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Parthenogenesis did not consistently evolve in insular populations of Ischnura hastata (Odonata, Coenagrionidae)

M.O. Lorenzo-Carballa[1,2], C. Hassall[3], A. C. Encalada[4], I. Sanmartín-Villar[1], Y. Torres-Cambas[5], A. Cordero-Rivera[1]

[1] ECOEVO Lab, EUE Forestal, Universidade de Vigo, Campus Universitario, 36005 Pontevedra, Spain.

[2] Institute of Integrative Biology, Biosciences Building, Crown Street, University of Liverpool, Liverpool L69 7ZB, UK.

[3] School of Biology, University of Leeds, Leeds LS2 9JT, UK

[4] Laboratorio de Ecología Acuática, COCIBA, Universidad San Francisco de Quito, Quito, Ecuador.

[5] Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad de Oriente, Patricio Lumumba s/n, Santiago de Cuba, Cuba.

Corresponding author: Adolfo Cordero-Rivera; email: adolfo.cordero@uvigo.es; phone: +34986801926

Running head: Sexual insular populations of Ischnura hastata

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Abstract

1. The evolutionary advantages that have driven the evolution of sex are still very much debated, and a number of benefits of parthenogenesis over sexual reproduction have been proposed. In particular, parthenogenetic individuals are thought to exhibit higher probabilities of establishment following arrival in new, isolated habitats such as islands.
2. One notable example of parthenogenesis occurring in islands is the damselfly Ischnura hastata, an American species that has colonized the Azores archipelago, where the populations consist only of females. This is the only known example of parthenogenesis within the insect order Odonata.
3. Here, we studied two island populations of I. hastata, one at the Galapagos and one at Cuba, to test whether island colonisation is consistently associated with parthenogenesis in this species. Field capture-mark-recapture studies and laboratory rearing of field collected eggs were undertaken in both areas.
4. We found that sex ratios in the field were heavily female biased among mature individuals; however, fertility rates of field collected eggs were high, and the sex ratios in the laboratory did not differ from 1:1. Data from laboratory rearing showed that shorter larval development times and shorter adult lifespans in males result in protandry, which might explain the skewed sex ratios in the field.
5. Our findings are consistent with sex differences in key demographic parameters which could predispose I. hastata to parthenogenesis. However, the Azores population of I. hastata remains the only documented case of asexual reproduction in this insect group.

Keywords: Zygoptera, damselfly, protandry, evolution of sex, asexual reproduction, islands, geographical parthenogenesis

Introduction

Explaining the maintenance and prevalence of sexual reproduction despite its numerous costs has been considered one of the primary challenges in evolutionary biology (Maynard Smith, 1978; Bell, 1982). Sex has been demonstrated to be advantageous in a landscape of biotic and abiotic interactions (van Valen, 1973; Bell, 1982; Ridley, 1995), and the process of recombination linked to sexual reproduction allows individuals to eliminate deleterious mutations (Kondrashov, 1988; Almbro & Simmons, 2014). However, the latter mechanism does not seem to be a general explanation for the wide distribution of sexual reproduction in nature (Keightley & Eyre-Walker, 2000). Despite all these benefits, a number of animal groups have reverted to (or exhibit the potential for) asexual reproduction. Among the different types of asexual reproduction found in nature, parthenogenesis (i.e. the development of a non-fertilized egg, which usually results in the production of all-female offspring) is found in most of the major animal groups, and it is quite common among rotifers, arthropods and nematodes (Suomalainen et al., 1987). The principal benefits of parthenogenesis lie in avoiding the two-fold cost of sex: in contrast with sexual organisms, in which males do not contribute directly to the next generation, in asexual species, each member of the population is capable of producing offspring. This implies that asexual populations will have an intrinsic capacity to grow more rapidly in each generation (West et al., 1999). However, this is balanced against the need to eliminate deleterious mutations, which are expected to accumulate in clonal organisms and which may more than negate the benefits of unisexual reproduction (Archetti, 2004).

One potential driver for the evolution of parthenogenesis is the greater probability of successful establishment in new habitats by uniparental species (known as "Baker's law";

Baker, 1955). This principle was derived from studies of plants that are either self-compatible or self-incompatible, but can be applied readily to parthenogenetic animals. Subsequent theoretical models have attempted to define the co-evolutionary relationships between dispersal and selfing/parthenogenesis (Cheptou & Massol, 2009; Massol & Cheptou, 2011), and have called into question Baker's general principles (Baker, 1955). Rather than a link between dispersal and asexual reproduction, which would enhance colonisation success following arrival in a new site, these models would predict dispersal to be associated with sex: for example, if there is temporal heterogeneity in habitat quality (e.g. plant pollinators or males are lost), asexual species would not experience fitness losses to the same extent as sexual species, and therefore asexuals would experience a lower selection pressure towards dispersal. Sexual species, on the other hand, would be impacted to a greater extent by reductions in pollinators or mates, and hence, dispersal would evolve in sexually reproducing species due to the benefits in escaping low quality habitats (Cheptou Massol, 2009). Thus, it seems more likely that parthenogenesis is a trait acquired secondarily after dispersal, rather than a part of a "syndrome" of related traits (Massol & Cheptou, 2011). However, other recent work has supported the hypothesis that parthenogenesis may represent a significant advantage in pioneer species that allows the exploitation of new areas, but only by so-called "general purpose genotypes" (Vrijenhoek & Parker, 2009). The occurrence of parthenogenetic populations on islands, whether directly through enhanced establishment of asexuals or secondarily through the evolution in situ of parthenogenesis from sexual colonisers, is one example of "geographic parthenogenesis" (originally described by Vandel, 1928), which designates the different characteristics of the distributions of sexual and parthenogenetic populations (Peck et al., 1998).

In contrast with most of the insect groups in which parthenogenesis is commonly found (Suomalainen et al., 1987), sexual reproduction is prevalent among the Odonata (dragonflies and damselflies), and only one example of parthenogenetic reproduction is known: the populations of Ischnura hastata on the Azores islands in the Atlantic Ocean (Cordero-Rivera et al., 2005). This is a species indigenous from the Americas, where it is widely distributed across the United States, Canada, Mexico, Central and South America (Colombia and Venezuela), the Caribbean and the Galapagos (Garrison et al., 2010). According to this widespread distribution, population genetic analyses of sexual and parthenogenetic I. hastata have found little genetic differentiation and no significant population structure across the species' range in North America, which confirms the high dispersal ability of the species. The genetic data available does not allow to identify the geographic origin of the asexual population; however genetic analyses do support a recent single long distance dispersal event, followed by a demographic expansion, as the most likely hypothesis explaining the colonization of the Azores (Lorenzo-Carballa et al., 2012). I. hastata has been captured on aeroplane-mounted nets at an altitude of 300 m (Dunkle, 1990), and the species has also been recorded over open ocean 100 miles from shore in the Gulf of Mexico (Geijskes, 1967). The colonization of the Azores could have thus occurred passively through the air currents associated with the Gulf Stream which, coupled with a great distance, renders the return journey back to the American continent very difficult (Lorenzo-Carballa et al., 2012).

The geology and geography of islands have been shown to be important in the evolution of odonates in the Pacific (Jordan et al., 2005) and Indian (Dijkstra, 2007) Oceans; but I. hastata is the only known example of an odonate that has responded to isolation on islands by turning to obligate parthenogenesis. Being the only known example of asexual reproduction in its order, the species offers a valuable opportunity to test fundamental theories concerning the evolution of sex, dispersal and geographical parthenogenesis. Here, we selected two island populations of I. hastata with different degrees of connectivity to the continent: Cuba, which is only ~200 km far from Florida, and the Galapagos islands, which are as isolated from mainland as the Azores. We used a combination of field work (capture-mark-recapture, CMR) and laboratory rearing methods to study the reproductive behaviour and examine the population dynamics of this species in both areas. Under parthenogenesis we would expect field CMR to show an absence of males and mating, and we would expect laboratory rearing to show unisexual clutches and also absence of males throughout generations. Further insights into the nature of parthenogenesis may also be obtained from these experiments: parthenogenetic reproduction could be favoured in populations showing reproductive asynchrony/protandry, which can be tested for using both CMR and laboratory rearing; and male-biased mortality in either adults or larvae, for which we can test using CMR and laboratory rearing, respectively; would also favour asexual reproduction. Hence, these complementary approaches allow us not only to comprehensively test for the presence of parthenogenesis, but also quantify the ecological and evolutionary factors that could drive its evolution. If parthenogenesis is associated with the colonization of remote islands in this species, we would expect it to occur with higher probability in a remote archipelago such as the Galapagos, whereas competition with nearby sexual conspecifics would preclude the establishment of a purely parthenogenetic population in Cuba.

Materials and Methods

Description of study sites, mark-recapture and behavioural observations

Cuba: Ischnura hastata flies throughout the year in this island, with adults having been observed in both the dry (November-March) and wet season (April-October). The species is found mainly in temporary ponds, but other (larger) ponds remain with water all the year, serving as sources for colonization of the temporary ponds (Y. Torres-Cambas personal observations). For the present study, we selected a farm pond in Alfredo Álvarez Mola, in the province of Camagüey (21.183°N, 77.551°W altitude: 111 m). This is an agricultural landscape in which other ponds exist, that are used as drinking troughs for cattle. The pond is 20-60 m wide and it is surrounded by grass and only a few trees (Figure S2). It has a rich odonate fauna, with several odonate species were observed during the study period: Ischnura capreolus, I. ramburii, Lestes tenuatus, Orthemis ferruginea, Erythemis vesiculosa, E. plebeja, Micrathyria hageni, Miathyria simplex, Erythrodiplax umbrata, E. justiniana and Crocothemis servilia. Fieldwork was carried out over the course of 19 days between 26 May and 26 June 2012 by 4 people during the first three days and 1-2 people for the remainder of the study, for an average of 2 h 39 min per day (over 48 h of observations in total); starting between 8:00 and 9:00 and ending around 17:00, with an interval in the hottest hours (12:00-16:00), due to total absence of odonate activity. Fieldwork was interrupted between 7 and 11 June and between 15 and 21 June, due to sampling carried out in other areas of the island.

Galapagos: I. hastata is the only damselfly found at the Galapagos. Adults of the species have been observed in the archipelago in January-May and December (Peck, 1992); but they have been also found in June, September and October, hence it is likely that the species flies throughout the year in the islands (A. Cordero-Rivera and A.C. Encalada, personal observations). Fieldwork for the present study was carried out on the island of San Cristóbal. After a first preliminary survey in the island, we selected one artificial, almost circular, pond inside the “Hacienda El Cafetal” (89.539°W, 0.925°S, altitude: 280 m). The pond has a diameter of 25 m and it is surrounded by secondary forest, and isolated from other suitable breeding areas, except for a small stream where a few I. hastata were also found (Figure S2).

Other species found in the pond were Rhionaeschna galapagoensis, Anax amazili, Erythemis vesiculosa, and Tramea cf. cophysa. Fieldwork was carried out daily between 20 February and 6 March 2014 by 2-3 people, for an average of 2 h 40 min per day (over 48 h of observations in total), starting at 9:00-11:00 and ending at around 14:00.

All adult I. hastata found in the shore up to a distance of about 100 m in the surrounding grassy areas were captured with a hand net. Individuals were individually marked by writing a number on their right hind wing with a permanent marker, sexed and aged. Three categories were used for age: "teneral" - newly emerged animals, up to two days old; "young" - animals with orange (females) or pale yellow (males) thoracic and abdominal coloration, between 2-5 days old; and "mature" - animals showing the coloration typical of sexually reproducing individuals, grey-brown in females, bright yellow with black markings and bright green thoracic marks in males (Figure S1). Focal observations on behaviour were conducted by walking along the shore and the nearby grassy areas to detect ovipositing females and matings.

Collection of eggs and measures of fecundity and fertility

Mature I. hastata females were collected in the studied ponds and placed in plastic containers with moist filter paper for 2-3 h to encourage them to oviposit. Females from Cuba (n=33) represent a single group collected on 23-26 June 2012. The Galapagos females (n=22) represent two groups of clutches which, for logistical reasons, had to be maintained at 4 °C to avoid the hatching of larvae before the field work finished. The first group (n=10) was collected on 25-27 February 2014 and refrigerated for 4 weeks, whereas the second group (n=12) was collected on 4-6 March 2014 and refrigerated for 2 weeks. In the laboratory, all the eggs were maintained at room temperature (20-25 ºC).

After hatching of larvae, the number of eggs per clutch was counted under a binocular microscope to measure fecundity and fertility. Fertility was determined by scoring the percentage of eggs that hatched. Dark eye spots are visible in the embryos about 12 days old. If no such eye spot was found in a non-hatched egg, then this was scored as sterile. We used the eye-spot rather than hatching as a criterion to determine the percentage of fertile eggs, to minimize possible effects of laboratory rearing conditions (Fincke 1984).

Analysis of CMR data

The recapture histories of marked animals were analysed using Cormack-Jolly-Seber models (CJS) as implemented in MARK 8.0 (White & Burnham, 1999). These models allow separate estimation of survival (**Phi**) and recapture (**p**) probabilities. We used Akaike's Information Criterion (AIC) to select the best models to explain the variability contained in the dataset (Burnham & Anderson, 1998; White & Burnham, 1999). We tested the goodness-of-fit of a model with two groups (g; males and females), daily variation in recapture and survival probabilities (time-dependent model, t) and interaction between both parameters. This model is known as the saturated model, **Phi**(g*t) **p**(g*t) using the notation of Lebreton et al. (1992). The asterisks indicate interaction between sex and time, i.e., parameters for both sexes may change over time in different ways.

We calculated the goodness-of-fit of the saturated model, using program RELEASE (Burnham et al., 1987) from within MARK. For the Galapagos dataset, TEST2, which is useful to test the assumption of equal catchability of marked animals (Burnham et al., 1987) was highly significant (χ^2=112.31, d.f.=67, p<0.001), while TEST3, which tests the hypothesis that all animals alive on day (i) have the same probability to survive to day (i+1) was not significant (χ^2=36.02, d.f.=46, p=0.855). Consequently, there is evidence that recapture probability was not homogeneous, which could be due to the effect of age on catchability. To test this, we analysed data in two groups: the first group included all immature animals (teneral and young), and the second included all the mature individuals marked. The time-dependent saturated model by age groups (CJS) met the assumptions of both equal catchability and equal survivorship across individuals (immature: TEST2, χ^2=4.20, d.f.=19, p=0.999; TEST3, χ^2=11.54, d.f.=26, p=0.994; mature: TEST2, χ^2=55.50, d.f.=47, p=0.185; TEST3, χ^2=61.75, d.f.=60, p=0.413), and was used to further refine parameter estimation.

The recapture rate of immature individuals was almost zero in the Cuban population (see Table 1). Therefore, we excluded these individuals from the analysis, and no age effects could be studied. Results of goodness of fit tests, indicate that a model without age groups is appropriate to describe the variability in the dataset (Total TEST2 and TEST3, χ^2=30.89, d.f.=42, p=0.897), and was used as the starting point for model selection.

Models were ranked based on the Akaike's Quasi-Criterion of information, corrected by the estimation of overdispersion (QAIC$_c$). The extra-multinomial variance factor (c-hat) was estimated from the saturated model, by dividing the value of the deviance of this model by the mean deviance estimated from the bootstrap procedure in MARK. The resulting value was used to correct parameter estimation.

Laboratory rearing

After hatching, larvae were randomly assigned to two treatments, common or individual rearing. Common rearing involved a maximum of 25 larvae placed in a plastic container of 30 x 18.5 x 7 cm, with strips of filter paper provided as perching substrate. Individual rearing was performed by randomly selecting 50 newly hatched larvae from each female and placing them in plastic cups of 3.5 cm diameter and 4.5 cm height.

Larvae from Cuba were fed daily with Artemia sp. nauplii ad libitum, and when they reached a body length of approximately 0.5 cm, the diet was also supplemented with Tubifex sp. Due to a shortage of food supply, the larvae from Galapagos were fed only with Artemia every 2 days.

Last instar larvae were maintained in plastic containers with wooden sticks as emergence support. One day after emergence, adults were measured (from head to tip of abdomen, to the nearest 0.1 mm), sexed and individually marked with a permanent marker; and introduced in insectaries of 50 × 50 × 50 cm. Culture bottles with Drosophila melanogaster were added to supply food. The shortage of food experienced by the larvae from Galapagos resulted in high larval mortality. Therefore, to estimate the sex ratios for this sample, we sexed larvae at 90-110 days of age, when the ovipositor rudiments are clearly visible in most females.

Sample sizes, sampling dates, and larval survivorship are shown in Table 2. The two groups of Galapagos females yielded similar fertility values (0.714±0.06 versus 0.756±0.07, GLM with binomial errors corrected for overdispersion; deviance ratio=0.65, p=0.430), and are therefore analysed jointly.

Analysis of laboratory data

Variation in fecundity was analysed using generalised linear models with Poisson error structures for the count data. Variation in sex ratios was analysed using binomial models to test for deviations from 1:1 ratios with the software GenStat (GenStat, 2015). In the Cuban population, development time of larvae was monitored and recorded to the nearest day, based on the date of hatching and date of emergence. A Cox proportional hazards model was used to test for a difference in development time between sexes and between rearing conditions, with sex as a fixed effect and with the model stratified for rearing condition such that group and individual reared animals had different baseline hazards. In addition to larval development time, adult lifespans of all animals in the Cuban rearing experiment were recorded to the nearest day and were also compared between sexes and rearing conditions (individual vs. common rearing). Cox models did not violate the assumption of proportional hazards for development time (χ^2=3.98, p=0.05) or adult lifespans (χ^2=1.29, p=0.26). Throughout the text, data are presented as mean±SE.

Results

Sex ratios in the field

More females than males were marked (and observed) in the two populations studied. The proportion of males overall was only 20.7% in the Cuban population and 42.8% in the Galapagos population, in both cases a sex-ratio significantly deviated from 1:1 (Table 1). Nevertheless, sex-ratio was not significantly different from parity among teneral individuals in both populations, and in Cuba also among young individuals; which suggests that sex ratio among larvae is not biased, but becomes female-biased when animals mature.

The proportion of sexes showed high daily variation over the study period in both populations. In Cuba, males represented less than 20% in the first week, to become predominant in the middle of the study (up to 64% on 12 June) and again reached low values at the end of the study (Figure 1A). Due to the low recapture rate in this population, including resighted individuals does not change these patterns. In Galapagos, daily sex-ratio oscillated between 24.8 and 55.1% males among the newly marked specimens (Figure 1B). These values were similar (24.3 to 51.8%) when all the individuals observed in a particular day are considered (newly marked+recaptured). Females were clearly more common during the first week, but sex-ratio became more balanced in the second week.

Survivorship in the field

The recapture rates were very low in both populations (Table 1). In Cuba 85.8% of males and 86.7% of females were never resighted. Only 31 males were recaptured once and 3 twice. We resighted 94 females once, 22 twice, 5 were seen three times and 1 five times. In the case of the Galapagos population, 69% of females and 74% of males were never resighted (Table 1). Most resighted males were recaptured only once (136), 26 were seen twice, 8 three times, 3 four times, and 1 six times. In the case of females 191 were recaptured once, 60 twice, 18 three times, and 7 four times.

Cuba: Results of model selection for mature specimens indicate that the best model to explain the variability in the data is **Phi**(t) **p**(t) (Table S1). This model suggests that both survival and recapture probabilities varied over time, but were similar for both sexes, and is highly supported ($\Delta QAIC_c>4.5$ for other models). Nevertheless, given the low and unequal resighting rates (due to the interruption of sampling), some parameters could not be estimated. From this model, mean daily survival was estimated as 0.547±0.105, and recapture rate 0.217±0.009. Using the formula in Cook et al. (1967) [Lifespan= $-1/\log_e$ (survival)] we can estimate expected mature lifespan as only 1.7 days. Predation by I. ramburii was the only cause of mortality directly observed.

Galapagos: For this population there was heterogeneity in catchability, related to age at marking (see Methods), and therefore we analysed immature (young and teneral) and mature specimens separately. For immature individuals, the most supported model by the data is the reduced model, with constant recapture and survival probabilities for both sexes [**Phi**(.) **p**(.)] (Table S2). From this model, estimates are Phi= 0.839±0.040 and p=0.073±0.015. As indicated above, resighting probability was extremely low for this age class (see also Table

1). Nevertheless, models **Phi**(g) **p**(.) and **Phi**(g) **p**(g) are also well supported by the data (ΔQAICc<2, Table S2), and thus there is low evidence for a sex effect on survival and recapture probabilities before sexual maturation, and no evidence for a time effect. Parameter estimation from the second most supported model [**Phi**(g) **p**(.)] indicates a survival rate of 0.791±0.061 for males and 0.859±0.043 for females (Table S2).

The best model to explain data variability in the case of mature individuals is **Phi**(g) **p**(t) (Table S3). Our data therefore suggest that both sexes differ in survival but not in recapture rates, and that recapture rates varied over time. Parameters estimated from this model are shown in Table S4. Survivorship is estimated as slightly higher (0.792±0.016) for females compared to males (0.739±0.022). Recapture rates were low (11-36% daily), but higher than for young specimens. Expected mature lifespan was 3.3 days for males and 4.3 days for females. The main cause of mortality directly observed was predation by spiders: we found 8 specimens captured in spider webs, which were common on the shore of the pond. No attacks by birds were observed.

Mating behaviour

In Cuba, no matings of I. hastata were observed during the study period, in contrast with the other odonate species found in the same pond, whose reproductive activity was commonly observed. I. hastata males and females were mostly found perching in the grassy areas around the pond, mainly foraging, but no copulations or tandem attempts were observed.

In Galapagos, we observed only one mating pair, on our first visit to the pond (20th of February, at 9:50 h). Attempts by males to seize females in tandem were very rarely observed (usually none, and never more than one per day), but ovipositing females were commonly seen. When searching for individuals in the surroundings of the pond, some were found foraging in grassy areas but no copulations were observed.

Sex ratio and fecundity in the laboratory

Cuba: From the 33 clutches collected in the field, three were completely sterile, and were excluded from subsequent analyses. The remaining 30 females laid between 39 and 248 eggs, with a mean of 124.1±11.9, and a fertility rate of 0.808±0.046. Larvae hatched on average after 13.8±0.23 days. Larvae reared in groups resulted in 253 adults (135 males and 118 females; sex-ratio 53.4% males), a sex-ratio not significantly different from 1:1 (test for one proportion, z=1.069, p=0.285). Larvae reared individually produced 65 males and 68 females, almost a 1:1 sex-ratio (48.9% males, z=-0.260, p=0.795).

Galapagos: The sample of 22 females laid between 54 and 356 eggs, with a mean of 171.5±17.9 eggs, a value significantly higher than for females from Cuba (GLM with Poisson errors corrected for overdispersion, deviance ratio=5.33, p=0.025). The average fertility was not significantly different from the Cuban sample (0.733±0.041; GLM with binomial errors corrected for overdispersion, deviance ratio=2.40, p=0.127). Due to the fact that these clutches were maintained at 4 ^{o}C during the first weeks (see Methods), eggs hatched 35.8±0.7 days after oviposition. A total of 167 larvae were sexed (77 reared in groups and 90 alone). Males represented 71.1±6.2 (11) % of larvae reared in groups but only 59.0±6.2 (11) % in the case of larvae reared individually. The type of rearing had a significant effect on sex-ratio (GLM with binomial errors, deviance ratio=3.94, p=0.047). Sex-ratio was male biased in the first case (z=2.466, p=0.014) but not significantly so in the second (z=0.404, p=0.686). The shortage of food resulted in high mortality rates among larvae, and thus only 22 individuals completed development and emerged as adults, from which 18 were females (a sex-ratio of only 18.2% males; z=-2.985, p=0.003), which might reflect sexual differences in cannibalism and/or differences between sexes in their response to the experienced food shortage.

Development rates and survivorship in the laboratory

In the laboratory rearing experiment on Cuban I. hastata, the duration of larval development was shorter in males (74.9±1.7 days, n=114) compared to females (88.4±1.7 days, n=123), with a strong effect of sex (z=5.857, p<0.001; Figure 2). Females reared in groups emerged after 77.8±2.3 days, whereas females reared individually emerged after 100.0±1.2 days. Males reared in groups emerged after 64.8±2.0 days, while males reared individually emerged after 87.7±1.7 days. The differences in development time observed between treatments reflect the effects of cannibalism among larvae in the common rearing treatment. The shorter development times in males produced adult laboratory populations that showed protandry in both rearing conditions (Figure 3).

After emergence, mean adult longevity was 4.0±0.3 days for males and 6.9±0.8 days in females. Cox proportional hazards analysis showed a significantly greater survivorship in females compared to males (z=3.397, p=0.001; Figure 4). The very small number of adults that emerged from the Galapagos sample (n=22) does not allow us to carry out the same analysis on these data. No mating pairs were obtained in the laboratory for any of the two populations.

Discussion

Our results from field mark-recapture indicate that sex-ratio is highly variable in the two insular populations of I. hastata studied, although females were clearly more abundant on most days in both cases, with an overall sex-ratio of only 21% (Cuba) and 43% (Galapagos) males. We found also evidence for lower survivorship of males relative to females in the

Galapagos population. Lower male survival was also observed in the sample from the Cuban population reared in the laboratory, in which also shorter larval developmental was found for males. Laboratory breeding shows that all I. hastata females who produced more than three descendants had both sexes in their progeny, and in the case of the Cuban sample, the sex ratio was not significantly different from 1:1. Therefore, there is no indication that parthenogenesis occur in any of these populations, in contrast with the parthenogenetic population at the Azores, where males have never been found (Cordero-Rivera et al., 2005; Lorenzo-Carballa & Cordero-Rivera, 2009).

Female-biased adult sex-ratios are very unusual in the Zygoptera (reviewed by Cordero Rivera & Stoks 2008), whose males are clearly more common around water bodies. In the case of I. hastata, our results of combined field work and laboratory rearing suggest that these female-skewed adult sex ratios are the result of lower male survivorship and protandry. Our data from CMR studies indicate the sex-ratio is not skewed during the larval development in both populations, and therefore the number of males and females is similar at the start of sexual maturation. Nevertheless, males emerge earlier in the season and suffer from higher mortality rates, so that the sex-ratio becomes female-biased among mature adults. This bias was particularly high for the Cuban population, where about 85% of the mature individuals were females (Table 1), and there was a high rate of entry of mature females into the population (low recapture rates).

One explanation for the field skewed sex-ratios is that I. hastata males suffer higher predation rates, due to their conspicuous coloration (see Figure S1). However, rearing of the species in the laboratory (i.e. no predation) has shown clear differences in adult survivorship between sexes; indicating that there is an intrinsically lower survival in males regardless of extrinsic mortality pressure (Figure 4). Physiological differences between the sexes could explain the differences in mortality: for example, males and females of the damselfly Hetaerina americana show differences in immune response, with newly emerged females having higher levels of phenoloxidase (i.e. better capacity to deal with parasites and pathogens) than males (Jiménez-Cortés et al. 2012). Alternatively, males may just disperse in a higher proportion, resulting in lower recapture rates. Models which include differences between sexes in recapture rates are not among the most supported, except for Galapagos, where model Phi(g) p(g) is the third most supported (Tables S1-S3). Given that CMR studies cannot distinguish between mortality and permanent emigration, if a significant fraction of males disperse from their natal pond, these will be considered as “dead”. The data available do not allow to evaluate the dispersal hypotheses, as our study was limited to only one pond in each island. To test this, CMR studies would need to be carried out simultaneously at several neighbour ponds to investigate inter-pond movement.

Laboratory rearing of I. hastata from Cuba has shown that the development time of male larvae was significantly shorter than that of females. These differences in development time resulted in males beginning to emerge earlier (Figure 3). The observed pattern of protandry may be an incidental by-product of sexual size dimorphism in this species, where males are smaller than females (Matsuura, 2006; Teder, 2014). Alternatively, protandry would be favourable for I. hastata males if females are monandrous, as it would increase male mating success (i.e. the mate opportunity hypothesis; see Morbey & Ydenberg, 2001). In fact, it has been proposed that this species is monandric, based on behavioural characteristics such as rarity of mating in the field, and that (when observed) mean duration of copulation is very short (Rowe, 1978; Fincke, 1987; Robinson & Allgeyer, 1996). Accordingly, the duration of the mating observed in Galapagos was shorter than 10 minutes, and two matings recorded in Cuba in 2010 (Y. Torres-Cambas, personal communication) lasted also very shortly (less than one minute from the time they were first spotted). However, and even if the species is really monandric, with the lower male survivorship observed, protandry would only be advantageous for males if the encounter rates are high (Degen et al., 2015). In this scenario, a high dispersal of individuals between ponds, and/or multiple generations overlapping in a population, would help to alleviate the effects of reproductive asynchrony. High dispersal of individuals would also explain the low recapture rates.

In any case, the observed protandry must be taken into account when studying the sex-ratio of I. hastata in the field; as the proportion of sexes will vary depending on the time in the emergence period at which a population is sampled. For example, at the end of fieldwork in Cuba, on 25th of June, when the sex-ratio was clearly female-biased in our study pond (Figure 1A), we sampled a nearby pond and found a more balanced sex ratio of 33 females and 39 males. Male-biased sex ratios among newly emerged individuals and female-biased mature populations have been previously reported in Florida, whereas in a population from Mexico the sex-ratio was close to 1:1 (Cordero-Rivera et al. 2005).

During the study period we did not observe any mating pair in Cuba, only one was observed in the Galapagos population; and no mating pairs were obtained in the laboratory. This is another unusual attribute of I. hastata, whose reproductive behaviour remains largely unknown. Mating in this species may happen at unusual places, at unusual times of the day (e.g. like in the coenagrionid Mortonagrion selenion; Naraoka, 2005), or for very short periods of time (see above). However, after visiting the Galapagos pond at different times of the day (one day very early in the morning a few minutes after sunrise [6:00 to 11:30], and another day at the end of the afternoon [14:49 to 17:12]), we were unable to provide any evidence of unusual mating times. Certainly, the very high proportion of females that were caught alone but carried sperm to fertilize their eggs (91% across the two studied populations), supports the idea that mating occurs regularly. Genetic analyses of the sperm stored by females, to assess whether they are monandric or not; as well as further field observations (which could be supplemented with video recording in the laboratory) are needed to uncover I. hastata mating behaviour.

Although our results do not suggest that sperm limitation is more frequent in I. hastata than in other species of the genus (e.g. Sánchez-Guillén et al., 2013), the fact that 3 out of 33 females collected in Cuba laid only sterile eggs, suggests that a certain degree of mating limitation might be possible.

Altogether, the observed female-skewed sex ratios, potential reproductive asynchrony as a result of protandry, and the fact that females in the laboratory remained unmated, suggest that mate limitation may be important in this species. This mechanism might act as a “driver” for occasional parthenogenesis in I. hastata, as asexual reproduction might be favoured in isolated populations which lack immigrating males, and where late-emerged females may not be able to find mates (Gerritsen, 1980; Schwander, 2010). Therefore, the establishment of an obligate parthenogenetic lineage in the core of the species’ distribution area in America, or in an island close to mainland such as Cuba, might be largely impeded by competition with arriving sexual conspecifics, habitat instability and/or different levels of parasitism (Lorenzo-Carballa et al 2010).

The question on why parthenogenesis occurs only in the Azores but not in another remote archipelagos like the Galapagos, remains yet a puzzle. Certainly, the Azores population, being situated at least 3,300km from the rest of the geographical range, is more isolated when compared to around 1,000-2,000km of distance to the species’ geographic range for the Galapagos population. One feature of note that differs between these two locations is the impact of the El Nino effect on the Galapagos, which causes large increases in temperature and precipitation approximately every four years. The Azores, on the other hand, experience a relatively stable climate where diurnal variation is typically greater than interannual variation; and the incidence of parasitism, predation and competition in this archipelago is also very low (Lorenzo-Carballa et al 2010). Traditional evolutionary theory suggests that parthenogenetic populations are more successful in stable habitats with low number of biological interaction, such as the Azores (Cuéllar 1977; Glesener and Tilman 1978). However, this discussion should be taken as provisional given the small number of sites that have been surveyed, and there could be substantial insights gained from a more comprehensive spatial survey of the demography of I. hastata across its insular range. Until such a survey is conducted, and despite the intensive study described here, the Azorean populations of this species remain the only known case of parthenogenetic reproduction among the Odonata (Lorenzo-Carballa et al., 2010).

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Contribution of authors

Project design: ACR and MOLC; data collection: ACR, MOLC, AE, ISV, YTC; data analysis: ACR, MLOC, CH; paper writing: all.

References

Almbro, M. & Simmons, L.W. (2014) Sexual selection can remove an experimentally induced mutation load. Evolution, **68**, 295–300.

Archetti, M. (2004) Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. Journal of Evolutionary Biology, **17**, 1084–1097.

Baker, H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. Evolution, **9**, 347–349.

Bell, G. (1982) The masterpiece of Nature: the evolution and genetics of sexuality. University of California Press, Berkeley.

Burnham, K.P. & Anderson, D.R. (1998) Model selection and inference. A practical information-theoretic approach. Springer, New York.

Burnham, K.P., Anderson, D.R., White, G.C., Brownie, C. & Pollock, K.H. (1987) Design and analysis methods for fish survival experiments based on release-recapture. American Fisheries Society Monographs, **5**, 1–437.

Cheptou, P.O. & Massol, F. (2009) Pollination fluctuations drive evolutionary syndromes linking dispersal and mating system. The American Naturalist, **174** (1), 46-55.

Cook, L.M., Brower, P.P. & Crozer, H.J. (1967) The accuracy of a population estimation from multiple recapture data. Journal of Animal Ecology, **36**, 57–60.

Cordero-Rivera, A., Lorenzo-Carballa, M.O., Utzeri, C. & Vieira, V. (2005) Parthenogenetic Ischnura hastata (Say, 1839), widespread in the Azores Islands (Zygoptera: Coenagrionidae). Odonatologica, **34**, 1–9.

Cordero-Rivera, A. & R. Stoks. (2008). Mark-recapture studies and demography. In Dragonflies: model organisms for ecological and evolutionary studies (ed. by A. Córdoba-Aguilar). Oxford University Press, Oxford, pp. 7–20.

Cuéllar, O. (1977). Animal parthenogenesis. Science, **197**, 837-843.

Degen T., Hovestadt T., Mitesser O. & Hölker F. (2015) High female survival promotes evolution of protogyny and sexual conflict. PLoS ONE, **10(3),** e0118354 doi:10.1371/journal.pone.0118354

Dijkstra, K.B. (2007) Gone with the wind: westward dispersal across the Indian Ocean and island speciation in Hemicordulia dragonflies (Odonata: Corduliidae), Zootaxa **48**, 27–48.

Dunkle, S.W. (1990) Damselflies of Florida, Bermuda and the Bahamas. Scientific Publishers, Gainesville.

Fincke, O. M. (1984). Sperm competition in the damselfly Enallagma hageni Walsh (Odonata: Coenagrionidae): benefits of multiple mating to males and females. Behavioral Ecology and Sociobiology **14**, 235–240.

Fincke, O.M. (1987) Female monogamy in the damselfly Ischnura verticalis Say (Zygoptera: Coenagrionidae). Odonatologica, **16**, 129-143.

Garrison, R.W., Ellenrieder, N. von & Louton, J.A. (2010) Damselfly genera of the New World. Johns Hopkins University Press, Baltimore.

Geijskes, D.C. (1967) Libellen gevonden op zee in het Caribisch gebied. Entomologische Berichten, **27**, 221–223.

GenStat. (2015) GenStat for Windows. 18th edn. VSN International Ltd., Oxford.

Gerritsen, J. (1980) Sex and parthenogenesis in sparse populations. The American Naturalist, **115 (5)**, 718-742.

Glesener, R. R. and Tilman, D. (1978). Sexuality and the components of enviromental uncertainty: clues for Geographic Parthenogenesis in terrestial animals. The American Naturalist, **112**, 659-673.

Jiménez-Cortés, J. G., Serrano-Meneses, M. A., & Córdoba-Aguilar, A. (2012) The effects of food shortage during larval development on adult body size, body mass, physiology and developmental time in a tropical damselfly. Journal of Insect Physiology, **58(3)**, 318-326.

Jordan, S., Simon, C., Foote, D. & Englund, R.A. (2005) Phylogeographic patterns of Hawaiian Megalagrion damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. Molecular Ecology, **14**, 3457–3470.

Keightley, P.D. & Eyre-Walker, A. (2000) Deleterious mutations and the evolution of sex. Science, **290**, 331–334.

Kondrashov, A.S. (1988) Deleterious mutations and the evolution of sexual reproduction.

Nature, **336**, 435–440.

Lebreton, J.D., Burnham, K.P., Clobert, J. & Anderson, D.R. (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs, **62**, 67–118.

Lorenzo-Carballa, M.O. & Cordero-Rivera, A. (2009) Thelytokous parthenogenesis in the damselfly Ischnura hastata (Odonata, Coenagrionidae): genetic mechanisms and lack of bacterial infection. Heredity, **103**, 377–384.

Lorenzo-Carballa, M.O. & Cordero-Rivera, A. (2014) Odonates. In The Tree of Life (ed. by Vargas, P. & Zardoya, R.). Sinauer, Sunderland, pp. 352–363.

Lorenzo-Carballa, M.O., Cordero-Rivera, A. & Andrés, J.A. (2010) Islands and parthenogenesis: genetical and ecological correlates of asexual reproduction in Ischnura hastata (Insecta: Odonata, Coenagrionidae). In Islands and Evolution, Recerca (ed. by Pérez Mellado, V. & Ramon, C.). Institut Menorquí d'Estudis, pp. 281–307.

Lorenzo-Carballa, M.O., Hadrys, H., Cordero-Rivera, A. & Andrés, J.A. (2012) Population genetic structure of sexual and parthenogenetic damselflies inferred from mitochondrial and nuclear markers. Heredity, **108**, 386–395.

Massol, F. & Cheptou, P.O. (2011) Evolutionary syndromes linking dispersal and mating system: the effect of autocorrelation in pollination conditions. Evolution, **65,** 591-598.

Matsuura, K. (2006) Early emergence of males in the termite Reticulitermes speratus (Isoptera: Rhinotermitidae): protandry as a side effect of sexual size dimorphism. Annals of the Entomological Society of America, **99,** 625-628.

Maynard Smith, J. (1978) The evolution of sex. Cambridge University Press, Cambridge.

Morbey Y.E. & Ydenberg R.C. (2001) Protandrous arrival timing to breeding areas: a review. Ecology Letters, **4 (6),** 663-673.

Naraoka, H. (2005) Fluctuations of the daily activity and the reproductive behaviour of Mortonagrion selenion (Ris) [In Japanese]. Tombo, **47**, 53–57.

Peck, S. B. 1992. The dragonflies and damselflies of the Galapagos Islands, Ecuador (Insecta: Odonata). Psyche, **99**, 309–321.

Peck, J.R., Yearsley, J.M. & Waxman, D. (1998) Explaining the geographic distributions of sexual and asexual populations. Nature, **391**, 889–892.

Ridley, M. (1995) The Red Queen: sex and the evolution of human nature. Penguin Books, New York.

Robinson, J.V. & Allgeyer, R. (1996) Covariation in life-history traits, demographics and behaviour in ischnuran damselflies: the evolution of monandry. Biological Journal of the Linnean Society, **58**, 85-98.

Rowe, R.J. (1978) Ischnura aurora (Brauer), a dragonfly with unusual mating behaviour (Zygoptera: Coenagrionidae). Odonatologica, **7**, 375–383.

Sánchez-Guillén, R.A., Córdoba-Aguilar, A. & Cordero-Rivera, A. (2013) An examination of competitive gametic isolation mechanisms between the damselflies Ischnura graellsii and I. elegans. International Journal of Odonatology, **16**, 259–267.

Schwander, T., Vuilleumier, S., Dubman, J. & Crespi, B.J. (2010) Positive feedback in the transition from sexual reproduction to parthenogenesis. Proceedings of the Royal Society B: Biological Sciences, **277**, 1435-1442

Suomalainen, E., Saura, A. & Lokki, J. (1987) Cytology and evolution in parthenogenesis. CRC Press, Boca Ratón, Florida.

Teder, T. (2014) Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. Functional Ecology, **28**, 479–486.

Valen, L. van. (1973) A new evolutionary law. Evolutionary Theory, **1**, 1–30.

Vandel, A. (1928) La parthénogénèse géogràphique: contribution à l'étude biologique et cytologique de la parthénogénèse naturelle. Bulletin Biologique de la France et de la Belgique, **62**, 164–231.

Vrijenhoek, R.C. & Parker, J.E.D. (2009) Geographical parthenogenesis: General purpose genotypes and frozen niche variation. In Lost Sex: the evolutionary biology of parthenogenesis (ed. by Schön, I., Martens, K. & van Dijk, P.J.). Springer, Dordrecht, pp. 99–131.

West, S. a, Lively, C.M. & Read, a F. (1999) A pluralist approach to sex and recombination. Journal of Evolutionary Biology, **12**, 1003–1012.

White, G.C. & Burnham, K.P. (1999) Program MARK: Survival estimation from populations of marked animals. Bird Study, **46 Supplem**, 120–138.

Figures

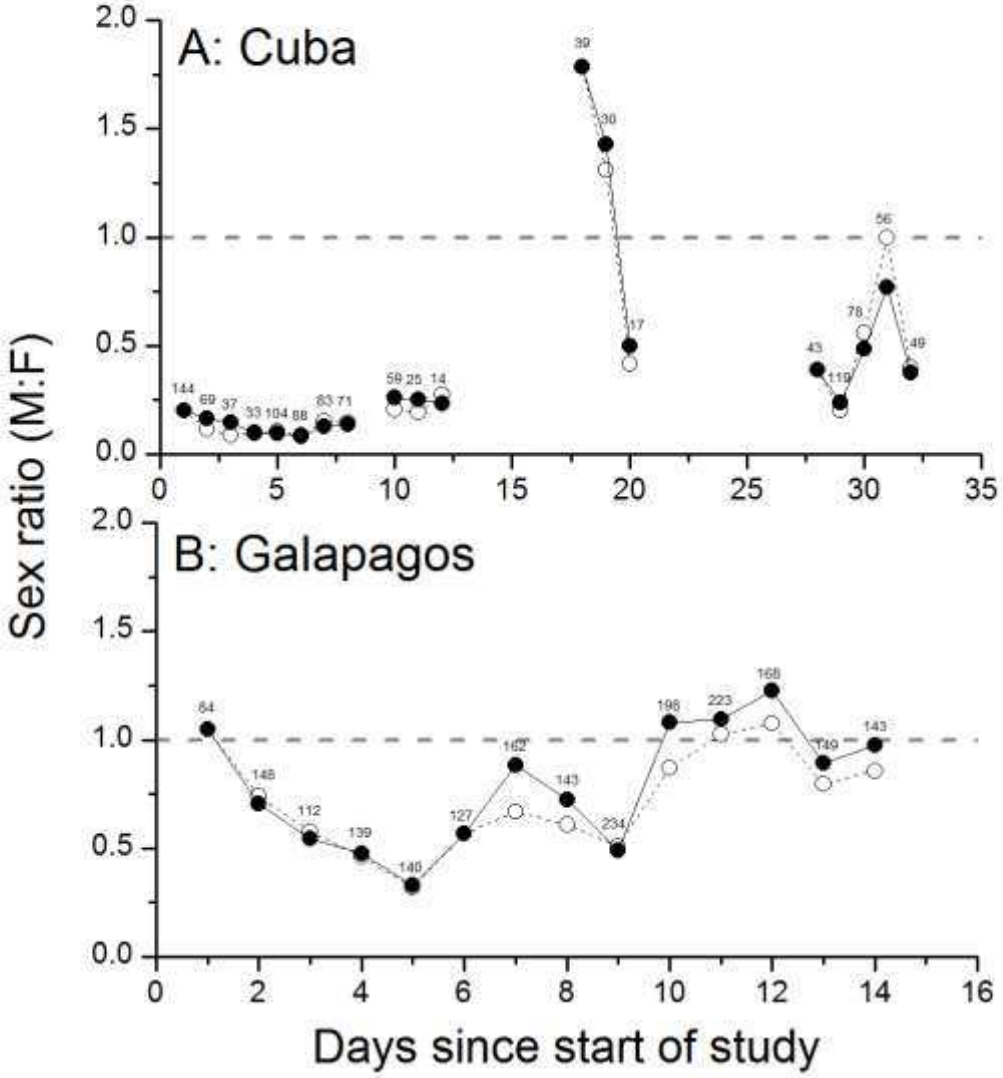

Figure 1: Daily sex-ratio variation in (A) Cuba and (B) Galapagos. We estimated sex-ratio from the newly marked animals (filled symbols, solid line) or from all specimens seen in a particular day (resighted; including marked and recaptured, open symbols, dotted line). Numbers above the points indicate the total number of animals sighted on each day.

Horizontal dashed line indicates 1:1 sex ratio, points above are male-biased and those below are female-biased.

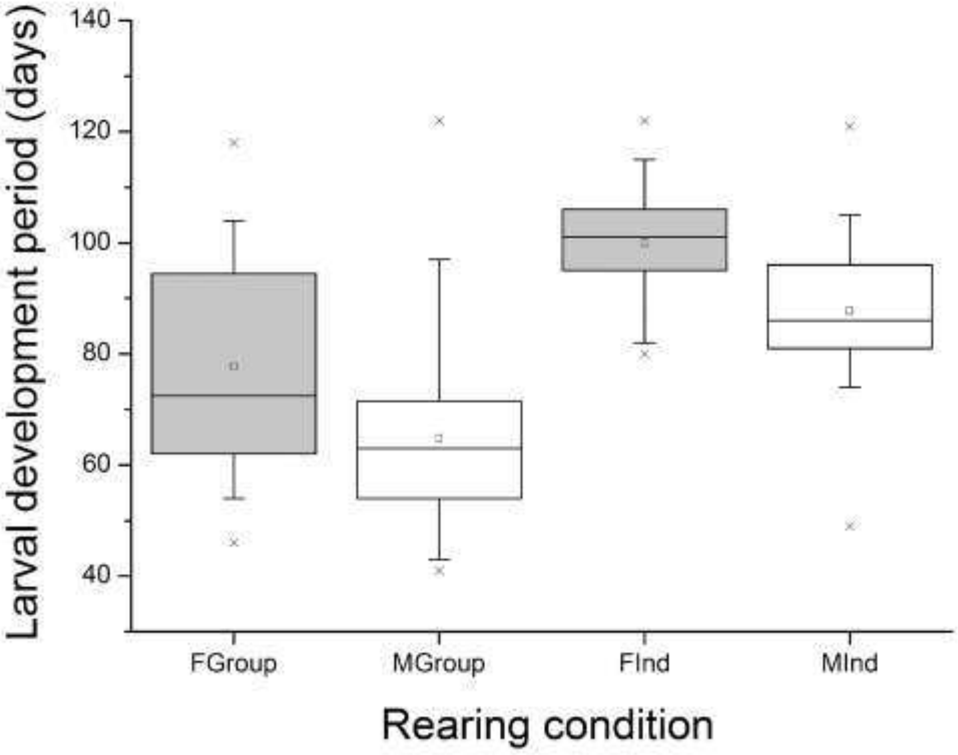

Figure 2: Variation in larval development time between females (F) and males (M) reared in groups (Group) or individually (Ind) in the progeny of field-caught female Ischnura hastata from Cuba. Boxes show the 25th, 50th, and 75th percentile values, whiskers show 5th and 95th percentiles, squares show means, and crosses show 1st and 99th percentiles.

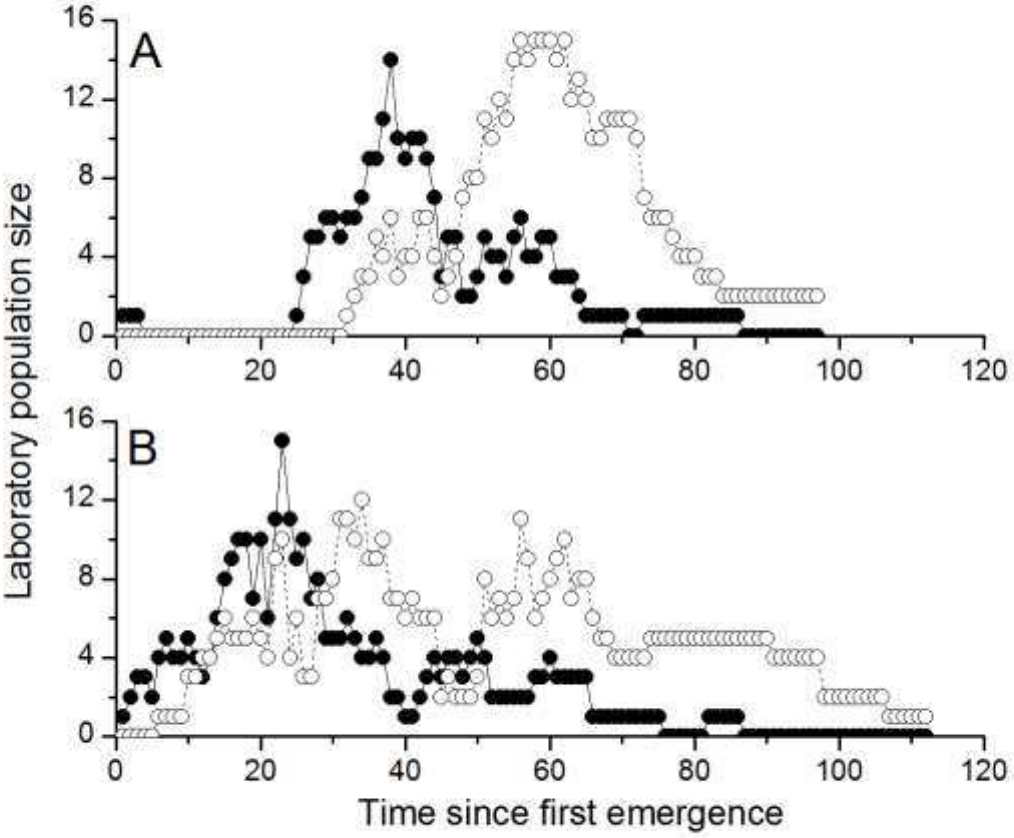

Figure 3: Laboratory adult population dynamics of Ischnura hastata from Cuba, showing protandry when animals are reared (A) individually, and (B) in groups. Filled symbols and solid line denote males, open symbols and dashed line denote females. All eggs were laid at the same date 49 days before the first emergence for individually-reared animals, and 54-57 days before the first emergence for group-reared animals.

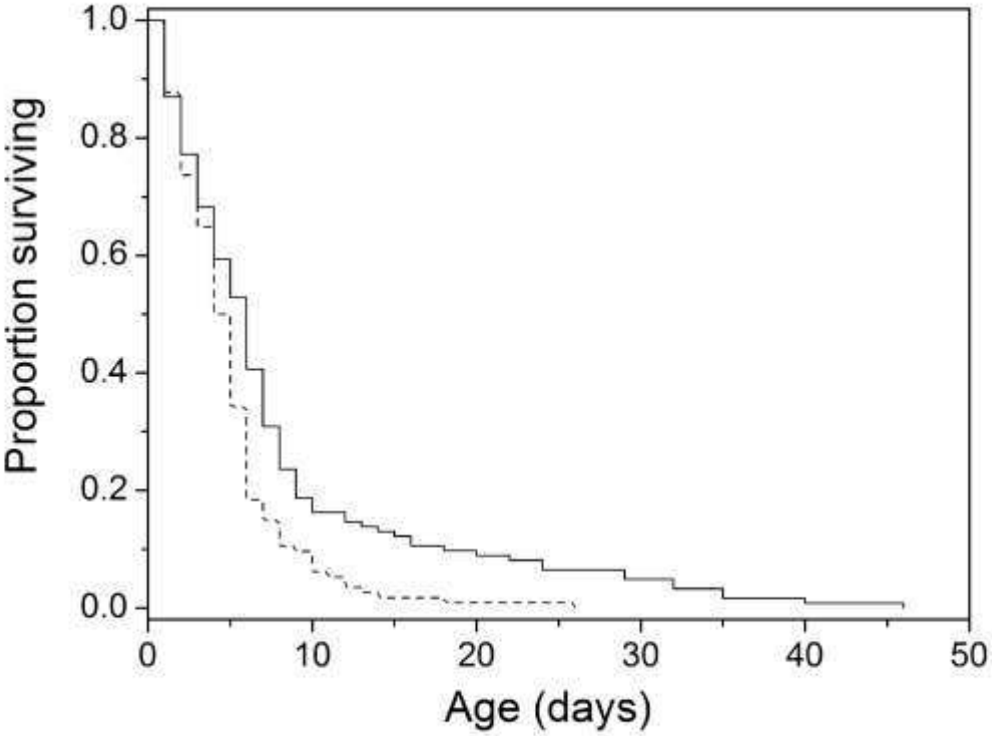

Figure 4: Differences in mortality between males (dotted line) and females (solid line) of Ischnura hastata from Cuba in the laboratory.

Table 1: Number of Ischnura hastata individuals marked (and recaptured) in each population by sex and age group at marking. Sex ratio is calculated as marked males/total. The p-value of H_0=equal sex-ratio is presented.

Population	**Age**	**Females**	**Males**	**Total**	**Sex-ratio**	**p-value**
Cuba	Teneral	44 (1)	40 (1)	84 (2)	0.476	0.663
	Young	27 (1)	33 (1)	60 (2)	0.550	0.439
	Mature	847 (120)	167 (32)	1014 (152)	0.165	<0.001
	Total	918 (122)	240 (34)	1158 (156)	0.207	<0.001
Galapagos	Teneral	28 (2)	26 (5)	54 (7)	0.481	0.785
	Young	180 (37)	100 (16)	280 (53)	0.357	<0.001
	Mature	678 (237)	537 (153)	1215 (390)	0.442	<0.001
	Total	886 (276)	663 (174)	1549 (450)	0.428	<0.001

Table 2: Structure of laboratory rearing experiments of Ischnura hastata. Under rearing condition, N refers to the number of females whose progeny was included in the experiments.

Population	**Date of egg collection**	**Rearing condition**	**N larvae**	**N adults**
Galapagos	25-27 Feb & 4-6 March 2014	Individual (N=11)	550	*
		Common (N=11)	500	*
Cuba	23-26 Jun 2012	Individual (N=10)	496	133
		Common (N=14)	539	154

* Due to high mortality, the few remaining larvae were reared together in their last month. A total of 22 adults emerged from this sample (see main text).