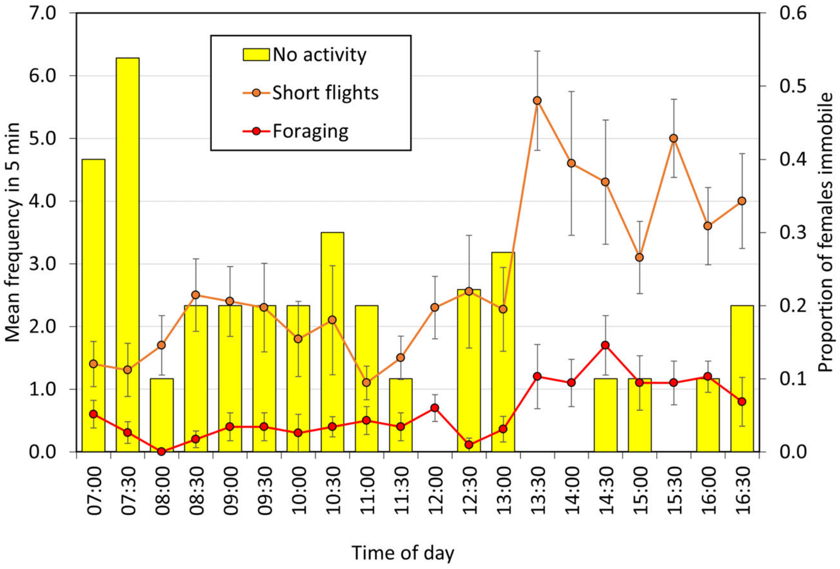

FIGURE 1 Diel activity of mature females ($N = 203$) perched near the shoreline of El Chapín ponds and observed in 5-min periods in January 2019. Note that the proportion of females remaining immobile (no activity) for all the focal period (right vertical axis) was higher in the early hours, and both short flights (spontaneous or due to interactions with conspecific or other insects) and foraging flights (clearly directed to a potential prey) increased after 13:00 h. Error bars indicate SE.

Diel activity

Activity was checked on 17 Jan 2019 at 6:00 (sunrise was at 6:08), and on four days between 6:30 and 7:00, when temperatures were 22–26°C. In all cases, damselflies did not fly spontaneously and only changed perch—with difficulty—if disturbed. Activity started at around 7:00.

In 2019, 17.2 ± 3.1% of females remained perched during the 5-min focal observation period, showing increased activity over the day, as can be seen by the number of foraging flights and short flights (Figure 1). Damselflies were observed capturing small dipterans, but also one spider and at least on three occasions they were observed capturing aquatic bugs (*Mesovelia* sp.) from the water surface.

In 2020, the percentage of individuals that remained perched over the 10-min focal observation was 6.3 ± 1.9% for mature females, 12.3 ± 3.4% for young females and 7.7 ± 2.6% for mature males. In total, 11 mature females, 10 young and 10 mature males did not fly

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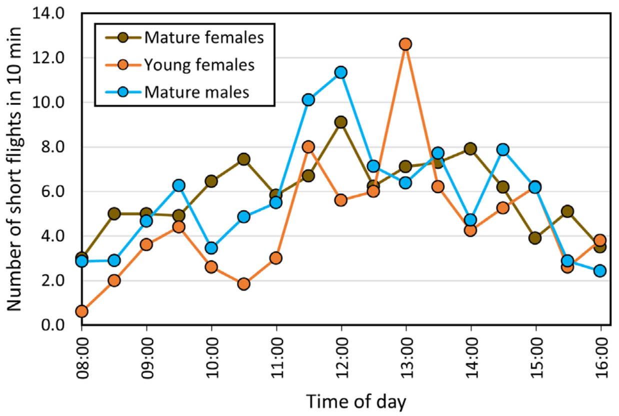

FIGURE 2 The mean number of short flights (less than 1 m) of focal mature (*N* = 174) and young (*N* = 83) females and mature males (*N* = 129) recorded in 10 min of focal observations in January 2020. Individuals were observed in the paddy fields around the ponds and the shoreline. Standard errors averaged 1.3–1.9 and are not presented for easy visualisation.

during their focal observation. These frequencies are not significantly different among groups (Contingence $Chi^2 = 2.52$, $df = 2$, $p = 0.284$). Flying activity averaged 5.92 ± 0.40 flights/10 min in mature females, 4.62 ± 0.68 in young females and 5.72 ± 0.62 in mature males, and peaked between 11:30 and 13:30 (Figure 2). In fact, some mature females remained motionless only between 8:00–10:00 and 14.30–16:00. These values are not significantly different using a Generalised Linear Model with Poisson distribution and log link (deviance ratio = 2.06, $p = 0.128$). Long flights (over 1 m) were rare, with an average of 0.17 ± 0.04 flights/10 min in mature females, 0.07 ± 0.03 in young females and 0.39 ± 0.09 in mature males. Males showed a higher frequency of long flights (GLM with Poisson distribution, deviance ratio: 15.28, $p < 0.001$). Grooming activities were observed with low frequency throughout the day.

Sexual behaviour

Interactions between males and females were extremely uncommon. In fact, none of the 203 focal mature females observed in 2019 mated, and only one mating was observed involving one of the 174 focal mature and intermediate females in 2020 (Table 2). On average, males tried tandem 0.06 ± 0.06 times per hour with mature females in 2019 and 0.52 ± 0.16 times per minute with mature females and 0.28 ± 0.16 with young females in 2020. Tandem attempts were observed at any time of the day.

Females reacted to approaching males by using two different behaviours. Mature females (Figure 3d) performed a "refusal display" (sensu Utzeri, 1988), which consisted of shaking the wings and at the same time flexing the abdomen tip downwards (Figure 3f; sometimes only this last action if the female was approached while in flight). In contrast, this behaviour was never performed by young females (Figure 3a), who showed a "threat display" by opening the wings and flexing the abdomen tip upwards when perched or in flight (Figure 3e). Intermediate females (Figure 3b,c) showed both behaviours. Table 2 shows the frequencies of these and other behaviours including all male–female interactions observed during focal observations and opportunistic records. Young females sometimes started the interaction with a threat display and then confronted the male (Table 2). Threat and refusal displays were observed whenever a male approached a female and diminished in frequency in the afternoon (Figure 4).

Taking into account male–female interactions observed between 2018 and 2020, when we recorded all of them (*N* = 249, considering the copulations observed as cases of successful tandem), males attempted to grasp females in tandem in 35.7% of interactions, and were clearly more likely to attempt tandem (94.3%) and to get tandem or copula (84.8%) with females of intermediate age (Table 3). Once a male grasped a female in tandem, in all cases we observed clear rejection behaviour by females, which shook their body strongly or remained with their abdomen straight (non-collaboration) (Figure 5a,b). The males would invite copulation by moving their abdomen upwards, trying to get contact with female genitalia (Figure 5b). Tandems that did not end in copulation fell into short (mean: 1.60 ± 0.43 min, range: 0.02–7 min, *N* = 15) or long categories (mean: 71.05 ± 6.08 min, range: 20–139 min, *N* = 31). Eventually, females would show very strong reaction, turning their head and body to try to break the tandem, and were therefore released by males.

In total, we recorded 44 copulations (8 in 2018, 9 in 2019, 10 in 2020 and 17 in 2022), but only two since the start of the interaction. On average, timed copulations lasted 11.06 ± 2.85 min (*N* = 18; all but two already started when first detected) and occurred at any time of day (the earliest at 7:19 and the latest at 15:21). Female age was recorded in all copulations. Forty females showed intermediate colouration (Figure 5d,e), two had the thorax orange but rather dark (Figure 5f), indicating were about to change colour and two had mature colouration (but not old). This intermediate colouration lasted apparently only a few hours, at an estimated age of 4–5 days.

Male–male interactions were very brief and ended with a threat display or a short confrontation. Occasionally, one male tried to grasp another male in tandem. In nine cases the tandem was achieved (Figure 5c). Two of these tandems lasted 2 and 5 min, but the remaining seven lasted between and 35 and 155 min, with the active male insistently "inviting" the other male to mate. Female–female interactions were rarely observed (*N* = 11), and in all but one case, the approached female reacted with a refusal or threat display, like the behaviour in male–female interactions. This rejection behaviour was also observed when other insects (notably wasps) were flying nearby, and sometimes females showed refusal display to males flying nearby which apparently did not notice the presence of the female.

Oviposition was done by the female alone, on floating leaves and algae, and in emergent grass stems. We did not observe submerged oviposition. It took place mainly in the central hours of day, between 9:30 and 13 h (Figure 6).

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TABLE 2 Reactions of young and mature females of *I. hastata* to approaching males.

Female age	Refusal display	Threat display	Confronts male	Fly away	Attacks male	No reaction
Young	0	35	10	5	1	0
Intermediate	2	4	0	0	0	1
Mature	132	0	1	2	1	4

Note: Young females usually responded by a "threat" display, sometimes combined with male confrontation, here included under "confronts male". Mature females used a different "refusal" display. Test of the three first categories: $\text{Chi}^2 = 175.1$, $\text{df} = 4$, $p < 0.001$.

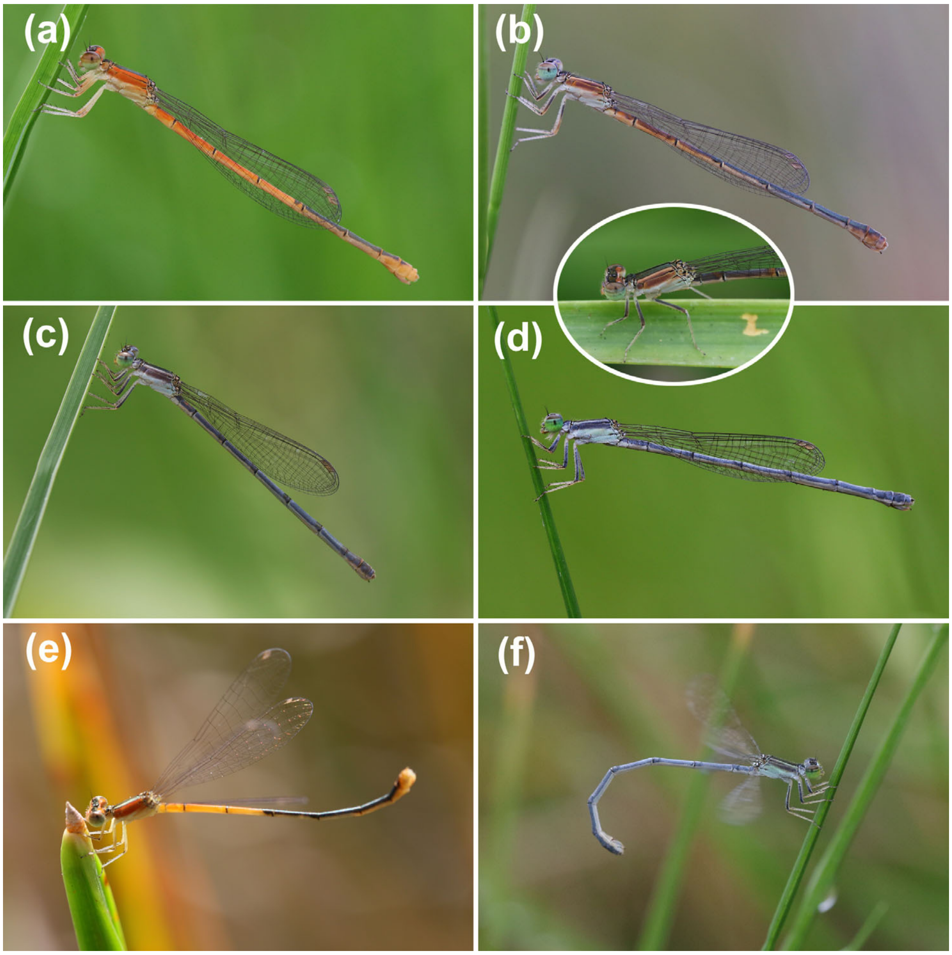

FIGURE 3 Ontogenetic colour changes in female *I. hastata*, and rejection behaviours against males. (a) Young orange female, 1–3 days of age; (b) intermediate female, 4–5 days of age (another example in the insert); (c) intermediate female slightly older than (b); (d) mature female with grey-bluish pruinescence, 7+ days of age; (e) threat display by young female, opening the wings and curving the abdomen upwards; (f) refusal display by mature female, curving the abdomen downwards and shaking the wings. Pictures by ACR.

DISCUSSION

Our results indicate that *I. hastata* females have a very short life expectancy, a fact that clearly limits polyandry. Most females apparently live just enough to mature a batch of eggs and then mate only once, and this occurs at a very short temporal window. Males were also very short-lived. However, given the high densities of our populations, the low number of copulations observed is intriguing.

As Table 1 indicates, adult lifespan is expected to fall between 1.0 and 7.6 days. Mature females lived on average only 2.4–3.3 days, which added to the time needed to achieve maturation (5 days) gives a total adult lifespan of about one week on average. These values confirm previous estimates obtained in populations of San Cristóbal island (Galápagos; expected mature lifespan 3.3–4.3 days) and Cuba (1.7 days) (Lorenzo-Carballa et al., 2017), but are lower than the expected lifespan of parthenogenetic *I. hastata* from the Azores (6.5 days, Cordero-Rivera et al., 2018). A recent review of mark-recapture studies in Odonata indicates that on average mature female Coenagrionids have a daily survival of 0.819 ± 0.028, which translates into 6.3 ± 0.3 days (Sanmartín-Villar & Cordero-Rivera, 2022).

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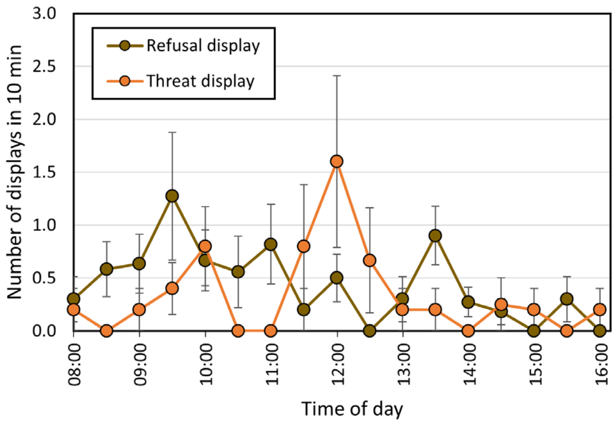

FIGURE 4 The frequency of threat display by young females and refusal display by mature females over the day. Values are mean ± SE.

Therefore, *I. hastata* is a species with a particularly short adult lifespan in its sexual populations.

One possible explanation for the low number of observations of mating pairs is that we might have missed the main peak of activity. Some damselflies mate very early in the morning, even before sunrise, like *Mortonagrion selenion*, whose mating activities are restricted to 4:00–8:00 h (Naraoka, 2005). However, our observations have clearly discarded this alternative for *I. hastata*: before 7:00 temperatures were low (even lower than 20°C) and the damselflies could not fly. This fact is also confirmed by the high proportion of focal individuals that did not fly at all when observed before 8:00 (Figure 1). Although we did not sample during the night, Figure 2 shows that activity clearly diminished at the end of the day, and therefore it is unlikely that mating could occur during the night (when temperatures drop again below 20°C).

Another alternative is that individuals disperse and mate far from the ponds, but this is also highly improbable, because the density of *I. hastata* becomes extremely low just at distances of 50 m from the ponds, or at least we could not find them (see also Lorenzo-Carballa et al., 2017). Furthermore, our data indicate that individuals move very little over the day. During 10-min focal observations, flights over 1 m were very rare, and individuals marked in one pond were almost always resighted in the same pond, even if the distance between ponds was as short as 50 m (data not shown). The small body size of this damselfly and the high temperatures achieved during the central hours of the day (up to 38°C in the shade), probably act as a limitation for mobility. In fact, it was common to observe that at midday the damselflies perched and oriented their body to be in the shade of their perch. Nevertheless, long-distance dispersal seems to occur even though passively, as *I. hastata* has been able not only to colonise the Galápagos Archipelago on the Pacific and all the main Caribbean islands, but also the Azores Archipelago on the Atlantic (Cordero-Rivera et al., 2005), and it shows very little genetic differentiation over large geographical areas (Lorenzo-Carballa et al., 2010, 2012).

A further explanation for the scarcity of observed matings is that protandry, which has been demonstrated to occur in *I. hastata* (Lorenzo-Carballa et al., 2017), results in reproductive asynchrony in these populations. Reproductive asynchrony would limit the number of encounters between receptive females and males. In fact, our focal observations indicate that these encounters are very rare. Some degree of protandry would be yet advantageous for males in the case of monandry (Morbey & Ydenberg, 2001) but given their low survivorship, this would be true only if dispersal of individuals between ponds is high (which does not seem to be the case) or if multiple overlapping generations occur, which would increase the number of male–female encounters. It is interesting to note that long flights (over 1 m) were more frequent in males than in females, indicating that males are actively searching for females, as expected if they mature before females.

Even though we accumulated over 230 h of field observations, the number of tandem attempts detected, and the number of copulations was extremely low. Some *Ischnura* species mate so frequently that mating wheels are very commonly recorded (Miller, 1987; Cordero-Rivera & Sánchez-Guillén, 2007). However, other *Ischnura* are considered monandric, because they are rarely seen mating, like *Ischnura verticalis* (Fincke, 1987). In fact, it has been suggested that there is a clade of small *Ischnura* from North America, which includes *I. hastata*, whose females are monogamous (Robinson & Allgeyer, 1996). We found clear evidence for a very narrow window of age when females are receptive to copulation. This occurs exactly when the juvenile orange colouration starts to change to the brownish colour of mature females (Figures 3b,c and 5d,e), and seems to last around 24 h, in agreement with laboratory observations of this species (MOLC pers. obs.). Incidentally, the only previously published picture of a copulation of *I. hastata* known to us also shows a female of intermediate age (see Figure 1 in Lorenzo-Carballa et al., 2010). All mature females that were observed in tandem refused to mate, but two of the 44 copulations we observed involved females with a mature colouration, albeit not old (i.e., they did not show pruinosity). Probably females mate at older age if did not mate at the “right” age, and this might explain these rare cases. Importantly, the change of thorax colouration is a honest signal of female receptivity, that males apparently learn to recognise, because they attempted to grasp females of intermediate age in 94% of cases, but only tried to form tandem in 24–31% of cases with young and mature females (Table 3). In polyandrous *Ischnura* species, which normally are also polymorphic, with some male-like females mimicking male behaviour, males are able to discriminate between female morphs, noticing the male-like morph more easily, but trying to mate with the gynochrome morphs more often (Piersanti et al., 2021). In the case of *I. hastata* only one female morph is known, but the immature and mature colourations are so different that males learn to discriminate between them, increasing their interest on intermediate females, which are more likely to be receptive to mate.

One surprising fact of male behaviour is the long duration of tandems with females unwilling to mate (Figure 5a,b). Males insistently showed clear invitation behaviour, by putting their abdomen upwards, as Figure 5b shows. Most of these pairs were first observed already in tandem, but even if we did not observe the complete behavioural

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TABLE 3 Behaviours observed when males and females of *I. hastata* interacted.

	Female age			
Attempted tandem	**Young**	**Intermediate**	**Mature**	**Total**
Yes	20	33	36	89
No	44	2	114	160
Tandem or copula success				
Yes	6	28	9	43
No	14	5	29	48
Proportion of tandem attempts	0.313	0.943	0.240	0.357
Proportion of tandem success	0.300	0.848	0.250	0.483

Note: The number of cases where males tried tandem, and the proportion of attempts that were successful were clearly related to female age. The results of contingence chi-square are also presented. Tandem attempt: Chi^2 = 61.8, df = 2, p < 0.001; tandem success: Chi^2 = 29.6, df = 2, p < 0.001.

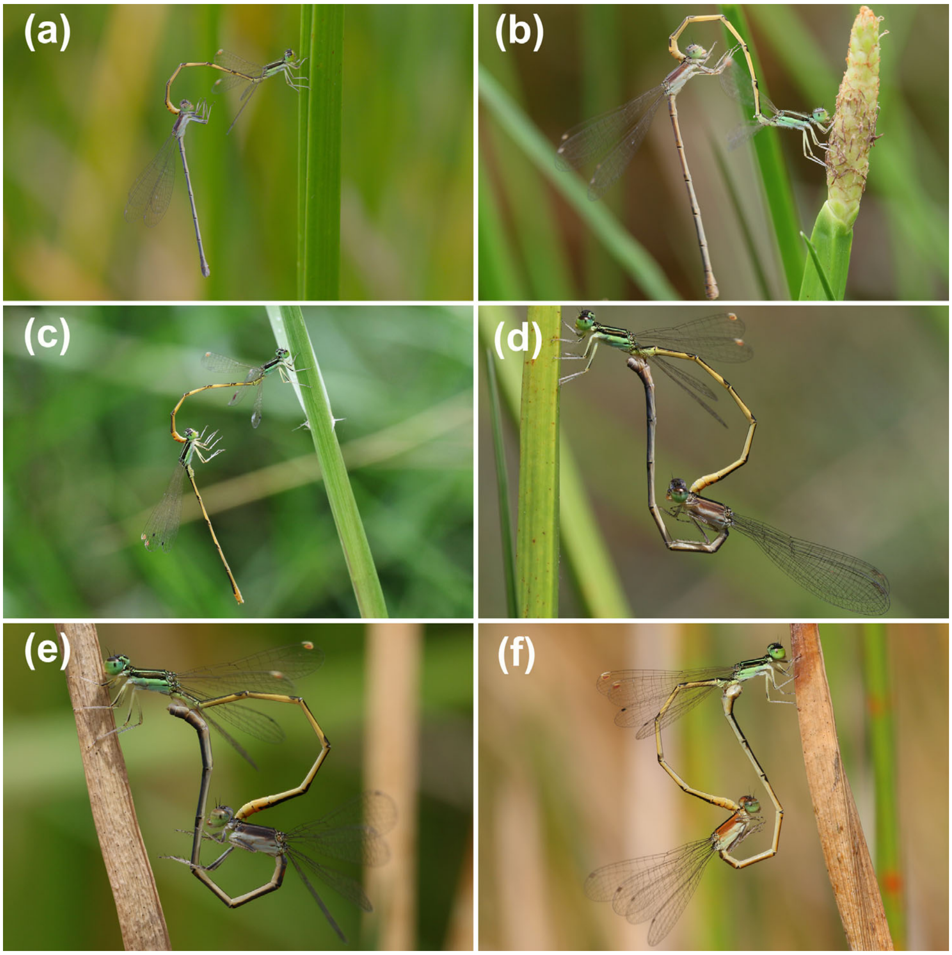

FIGURE 5 Reproductive behaviour of *I. hastata*. Males were more likely to try and to grasp in tandem females of intermediate age, as shown in (a) and (b). Females unwilling to mate remained with their abdomen straight (a), even when the male put his abdomen upwards trying to contact with female genitalia (b), for as long as 137 min. Male–male tandems were rare but could last up to 155 min (c). Copulation was observed with females of intermediate age (d, e), and rarely orange (f) or mature females. Pictures by ACR.

sequence, it was clear that some males retained females for very long periods (even more than 2 h), but only in one case, a long tandem (15 min) ended in copulation. Therefore, females in this species regulate mating frequency and cannot be forced to mate, that is, they are able to control the resolution of this sexual conflict (Fincke, 1997). This contrast with other odonates whose females accept to mate because the insistence of males is too costly for them, in a case of convenience polyandry (Cordero Rivera & Andrés, 2002). In another

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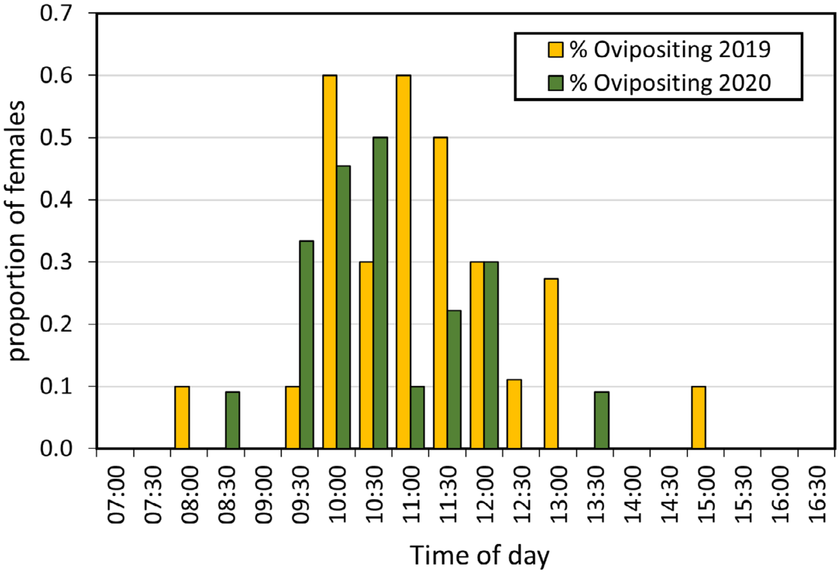

FIGURE 6 Diel distribution of oviposition behaviour by female *I. hastata*. In 2019 focal females were chosen among those perched near the shore of the ponds. In 2020 females were observed also in the paddy fields around the ponds.

coenagrionid it has been recently shown that females use a head turn display to indicate non-receptivity, and males were less likely to persist when females used this display (Xu & Fincke, 2022). In *I. hastata* female monogamy produces a scenario of extreme sexual conflict over mating rate, where males are very insistent, perhaps because their motivation to mate is very high once they have grasped a female, which is clearly a very unusual event in their life. Males even sometimes grasped other males and behaved in the same way, retaining "their" male for long time (even more than 2 h). This was not a common fact but might be a consequence of the scarcity of receptive females, selecting for male indiscriminate behaviour.

Copulations lasted about 20–30 min, a time comparable to other *Ischnura* species that apparently mate only once or very few times like *Ischnura aurora* (Rowe, 1978), or other coenagrionids of similar size (Guillermo-Ferreira & Del-Claro, 2012), but shorter than the monogamous *I. verticalis*, whose matings last around 40 min (Fincke, 1987). Polyandrous *Ischnura* mate for 1–6 h (Miller, 1987; Cordero, 1989, 1990; Cordero-Rivera & Andrés Abad, 1999; Sawada, 1999; Naraoka, 2011; Huang et al., 2012; de Almeida et al., 2018). These patterns add further support to the hypothesis of a correlation between life-history traits and behaviour in the genus *Ischnura* (Robinson & Allgeyer, 1996; Sánchez-Guillén et al., 2020).

In conclusion, short lifespan has been selected for female monandry in *I. hastata*, creating an intense sexual conflict for mating rate. Females can resist male attempts to mate, therefore controlling the resolution of the conflict. Whether occasional polyandry occurs in this species needs to be studied using molecular markers. If multiple mating by females is very rare, then the intensity of postcopulatory sexual selection by sperm competition should be very low. In general, male damselflies are able to remove sperm from the female genitalia (Cordero-Rivera & Córdoba-Aguilar, 2010). In this context, future studies aimed at testing if the ability of sperm removal is maintained in *I. hastata* males are of special interest.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data from this study are available as supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Results of model selection for the different ponds and years. Models are ranked by Quasi Akaike's Information Criterium corrected for c-hat.

Data S1. Supporting Information.

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