Anim. Behav., 1998, **55,** 185–197

Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata)

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(Received 21 January 1997; initial acceptance 17 March 1997; final acceptance 25 May 1997; MS. number: 5459)

Abstract. Female colour polymorphism is a perplexing characteristic of many damselfly species. In *Ischnura elegans* three female phenotypes occur, one of which has the same blue coloration as the male (androchromes) whilst the others are inconspicuous brown gynochromes (*infuscans* and *infuscans-obsoleta* morphs). By marking a natural population near Rome, Italy, we found that all female phenotypes have similar survivorship, but they differ in mating frequency. Androchromes represented 55% of females but were involved in 43% of matings, whereas *infuscans* females represented 27% of females and 40% of matings and the *infuscans-obsoleta* phenotype 18% of females and 17% of matings. Old androchromes stored significantly less sperm in their spermatheca than old gynochromes, suggesting that they had mated less often. The majority of mature androchromes were observed alone (54%) when the majority of gynochromes (82–84%) were mating. When live tethered conspecifics were presented to males, blue models (male and androchrome female) were less attractive than brown models (gynochrome females). In contrast, all female colour morphs and males were equally (highly) attractive to males when the models were dead. Androchromes were significantly larger than gynochromes. Our results indicate that androchrome females mate less often than gynochromes, which could be a means of avoiding unnecessary and costly matings, but some androchrome females failed to reproduce (mate or oviposit) probably because they were unable to mate at all. The different explanations for the maintenance of this polymorphism in *I. elegans* are discussed.

Colour polymorphism has attracted considerable attention from biologists, probably because colour morphs are conspicuous, and because an adaptive explanation for their maintenance in natural populations is not intuitive (Oxford 1993). Even more intriguing are colour polymorphisms restricted to one sex. In many butterflies, for example, females may be male-like or non-male-like (one or several morphs sometimes mimic non-palatable model species; Vane-Wright 1975; Turner 1978).

In the Odonata, female-limited colour polymorphism is widespread, especially in the family Coenagrionidae (Cordero & Andrés 1996). One of the female morphs is coloured like the male, and in some species also has the same patterning. These females therefore look exactly like males. The number of additional, non-male-like, female morphs ranges from one to three. Here we use the terms androchrome for the female morph that has the same coloration as males and gynochrome for all the other morphs, thereby shortening the terms proposed by Hilton (1987).

Four main hypotheses have been proposed to explain the maintenance of this colour polymorphism. The reproductive-isolation hypothesis proposes that androchromes benefit by avoiding matings with congeneric species, whereas gynochromes are commonly involved in heterospecific (sterile) matings. Greater predation pressure on the (supposedly) more conspicuous androchrome is proposed as the trade-off that maintains this

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0003–3472/98/010185+13 $25.00/0/ar970603

polymorphism (Johnson 1975; De Marchi 1990). This hypothesis assumes that interspecific matings are common, and predicts that androchromes do not live as long as gynochromes but mate less frequently with heterospecific males (Cordero 1992).

The male-mimicry hypothesis proposes that androchromes avoid male harassment because of their male-like appearance and, after first mating, avoid additional (unnecessary and long) matings, which allows them to allocate more time to feeding and egg maturation. Greater predation on androchromes is also proposed as the balancing mechanism (Robertson 1985). A second advantage of male-like females would be reduced male harassment during oviposition (Van Noordwijk 1978). These advantages would be frequency dependent because at high androchrome frequencies males would be selected to attempt to mate with all individuals, in order not to miss mating opportunities (Robertson 1985). The main assumptions of this hypothesis are: (1) mating takes a long time and is probably costly; (2) one mating is enough to fertilize lifetime egg production; and (3) males are unable to distinguish between androchromes and other males. This idea predicts a short life span, low mating frequency and long inter-mating interval for androchromes (Cordero 1992).

The density-dependent hypothesis proposes that female colour polymorphism is an adaptation to population density (Hinnekint 1987). Androchromes escape the attention of males because of their male-like appearance. This causes a mating disadvantage at low population density that becomes an advantage in dense populations (androchromes are less disturbed by males searching for mates). This hypothesis assumes that sex ratio is male biased in dense populations, and that androchromes are male-mimics. It predicts similar survivorship for andro- and gynochromes and density-dependent mating success in androchromes but not in gynochromes (Cordero 1992).

Finally, female morphs may be neutral to natural or sexual selection, and their frequencies maintained by an equilibrium between founder effects, mutation and genetic drift ('neutral' hypothesis: Fincke 1994). This idea specifically predicts that all morphs are equally successful in attracting or rejecting males (Fincke 1997). Consequently, all morphs should have the same fitness and therefore their frequencies should vary in a random way between populations.

Our aims in this paper are to test adaptive and non-adaptive hypotheses for the maintenance of female colour morphs in the damselfly *Ischnura elegans* Van der Linden, which was the original species for which the density-dependent hypothesis was proposed (Hinnekint 1987).

STUDY SPECIES AND METHODS

The Study Species

Ischnura elegans is probably the commonest damselfly in Europe, and has been the subject of several studies on larval development (Thompson 1987; Gribbin & Thompson 1990), demography (Parr 1965, 1973a, b; Parr & Palmer 1971; Parr & Parr 1972; Van Noordwijk 1978; Hinnekint 1987) and behaviour (Krieger & Krieger-Loibl 1958; Miller 1987a, b; Cordero et al., in press). To our knowledge, in all populations of *I. elegans* three female colour morphs are present: the androchrome morph (abbreviated to 'A' in the Results), which closely resembles the conspecific blue male in colour and pattern; and two brown gynochrome morphs, *infuscans* (abbreviated to 'I' in the Results) and *infuscans-obsoleta* (abbreviated to 'O'). The mature *infuscans* morph has the same pattern as males, but is brown where the male is blue. The *infuscans-obsoleta* morph also has brown markings when mature, but it has only a single black line on the thorax (males, androchromes and *infuscans* females have three; see D'Aguilar et al. (1985) and Askew (1988) for colour figures of female morphs). Furthermore, all female morphs change their colour markedly during their lives (Fig. 1). Male coloration changes from pale green to yellow, yellow-green, turquoise and finally blue. Male colour changes are therefore rather similar to those of androchrome females (Fig. 1).

The genetic basis of this polymorphism is unknown in *I. elegans*. Nevertheless, in the closely related species *I. graellsii* (see Carchini et al. (1994) for a study of the genetic distance between *I. elegans* and *I. graellsii*), with three female morphs, which match the morphs found in *I. elegans*, polymorphism is controlled by an autosomal locus with sex-limited expression and three alleles with a simple dominance hierarchy:

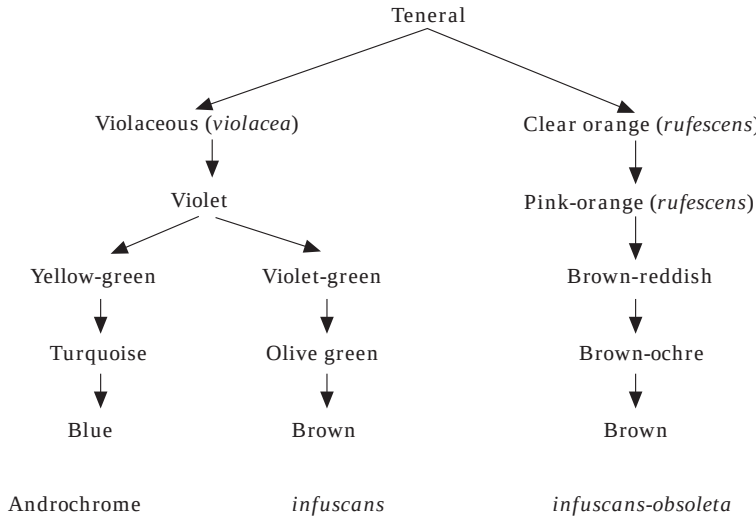

Figure 1. The relationship between female colour polymorphism and age-dependent colour changes in *I. elegans*.

androchrome>*infuscans*>*aurantiaca* (the equivalent morph of *infuscans-obsoleta*; Cordero 1990b). We are therefore confident that the same genetic system is applicable to *I. elegans*.

Methods

We studied a natural population of *I. elegans* that inhabited a small pond at Castel Porziano (Rome, Italy), using the marking–release–resighting method, from June to August 1994. Marking and observations were carried out by one to three observers, in sessions of 5 consecutive days, separated by 2 days without observations. The dates of sampling were 30 June, 1, 4–8, 11–15, 18–22, 25–28 July, and 1–3 and 5 August. Our observations lasted on average ± SE 3.6 ± 0.3 h per day (range 1–5.7 h, $N = 25$ days).

The study pond was of natural origin, formed by a spring, but had recently been enlarged to a perimeter of approximately 140 m. The dominant vegetation consisted of *Juncus*, *Typha* and *Digitaria* spp. Other species of damselflies at this site during the period of study were *Ceriagrion tenellum* (abundant), *Ischnura pumilio* (rare), *Coenagrion mercuriale* (rare) and *Erythromma viridulum* (rare). The pond was bounded by agricultural fields (clover), in which we searched occasionally for copulating pairs of *I. elegans*. It had a drainage channel, ending in a larger pond about 100 m away, which was not sampled.

We netted the insects, measured them (from the end of the abdomen to the head) to the nearest 0.1 mm, marked them with a number in black ink (Staedtler Pancolor 303 S pen) on the right hindwing and released them at the site of capture. At the time of marking and in each subsequent observation we recorded number, sex, thorax colour (an indicator of age, Van Noordwijk 1978), female morph, hour of observation and activity (in copula, tandem, feeding, oviposition, etc.).

We estimated age at marking, using thoracic colour as a cue. Age-groups were: teneral (0–1 days old; pale pink colour, violaceous or very clear orange, soft body and glistening wings); immature (2–5 days old; yellow-green, violet or pink-orange thorax); and mature (at least 6 days old; turquoise, blue, olive green, orange-ochre or brown thorax, deepening with age). We consider the last group as mature because the mean age at first copulation of females that were marked as tenerals ± SE was 6.1 ± 0.6 days, $N = 22$ (Cordero et al., in press). Females in the immature age-classes were sometimes seen in copula, probably because colour change was imminent (Hinnekint 1987).

We marked 1043 males (349 resighted at least once), 333 androchrome females (138 resighted), 181 *infuscans* females (78 resighted), 176 *infuscans-obsoleta* females (62 resighted) and 267 females of the *violacea* colour phase (47 resighted) that we could not assign to the androchrome or *infuscans*

phenotypes, because we never resighted them in their mature phase. Of the females that we first recorded as *violacea*, and that lived long enough to be assigned to a mature phenotype of coloration, 71% (106/149) were androchrome. If the proportion was similar in the group of the 267 above-mentioned females, then 190 females were androchrome and 77 were *infuscans*. Our ability to distinguish between androchrome and *infuscans* females during young phases was good, since only one female that we first recorded as androchrome (violet-blue thorax) was subsequently resighted as *infuscans* in her mature phase. We estimate the frequencies of the three female phenotypes in this population as: 333+190 androchromes (54.6%), 181+77 *infuscans* (27.0%) and 176 *infuscans-obsoleta* (18.4%). The ratio for females marked as mature adults was 101 androchromes (48.8%), 61 *infuscans* (29.5%) and 45 *infuscans-obsoleta* (21.7%). The two estimates are not significantly different ($\chi^2_2 = 2.49$, NS).

To test the ability of male *I. elegans* to distinguish between males and androchrome or gynochrome females, we carried out two experiments with models. The first experiment used live conspecific adults, which were tethered to the end of a *Juncus* stem by means of a copper wire approximately 30 cm in length and 0.1 mm in diameter wound around the thorax (Cordero 1989). This allowed the lure to be mobile, without damaging it. The experimental procedure consisted of presenting the model in flight at 10–15 cm in front of the test male, and allowing the model to perch in the immediate vicinity of the male. The response of the male was scored in four categories: (1) no response or fly away; (2) approach to the model without making contact with it; (3) attempt to grasp the model in tandem; or (4) successful tandem. We assume that these categories represent an increasing sexual interest towards the model. If a male did not respond within 5–10 s on the first presentation, we carried out a second presentation with the same procedure after approximately 1 min. If, after 5–10 s in the second presentation, no response was observed we abandoned the test. Males that did not respond were not considered in further analyses, because a closer examination revealed that the majority were feeding or were very young, and were therefore probably not searching for mates. Presentations were carried out between 25 July and 3 August. For each phenotype we used four individuals as lures (except for the *infuscans-obsoleta* phenotype, of which we could find only three models). Each model was used in 2–13 positive presentations on one morning (5–10 in the majority of cases). The experiment ended when 30 males had responded to each phenotype. It is unlikely that the same male had responded more than once to the model, since approximately half the males tested were already marked and therefore could be recognized individually. We displaced the model at least 2–3 m between two presentations, to avoid testing the same unmarked male twice.

The second presentation experiment was carried out with dead pinned animals, and took place on 29 and 30 August. The objective of this experiment was to differentiate the effect of body coloration from the effect of behaviour/smell of the model on the male's response. We used three models per colour phenotype and registered 10 responses per model, until we reached 30 per phenotype. Presentations were the same as those for live models.

To test the male-mimicry and density-dependent hypotheses we carried out nine transects, slowly walking round the perimeter of the pond and recording the number of solitary individuals and mating pairs observed in a strip of approximately 2 m. All the transects were carried out when reproductive activity was highest (1200–1300 hours), on 20 and 30 June, 1, 4, 6, 12, 18, 26 July and 1 August.

On 14 July, we carried out an experiment to see if ovipositing females were disturbed by mate-searching males (Van Noordwijk 1978). One mature dead female of each phenotype was pinned in the egg-laying posture to a floating *Typha* leaf (40 cm long and 8 mm wide). The androchrome female was in the centre and females were separated by 6–7 cm. We observed the set of females for 30 min when oviposition behaviour was at its highest (1600–1630 hours), and recorded male and female responses to the ovipositing models.

We did not see all matings, either because they took place at some distance from the water (Van Noordwijk 1978) or because they took place on days when we were not present. To check whether androchrome females mated less often than gynochromes, we captured and preserved 15 androchromes and 17 gynochromes in 70% ethanol. From females captured during the morning, we selected solitary unmarked females with an

enlarged abdomen and mature coloration. We dissected these females and measured the volume of sperm in their sperm storage organs by the methods described in Cordero & Miller (1992). The area of the sperm masses was measured with the image analysis program GlobalLab from Windows.

We analysed marking–recapture data by means of Jolly's method (as described in Begon 1979). We estimated the number of individuals present in the population and the daily survival rate. Throughout the text, values are presented as mean ± SE (N). Integer variables (e.g. number of copulations, longevity, etc.) were square-root transformed (after adding 0.5) and the proportions arc-sine square-root transformed before being entered into parametric tests.

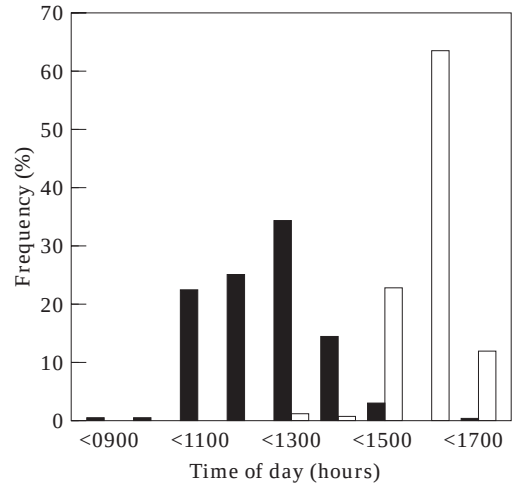

Figure 2. Daily distribution of copulations and ovipositions in *I. elegans* (time of first observation). ■: Matings, $N = 556$; □: ovipositions, $N = 150$.

RESULTS

Reproductive-isolation Hypothesis

We observed 547 copulations by marked females. *Ceriagrion tenellum* males showed interest in *I. elegans* females, and one male persistently tried to grasp our dead *infuscans-obsoleta* model, but we saw no interspecific mating. Therefore this idea seems unable to explain the maintenance of colour morphs in female *I. elegans*.

Male-mimicry Hypothesis

The first assumption of this idea (mating is very long) is supported by our observations. Mating took place early in the day (Fig. 2). The first mating was observed at 0854 hours, but on hot days, some matings started earlier (see also Miller 1987a). Some mating pairs remained in copula for 3–4 h. As female *I. elegans* can lay fertile eggs over several days after just one mating (A. Cordero, personal observation), the second assumption (one mating is enough for lifetime egg fertilization) is probably also true for *I. elegans*.

The third prerequisite (androchromes are male-mimics) is the basic assumption of this hypothesis. If that were true, then males should respond in the same way to androchrome and male models, but in a different way to the brown gynochrome models. Figure 3a shows the results of the experiment with living models. The majority of males tested responded positively to the brown models (I: approach: 12 males; tried to initiate tandem: 2; tandem: 16; O: 11:5:14), but simply approached the blue models (A: 20:1:9; male: 19:2:9). If we compare the sexual responses (tried to initiate tandem plus tandem) with the non-sexual response (approach), the differences are not significant, either between the two brown models ($\chi^2_1 = 0.075$, NS), or between the two blue models ($\chi^2_1 = 0.073$, NS). The blue models and the brown models differ significantly, however ($\chi^2_1 = 8.543$, $P < 0.01$).

The test with dead models produced very different results. The response of males (approach:tried to initiate tandem:tandem) was the same regardless of coloration (Fig. 3b): A: 5:3:22; males: 5:5:20; I: 3:8:19, O: 2:6:22. The comparison between the two blue models, brown models or blue versus brown indicates that the male's response was similar in all cases (NS).

We used 45 males that mated twice with mature females (*violacea* excluded) to test whether individual males prefer to mate with a particular female morph. Comparing the observed frequencies of double matings (AA: 13; AI: 9; AO: 5; II: 11; IO: 7; OO: 0) with the expected from the female ratio in all mating pairs (8.3:15.6:6.5:7.3:6.1:1.3), no significant deviation from the expected was obtained (chi-square goodness-of-fit test: $\chi^2_4 = 7.70$, NS).

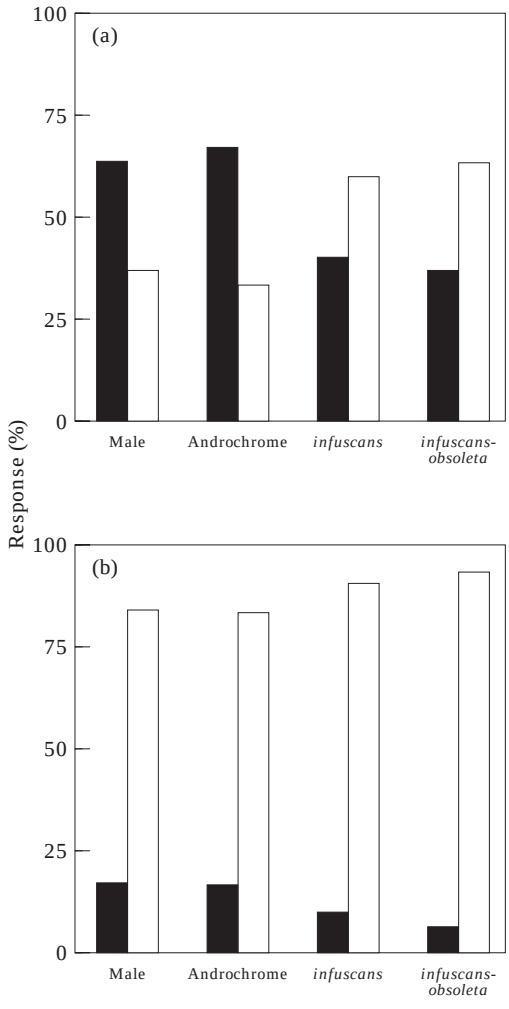

Figure 3. Results of presentation of models of males and three female morphs to mature males. (a) Living models; (b) dead models. Bars show the percentage of mature males that tried (or achieved) tandem with the model or simply approached to inspect it. ■: Approach; □: try tandem.

Van Noordwijk (1978) suggested that androchromes may be protected from male harassment during oviposition. Our results do not support this idea. During the 30-min observation period, two females started to oviposit at about 5 cm from our models and one male perched at 10 cm, but showed no interest in either female. At this time of day males were observed in the vicinity but apparently they were not searching for mates. Furthermore, we never observed males harassing ovipositing females.

This hypothesis predicts that androchromes should have a shorter life span because of selective predation. Table I shows the estimates of the mean and maximum life span, and the survival rate of the three female phenotypes and males. We could not detect significant differences in life span between female morphs. The estimates of daily survival rate based on Jolly's method also indicated no significant differences between phenotypes. The significant differences between morphs in the life-table analysis are an artefact caused by the exclusion of *violacea* females that did not live long enough to be scored as androchromes or *infuscans*. Their exclusion increases the life spans of these two phenotypes.

If androchromes are avoiding unnecessary matings they should mate less often than gynochromes (prediction 2). Three independent sources of evidence support this prediction. First, in the nine transects, the majority of mature *infuscans* and *infuscans-obsoleta* females (84 ± 4.0 and $82 \pm 14.1\%$) were observed in copula, but only $46 \pm 5.2\%$ of mature androchrome females were mating (Kruskal–Wallis test: $H_2 = 12.174$, $P < 0.01$). Second, out of 547 copulations, 235 (43%) involved androchrome females, 220 (40%) *infuscans* females and 92 (17%) *infuscans-obsoleta* females. The expected frequencies from the proportion of females in the population (see Methods) are 298.9 (55%) androchrome, 147.5 (27%) *infuscans* and 100.6 (18%) *infuscans-obsoleta*. The differences are highly significant ($\chi^2_2 = 50.03$, $P < 0.0001$). Third, old androchromes stored less sperm in their spermatheca than old gynochromes, suggesting they mated less often (Table II). Five young females had no sperm, and were probably unmated. Of these, three were androchromes, one *infuscans* and one *infuscans-obsoleta*. Only one old female was unmated, and it was an androchrome female. If this female is excluded, the differences between phenotypes are still significant ($t_{16} = 2.57$, $P < 0.05$).

The third prediction is that androchrome females should mate with longer intervals between copulations than gynochromes. We calculated the inter-mating interval for our sample of females that mated twice, and averaged the intervals for females that mated more than twice. Although androchromes remated after 3.9 ± 0.88 (32) days whereas gynochromes did so after 2.8 ± 0.26 (57) days, these differences are not significant ($t_{36} = -1.24$, NS).

Table I. Demographic parameters for the different phenotypes of *Ischnura elegans*

Variable	Male	Androchrome	*infuscans*	*infuscans-obsoleta*	*P*
Survival rate (life table)	0.797 ± 0.018 (15) (0.794)	0.831 ± 0.016 (14) (0.829)	0.854 ± 0.025 (12) (0.849)	0.771 ± 0.012 (7) (0.770)	<0.05
Survival rate (Jolly)	0.960 ± 0.118 (23) (0.877)	0.929 ± 0.113 (23) (0.832)	0.904 ± 0.071 (21) (0.856)	0.860 ± 0.095 (20) (0.776)	NS
Mean life span*	6.12 ± 0.24 (349)	6.16 ± 0.41 (138)	6.92 ± 0.55 (78)	5.35 ± 0.48 (62)	NS
Maximum longevity (days)	25	27	21	18	

Arithmetic mean ± SE (*N*) (geometric mean). In the life-table analysis we have included only estimates based on at least 10 individuals and we have excluded survival from day 1 (marking) to 2 (Cordero et al., in press). Kruskal–Wallis test.
*Only recaptured individuals are included.

Table II. The volume of sperm ($\bar{X} \pm$ SE (*N*) mm^3) stored in female *I. elegans* captured alone

Age group	Organ	Androchrome	Gynochrome	*P*
Young	Bursa copulatrix	0.0013 ± 0.001 (5)	0.0020 ± 0.001 (8)	NS
	Spermatheca	0.0025 ± 0.002 (5)	0.0031 ± 0.001 (8)	NS
	Total	0.0042 ± 0.003 (5)	0.0051 ± 0.001 (8)	NS
Old	Bursa copulatrix	0.0025 ± 0.0003 (10)	0.0023 ± 0.0005 (9)	NS
	Spermatheca	0.0048 ± 0.0006 (10)	0.0065 ± 0.0004 (9)	<0.05
	Total	0.0073 ± 0.0008 (10)	0.0088 ± 0.0007 (9)	NS

Young females are mature but still with intermediate body coloration (turquoise, olive-green or orange-ochre), whereas old females are fully mature. *P* after two-tailed *t*-test.

Density-dependent Hypothesis

The first assumption of this idea is that the sex ratio is male biased, especially in dense populations. The sex ratio in our population was clearly male biased (Fig. 4). The second assumption (androchromes are male-mimics) and the first prediction (similar survivorship between morphs) were tested in the previous section.

The main prediction of this hypothesis is that the density of males searching for mates determines androchrome mating frequency. Therefore there should be a positive correlation between the number of males present at the pond and the percentage of androchrome females that mate. Table III shows the results from the nine transects. Androchrome mating rate was positively correlated with male density only for sampling days when sample size was greater than five females ($r = 0.87$, $N = 7$ days, $P < 0.05$), but with the *infuscans* females it was not ($r = 0.08$, $N = 7$, NS; the *infuscans-obsoleta* females were so rare that we never sighted more than five mature females in one census).

The density-dependent hypothesis could maintain female morphs only if androchromes have a disadvantage at low male density. The only females that could suffer from this disadvantage are those that never mate. Table IV shows the mating frequencies of the female phenotypes. Only seven androchrome females that were marked as tenerals lived at least 6 days. Of these, three (43%) were never observed to mate or to oviposit. In contrast, all 11 *infuscans* females that were marked as tenerals and lived at least 6 days mated. Just two teneral *infuscans-obsoleta* females lived at least 6 days; one mated and oviposited and the other was observed to oviposit. These figures are too low to make a firm conclusion, but the percentage of teneral females that apparently failed to reproduce depended on their phenotype (comparison A:G; Fisher's exact test: $P = 0.031$). The same is true for the other two age classes (young: $\chi^2_2 = 9.0$, $P < 0.05$; mature: $\chi^2_2 = 11.3$, $P < 0.01$). Although the life span of *infuscans* females marked as mature individuals was the shortest of the three morphs, they had the greatest

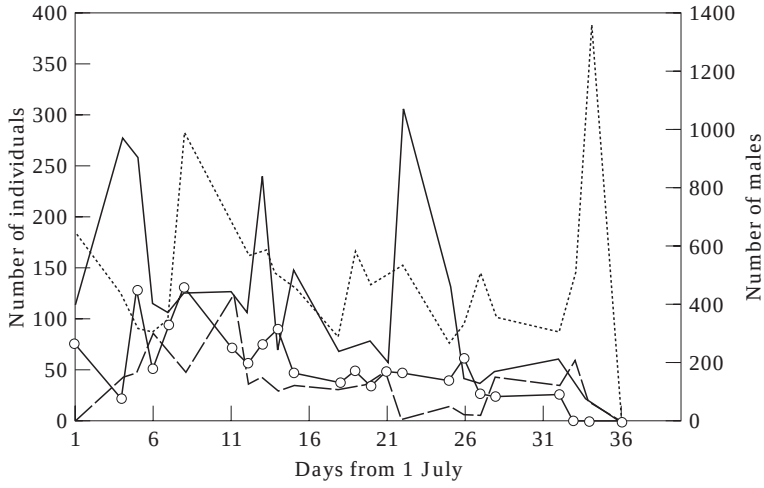

Figure 4. The seasonal variation in numbers of males and three female morphs, estimated from mark–recapture data (Jolly method). ——: Androchromes; —○—: *infuscans*; – – –: *infuscans-obsoleta*; · · · ·: males.

Table III. Number of individuals of each phenotype observed in copulation and alone during censuses

Day	Time of day (hours)	Males	Androchrome		*infuscans*		*infuscans-obsoleta*	
			Alone	In copula	Alone	In copula	Alone	In copula
20 June	1153–1228	125	10	19	1	10	0	5
30 June	1159–1230	42	5	3	1	7	0	2
1 July	1200–1242	32	11	8	4	9	1	3
4 July	1205–1231	19	6	3	0	3	0	1
6 July	1209–1310	34	15	5	0	9	0	3
12 July	1203–1312	53	13	9	2	7	1	0
18 July	1155–1238	34	2	2	1	5	0	3
26 July	1200–1237	43	4	3	1	5	0	0
1 August	1203–1240	16	1	3	1	2	0	0

Only mature females are included.

mating frequency (only four out of 61 failed to mate, compared with 26 out of 101 androchromes and five out of 45 *infuscans-obsoleta*).

Since we did not sample all reproductive activities on all days, some females could have mated without having been observed in copula. From Table IV we can estimate that at least 6% of *infuscans-obsoleta*, 10% of androchromes and 34% of *infuscans* females mated, even though they were not recorded in copula, since subsequently they were seen ovipositing. Since we apparently missed a greater proportion of matings by the *infuscans* females, the preceding conclusions remain unchanged: the reproductive failure of androchromes was greater than that of gynochromes. Note that, for the sample of females marked as mature individuals, 16% (16/101) of androchromes failed to reproduce (to mate or to oviposit), whereas only 2% (1/61) of *infuscans* and 7% (3/45) of *infuscans-obsoleta* females did ($\chi^2_2 = 9.38$, $P < 0.01$).

Neutral Hypothesis

If female morphs are neutral to selection then there should be no differences between them in any fitness correlate. The number of eggs laid is probably the best correlate of fitness, but it is impossible to measure under field conditions. Instead we can test this hypothesis by comparing

Table IV. Distribution of the number of copulations per individual and longevity in *I. elegans*

Phenotype	Marked as	Longevity	Number of copulations							Total
			0	1	2	3	4	5	6	
Male	Teneral	2.65 ± 0.17	382	24	5	0	0	0	0	411
	Young	3.31 ± 0.28	140	61	7	3	0	0	0	211
	Mature	2.48 ± 0.16	174	197	38	12	0	0	0	421
	Total	2.71 ± 0.11	696	282	50	15	0	0	0	1043
Androchrome	Teneral	4.07 ± 0.46	81 (77)	22	3	0	0	0	0	106
	Young	2.81 ± 0.34	55 (52)	56	9	3	1	0	1	125
	Mature	2.64 ± 0.35	26 (16)	60	12	1	1	0	1	101
	Total	3.15 ± 0.22	162 (145)	139*	24	4	2	0	2	333
infuscans	Teneral	8.28 ± 0.84	7 (4)	15	12	6	2	1	0	43
	Young	2.23 ± 0.31	18 (14)	45	11	2	1	0	0	77
	Mature	1.89 ± 0.25	4 (1)	47	10	0	0	0	0	61
	Total	3.55 ± 0.32	29 (19)	107	33	8	3	1	0	181
infuscans-obsoleta	Teneral	2.69 ± 0.30	90 (87)	19	2	1	0	0	0	112
	Young	1.53 ± 0.33	8 (7)	10	1	0	0	0	0	19
	Mature	2.58 ± 0.46	5 (3)	32	5	2	0	0	1	45
	Total	2.53 ± 0.23	103 (97)	61	8	3	0	0	1	176
violacea	Teneral	1.36 ± 0.06	246 (246)	20	1	0	0	0	0	267

Longevity is the $\bar{X} \pm$ SE number of days between marking and the last observation (longevity = 1 for individuals never resighted). In parentheses is the number of females that were never observed to mate or oviposit.

*One female whose age was not estimated is included in the total but not in the age groups.

traits that are correlated with female fecundity. Body size is an important determinant of female fecundity in many animals, including *Ischnura* damselflies (Cordero 1991). In our population both gynochrome females were of similar size (I: 29.57 ± 0.13 mm, $N = 120$; O: 29.66 ± 0.13, $N = 146$; $t_{264} = -0.53$, NS), but androchromes (29.96 ± 0.09, $N = 270$) were larger than gynochromes (29.62 ± 0.09, $N = 266$; $t_{534} = 2.73$, $P < 0.01$), suggesting they could lay more eggs.

In insects, body size is fixed at emergence and usually the first insects to emerge are larger. To test for differences in emergence of morphs, we compared date of marking of females that were marked as tenerals or inmature (*violacea* excluded). There were no significant differences (Kruskal–Wallis test: $H_2 = 5.444$, NS).

Oviposition is accomplished by the female alone, on floating vegetation, mainly between 1500 and 1700 hours (Fig. 2). The mean time of first observation of egg-laying behaviour was similar in all morphs ($F_{2,146} = 0.42$, NS). For females marked as mature individuals, the number of ovipositions was not significantly different between phenotypes (A: 0.30 ± 0.06 days of oviposition per female; I: 0.25 ± 0.07; O: 0.36 ± 0.09; Kruskal–Wallis test: $H_2 = 0.908$, NS), or for the young sample (A: 0.25 ± 0.05; I: 0.22 ± 0.06; O: 0.06 ± 0.05; $H_2 = 2.46$, NS), although it was for the teneral sample (A: 0.08 ± 0.03; I: 0.44 ± 0.13; O: 0.11 ± 0.04; $H_2 = 15.27$, $P < 0.001$).

DISCUSSION

The Reproductive-isolation Hypothesis

Our results indicate that this hypothesis cannot explain the maintenance of female morphs of *I. elegans*. Rare hybridization events could have contributed in the past to the maintenance of female morphs, but we could not detect any interspecific copulation in our population.

The Male-mimicry Hypothesis

This hypothesis assumes that matings are long and (presumably) costly. Because of their male-like appearance, androchromes can avoid this cost by mating only once and escaping further attention from males. In fact, mating was very long in our population. Miller (1987c) indicates that this species holds the record for length of copulation among odonates, reaching 7–8 h. Nevertheless, during the hours that gynochromes were copulating, androchromes did not engage in oviposition behaviour (Fig. 2); the only benefit they could obtain would be to dedicate more time to feeding and egg maturation than gynochromes. In addition, if predation to mating pairs were common, androchromes could suffer less predation than gynochromes (Robertson 1985). Nevertheless, we observed only two cases of spider predation to mating pairs. In both cases only the (gynochrome) female was captured.

Avoiding matings can be advantageous only if one mating is enough for lifetime egg fertilization. This is true for *I. verticalis* (Grieve 1937) and *I. graellsii* (Cordero 1990a). The same is probably true for *I. elegans*, because this species has a very similar biology (personal observation).

The main assumption of this hypothesis is that androchromes are male mimics. Our results give some support to this. Males confronted with live lures did not discriminate between androchrome females and other males, and showed greater sexual interest towards *infuscans* and *infuscans-obsoleta* females (Fig. 3). Nevertheless, when models were dead, all were highly attractive to the test males. The experiment with dead lures was made when the population density was very low. In these circumstances males might be more motivated to mate with any individual that they find, and are therefore more indiscriminate in their mating attempts. We think, however, that these results could be more easily explained if, for a perfect imitation, androchromes must have both male coloration and behaviour (or smell). Similar experiments with *C. tenellum* also indicated that males discriminate between female morphs only if the latter are alive, but all phenotypes are highly attractive if the models are dead (J. A. Andrés & A. Cordero, unpublished data). An explanation based on chemical mimicry between androchromes and males (i.e. live androchromes produced male-like pheromones whereas dead models did not) cannot be ruled out because odonate antennae bear sensory structures resembling those that detect chemicals in other insects (Miller 1987c). We interpret this result as a consequence of the behaviour/smell of our models, and predict that androchromes should be more similar to males than gynochromes in their general behaviour or smell, and especially in their refusal

behaviour when approached by males searching for mates (see also Utzeri 1988).

Van Noordwijk (1978) proposed that androchromes could be less disturbed by males searching for mates than are gynochromes during oviposition. In our population, this potential benefit for androchromes was not observed, because when females oviposited (in the afternoon and early evening), males were feeding and no copulation occurred (Fig. 2).

Our results did not support the prediction that androchromes would have a shorter life span. It is very unlikely that body coloration attracts predators, because the main predators of damselflies are spiders and frogs, and frogs attack any moving object rather than coloured objects. If predators were attracted by blue individuals, they would prey more on males because they are more common at water, and the attack rate to androchromes would be minimal.

With regard to the second prediction, our data indicate that androchromes mated less often than gynochromes. This is demonstrated by their lower mating frequency and by the smaller amount of sperm that old androchromes stored in their spermatheca. Males of *I. elegans* are able to remove sperm from the bursa copulatrix of the female during copulation, but they seem unable to remove sperm from the spermatheca (Miller 1987b). In this situation, sperm should accumulate in the spermatheca, but not in the bursa. In young mature specimens (those with thorax turquoise, olive-green or orange-ochre), androchromes also had less sperm, but differences were not significant, perhaps because the number of matings by these females was still low. It is interesting that four out of six females that had no sperm were androchromes, but these figures are too small for a statistical comparison. We think that androchromes were avoiding additional matings. This idea implies that once seized in tandem by a male, gynochromes will usually cooperate in mating, even if this is disadvantageous, otherwise androchromes would have no advantage. We therefore predict that males could mate forcibly with gynochrome females but almost never with androchromes, as was shown for *I. graellsi* in captivity (Cordero 1989; but see Fincke 1997).

The third prediction of the male-mimicry hypothesis (androchromes should mate with longer intervals) was not supported by our data.

The Density-dependent Hypothesis

This hypothesis also assumes that androchromes are male-mimics, and predicts a similar life span in all phenotypes, and a female mating rate positively correlated with male density.

When days with small sample size are excluded, androchrome mating rate was positively correlated with male density, whereas most gynochromes mated, irrespective of male density (Table III). The results of the census of 1 August contrast with this prediction because only four androchromes were observed and three were mating, precisely when the density of males was lowest. Nevertheless, we think that this data point is an outlier because we observed seven additional mature androchromes alone on the same morning (before starting the census) and no additional gynochrome female. There were significant differences between female morphs in mating frequency, differences that were not due to a shorter life span of androchromes, and can be explained only by an active refusal of additional copulations by this phenotype (see above).

It could be argued that the differences in mating rate that we observed could have been due to incorrect age estimation, that is, we scored androchrome females as mature when in fact they were still young, and therefore many of them could have died before mating. This is unlikely, because the sample of androchromes that we assumed were mature for the estimation of sperm volumes had a great number of mature eggs (the same is true for gynochromes). Furthermore, in the samples of females that were marked as tenerals (and whose age was therefore precisely known) proportionally fewer androchromes than gynochromes were observed mating and/or ovipositing.

Conclusions

Androchrome *I. elegans* females avoided unnecessary long matings; the time saved could enable them to mature more eggs. This behavioural mechanism, together with their larger size, could increase the reproductive success of this phenotype. In contrast, a greater proportion of androchromes than gynochromes failed to reproduce. This colour polymorphism is therefore not neutral in *I. elegans*.

Androchrome females of *I. graellsii* were also larger than gynochromes in one population

(Cordero 1992), but this is not the general rule in polychromatic damselflies (Cordero & Andrés 1996). A long period of high population density (when matings are longer (Cordero 1990a) and more costly) could therefore lead to an increase in the frequency of androchrome females. In fact, among all European Coenagrionids, *I. elegans* has probably the highest frequency of androchromes, although we do not know of any population where gynochromes have disappeared. We propose that this is because even in highly dense populations, there are periods of low male density, especially at the start and at the end of the flight season. During these periods, some androchromes could be unable to mate, and their reproductive success would be zero. In a state of evolutionary equilibrium, the mean reproductive success per phenotype should be the same, and this seems to be the case in our population, since the number of ovipositions per female was the same for all phenotypes.

Fincke (1994) concluded that in both *Enallagma hageni* and *E. boreale* the two colour morphs had the same mating frequency, lifespan and oviposition duration (see also Thompson 1989). She suggested that the colour morphs are neutral in these populations. Nevertheless, this interpretation seems premature (Cordero & Andrés 1996), because in *Enallagma*, androchromes are not male mimics, and, more important, oviposition is underwater and additional matings are advantageous for all female morphs, because females benefit from male help in escaping from the surface film after oviposition (Fincke 1986; Miller 1990).

We conclude that female colour polymorphism is an adaptive trait that is maintained mainly by density-dependent selection on androchromes in species with relatively long matings (probably many *Ischnura*). Other hypotheses are needed to explain why female morphs persist in species with short copulations. For instance, Grether & Grey (1996) suggested that female morphs may differ in their conspicuousness to prey rather than to predators, and therefore this polymorphism could be related to hunting efficiency. Future research should address the study of the genetic basis of colour polymorphism in more species, and test the assumptions as well as the predictions of adaptive hypotheses. The fact that long matings are costly is a crucial assumption of adaptive hypotheses that remains untested. The existence of more than one gynochrome phenotype, as in *I. elegans*, remains to be explained.

ACKNOWLEDGMENTS

We are very grateful to Michael T. Siva-Jothy and Ola M. Fincke for their comments on early versions of the manuscript. Additional comments by R. I. Vane-Wright, P. J. Mill and an anonymous referee greatly improved the manuscript. C.U. was supported by funds MURST 40% and 60%, and CNR. A.C. was supported by a travel grant from Xunta de Galicia.

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