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Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae)

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Female-limited polychromatism is frequent in many species of Odonata. *Ischnura elegans* has three colour morphs: one male-like coloured (androchrome) and two additional gynochrome brown morphs (*infuscans* and *rufescens-obsoleta* morphs). A total of 19 progenies obtained from once-mated females were reared in the laboratory in three generations. Results indicate that the colour morphs are controlled by the same genetic system as previously described for *I. graellsii*, i.e. an autosomal locus with female-limited expression and with three alleles with a hierarchy of dominance ($p^a > p^i > p^o$). Five interspecific crossings between female *I. graellsii* and male *I. elegans*, five crossings between hybrid females and male *I. elegans* and one crossing between female *I. graellsii* and a hybrid male further confirmed that the genetic system is the same in both species. A survey of morph frequencies in north-west Spain revealed that *I. elegans* shows high variability in androchrome frequency (4–91%) between nearby populations, whereas in *I. graellsii* androchromes never are the majority morph (5–40%). The highest androchrome frequency in *I. graellsii* was found in populations closest to a locality where both species have hybridized, and that now has the highest androchrome frequency of *I. elegans*. We hypothesize that *I. elegans* genes have been incorporated into the genome of *I. graellsii* resulting in increased androchrome frequency in the latter species. Low androchrome frequency in *I. elegans* seems also related to the influence of *I. graellsii* genes. Therefore, we suggest that hybridization between both taxa is contributing to the temporal maintenance of contrasting androchrome frequencies in nearby populations. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, **85**, 471–481.

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INTRODUCTION

Female-limited polymorphisms are widespread in odonates (Cordero & Andrés, 1996) and butterflies (Turner, 1978; Turner, 1983; Clarke *et al*., 1985; Herrell & Hazel, 1995; Nielsen & Watt, 2000), but appear also rarely in beetles (Bergsten, Toyra & Nilsson, 2001), copepods (Fava, 1988), lizards (Forsman & Shine, 1995; Galán, 2000) and hummingbirds (Bleiweiss, 1992). One important step in the study of these polymorphisms is to establish whether they are determined genetically. Moreover, if they are shared by closely related species, the study of their ecological requirements might contribute to explaining the maintenance of different morphs within one species (Oxford & Reillo, 1993). Two or more phenotypes will be maintained when their fitness curves cross for an environmental condition, owing to frequency-dependence or to spatial or temporal changes (Brockmann, 2001).

Two types of body coloration (male-like or androchrome and non-male-like or gynochrome) are common in many female damselflies. It has been proposed that this conspicuous polymorphism is maintained by

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hybridization between closely related species, counterbalanced by differential predation pressure. One explanation for this is that androchromes benefit by avoiding matings with males of similar species, while gynochromes commonly were involved in heterospecific sterile copulations (Johnson, 1975). A second explanation by Hinnekint (1987) suggests that androchromes are male mimics and avoid male harassment at high population density but suffer the risk of not mating at all at low population density. Inter- (Hinnekint & Dumont, 1989) and intra-annual (Cordero, Santolamazza Carbone & Utzeri, 1998) cycles in population density were hypothesized to contribute to the maintenance of this polymorphism. Recent work has nevertheless suggested that males are attracted to the commonest female morph in the population (Miller & Fincke, 1999; Van Gossum, Stoks & De Bruyn, 2001a; Fincke, 2004). In a signal-detection model Sherratt (2001) proposed that androchromes are not only more similar to males, but are also encountered more often by males, and suggested that these factors might combine to generate a balanced polymorphism. Field studies have revealed that female morphs in *Ischnura* differ in mating frequency and sperm stores (Banham, 1990; Cordero *et al*., 1998), egg load (Banham, 1990), and male avoidance techniques (Van Gossum, Stoks & De Bruyn, 2001b; Sirot *et al*., 2003). Furthermore, morphs do differ in fitness correlates in insectaries (Sirot & Brockmann, 2001; Van Gossum *et al*., 2001b). Comparative analyses suggest a relationship between reproductive behaviour and polymorphism (Robinson & Allgeyer, 1996), but no single hypothesis seems able to explain all these results (Andrés, Sánchez-Guillén & Cordero Rivera, 2002).

In this paper we study the inheritance of female colour morphs of the damselfly *I. elegans*, and compare the genetic system with the mechanism previously described for *I. graellsii* (Cordero, 1990): three alleles (p^a: androchrome, p^i: *infuscans*, p°: *aurantiaca*) co-occur with a hierarchy of dominance ($p^a > p^i > p^\circ$), each one coding for a different female morph. Androchromes have three possible genotypes (p^ap^a, p^ap^i, p^ap°), *infuscans* females (gynochrome) two (p^ip^i, p^ip°) and *aurantiaca* females (also gynochrome) only one ($p^\circ p^\circ$) (Cordero, 1990).

The genetic distance between *I. elegans* and *I. graellsii* based on DNA sequence variation of the mitochondrial cytochrome *b* and coenzyme II is only 0.196% (J. V. Robinson, pers. comm.). Furthermore, both species are very similar with respect to their ecology and morphology, but are rarely found in sympatry. *Ischnura elegans* is common all over Europe (including the Mediterranean countries) but is replaced by *I. graellsii* in the Iberian peninsula and North of Africa (Askew, 1988). Nevertheless in north-west Spain both species hybridize and produce fertile offspring (Monetti, Sánchez-Guillén & Cordero Rivera, 2002).

Hybridization and introgression are increasingly recognized as important factors in the diversification of both plants and animals, and provide an excellent opportunity to study evolutionary processes such as selection, gene flow and speciation (Harrison, 1993; Arnold, 1997; Dowling & Secor, 1997). Studies of population structure and hybrid zones have also indicated that natural hybridization is often common where conditions favouring both taxa are found in proximity and at the ecological limits of their distribution range (Dowling & Secor, 1997; Huxel, 1999). The simplest spatial pattern is where two populations overlap in a broad region of sympatry. If fertile F_1 hybrids are produced by some low rate of cross-mating, these will almost certainly backcross to one or other parental genotype. If the resulting backcrossed individuals then mate with the most similar parental genotype, then individual genes are rapidly introduced into the new genetic background (Barton, 2001).

The aim of this paper is therefore threefold. First, the mode of inheritance of female colour polymorphism will be studied in laboratory breeding of controlled crosses of both *I. elegans* and hybrids between male *I. elegans* and female *I. graellsii*, and back-crossings between hybrids and parental animals. Secondly, estimates of phenotypic morph frequencies will be determined for natural populations of *I. elegans* and *I. graellsii* in north-west Spain. Thirdly, combining knowledge of inheritance with field estimates of morph frequencies will enable us to discuss the maintenance of female colour polymorphism and outcomes of hybridization between *I. elegans* and *I. graellsii*.

STUDY ANIMALS AND METHODS

Female colour morphs of *I. elegans* and *I. graellsii*

Descriptions of phenotypical differences between males and the different female morphs of both *I. elegans* and *I. graellsii* and colour pictures of the different morphs can be found in several publications and identification guides (e.g. Askew, 1988). Three mature female morphs occur in both *I. elegans* and *I. graellsii* (Fig. 1): one blue androchrome and two brown gynochromes (Parr, 1965; Cordero, 1990). Mature androchromes of both species are a good mimic of the male blue phenotype, and can only be distinguished by visual examination of external genitalia or by the wider abdomen of mature females. The differences between the two mature gynochromes (*infuscans* and *rufescens-obsoleta* in *I. elegans* and *infuscans* and *aurantiaca* in *I. graellsii*) are minor and involve the absence of black humeral stripes on the thorax in

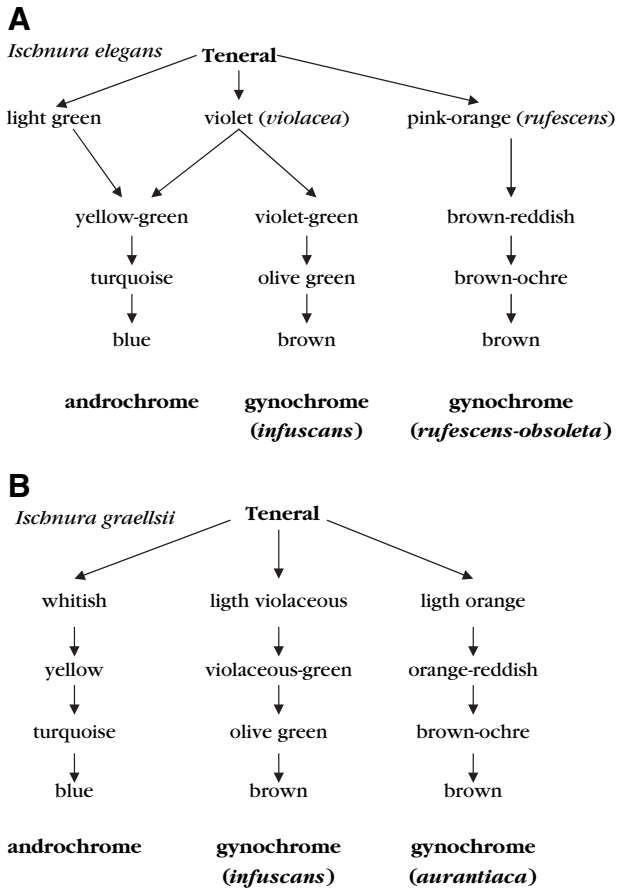

Figure 1. The relationship between colour polymorphism and age-related colour changes in females of *Ischnura elegans* and *I. graellsii*. Italic names refer to names used to designate colour phases in the literature (Parr, 1999).

rufescens-obsoleta and *aurantiaca*, while the pale parts of the thorax are slightly darker in *infuscans*. All female morphs change their colour markedly during their lifespan in an irreversible way (Parr, 1965; Cordero, 1987; Hinnekint, 1987; Cordero *et al*., 1998).

Until this study all androchrome and *infuscans* females of *I. elegans* were thought to pass through an immature violet phase (*violacea*) (Cordero *et al*., 1998). However, in this study (see below) we found that 25% of androchrome females did not pass through a violet phase, but instead developed a light green thorax, which changed to turquoise and blue (Fig. 1). Importantly, we note that this change in body coloration is identical to the changes observed in males (Parr, 1973).

Inheritance of colour morphs in *I. elegans*

Freshly emerged individuals of *I. elegans* were collected in a coastal lagoon at Louro (A Coruña, northwest Spain) in September 1999 and May 2000. All individuals were between 0 and 1 day old, hence immature and virgin. In the laboratory, animals were individually marked with a number on their wings using permanent markers, sexed and housed in insectaries (dimensions $50 \times 50 \times 50$ cm; 15–20 animals per insectary). Insectaries were lined internally with aluminium foil, to minimize escape reactions (animals tend to concentrate on the glass, where the visual sensation is of open space) (Johnson, 1975; Cordero, 1990), provided with numerous wooden sticks serving as perches and with small plastic containers filled with water (covered with a net to avoid drowning of animals) to guarantee humid conditions. Insectaries were at room temperature (20–26 °C) and received natural, indirect light through windows and additional artificial light. Culture bottles of *Drosophila melanogaster* were added to provide food. For more information on rearing methods see Van Gossum, Sánchez-Guillén & Cordero Rivera (2003).

When mature, males (> 6 days) and females (8–10 days) were put together and copulating pairs were isolated to avoid further matings of the focal female. After copulation, females were maintained during 3–4 h in oviposition glasses with a sheet of humid filter paper as oviposition substrate (Cordero, 1990). In order to maximize the number of eggs laid, mated females were given the opportunity to lay eggs daily during their entire life span (Cordero, 1991). Eggs and larvae were maintained in covered Petri dishes using tap water purified with active coal during the first generation, and spring water during the following generations. Larvae emerged after approximately 15 days. Newly emerged larvae were transferred to plastic boxes of $32 \times 20 \times 5$ cm with strips of filter paper as a substrate. Water in these boxes was changed every three days.

Larvae were fed nauplii of *Artemia salina* until they reached a length of approximately 5 mm; thereafter they were additionally fed with *Lumbriculus* and *Tubifex* (Cordero, 1990). When larvae reached the last instar, rearing containers were covered with a plastic net with numerous sticks as emergence substrate. One day after emergence, animals were individually marked with a number on the wing. Thorax colour was recorded daily, to distinguish between androchrome and *infuscans* females. Unfortunately many females died during their immature phase, a result that commonly occurs in damselflies maintained in the laboratory (Cordero, 1994), and which reduced sample size for phenotype segregation. We reared a total of 211 females from the first generation, but due to mortality in immature phases we obtained the phenotype of only 148 females. These figures were 139 (105 with known phenotype) for the second generation and 72 (all with known phenotype) for the third generation.

We obtained 11 crossings in the first generation, and four in the second and third generations. One of the crosses of the first generation (female 9 in Table 1) was obtained with a virgin female from a pond at Walenhoek (Niel, Belgium), because the *rufescens-obsoleta* morph is rare in north-west Spain (Table 3). Furthermore, two mated *rufescens-obsoleta* females were collected in the field in Spain and their progeny was mated in the second and third generations to obtain information on the genetic inheritance for this morph.

Hybrid crossings

Hybridization between *I. elegans* and *I. graellsii* is unidirectional: only female *I. graellsii* mate with male *I. elegans*. The reverse mating is impeded due to mechanic incompatibility (Monetti *et al*., 2002). We obtained five hybrid crossings with females of *I. graellsii* collected as teneral specimens in field populations in norht-west Spain and males of *I. elegans* from the first laboratory crosses. Back-crosses were obtained by mating four hybrid females with male *I. elegans* from the second laboratory generation, and a hybrid male of the first generation with one *I. graellsii* female.

Field data on morph frequencies

Between 2000 and 2002 we sampled individuals from seven localities to obtain morph frequencies for *I. elegans*. Furthermore, data on morph frequencies in five populations of *I. graellsii* during 2002–03 and published data on previously studied populations of this species were gathered (Cordero, 1990; Cordero Rivera & Egido Pérez, 1998; Andrés, Sánchez-Guillén & Cordero Rivera, 2000). As males and androchromes

Table 1. Frequencies of female morphs in progenies of three generations of *Ischnura elegans*. Bold letters after the female code indicate colour morph (A, androchrome; I, *infuscans*; O, *rufescens-obsoleta*). Individuals of the second and third generation are named after their mother's code adding an ordinal. Genotypes were deduced from a system with one autosomal locus, three alleles and a hierarchy of dominance ($p^a > p^i > p^o$). Observed frequencies indicate the number of females of each morph obtained per family. In parentheses we show an estimation of the number of females of each morph including those that did not achieve sexual maturation (see the text for further explanation)

	Parental genotypes		Individuals			Expected (%)			Observed				
Crossing	♀	♂	♂	♀	%♂	A	I	O	A	I	O	χ^2	*P*
First generation													
♀1 **A** × ♂1	$p^a p^?$	$p^a p^a$	33	39	46	100			35 (39)			–	–
♀2 **I** × ♂1	$p^i p^{i/o}$	$p^a p^a$	24	26	48	100			19 (26)			–	–
♀3 **A** × ♂2	$p^a p^{i/o}$	$p^i p^i$	14	15	48	50	50		4 (8)	3 (7)		0.14	0.705
♀4 **A** × ♂2	$p^a p^i$	$p^i p^i$	14	12	54	50	50		3 (8)	0 (4)		3.00	0.083
♀5 **A** × ♂3	$p^a p^a$	$p^a p^i$	75	53	59	100			23 (53)			–	–
♀6 **O** × ♂3	$p^o p^o$	$p^a p^i$	21	25	46	50	50		4 (12)	7 (13)		0.82	0.366
♀7 **I** × ♂4	$p^i p^{i/o}$	$p^a p^i$	14	11	56	50	50		5 (6)	5 (5)		0.00	1.000
♀8 **I** × ♂4	$p^i p^{i/o}$	$p^a p^i$	24	25	49	50	50		6 (18)	2 (7)		2.00	0.157
♀9 **I** × ♂5	$p^i p^o$	$p^a p^o$	11	5	69	50	25	25	3 (3)	0	2 (2)	1.80	0.407
♀10 **A** × ♂6	$p^a p^?$	$p^? p^?$	17	16	52	100			12 (16)			–	–
♀11 **A** × ♂6	$p^a p^?$	$p^? p^?$	35	29	55	100			15 (29)			–	–
Second generation													
♀A.1 **A** × ♂C	$p^a p^o$	$p^a p^i$	36	49	42	75	25		25 (33)	13 (16)		1.72	0.190
♀A.2 **O** × ♂9.2	$p^o p^o$	$p^a p^i$	49	54	48	50	50		15 (19)	24 (35)		2.08	0.150
♀7.1 **A** × ♂B.1	$p^a p^i$	$p^i p^o$	18	17	51	50	50		3 (7)	6 (10)		1.00	0.317
♀9.1 **O** × ♂B.3	$p^o p^o$	$p^a p^o$	18	19	49	50		50	6 (6)		13 (13)	2.58	0.108
Third generation													
♀C11 **A** × ♂9.1.1	$p^a p^o$	$p^o p^o$	40	35	53	50		50	14 (14)		21 (21)	1.40	0.237
♀C12 **A** × ♂9.1.2	$p^a p^a$	$p^a p^o$	10	9	53	100			9 (9)			–	–
♀C13 **I** × ♂9.1.2	$p^i p^o$	$p^a p^o$	9	8	53	50	25	25	4 (4)	2 (2)	2 (2)	0.00	1.000
♀9.1.3 **O** × ♂9.1.2	$p^o p^o$	$p^a p^o$	25	20	56	50		50	10 (10)		10 (10)	0.00	1.000

Individuals with codes A and B are from the progenies of two field-collected females with *rufescens-obsoleta* phenotype. Male C lost his mark, and therefore his family is unknown.

are not easily distinguished at a distance, morph frequency estimates were determined after collection of all observed adults with a net. Individuals were either marked or retained until the end of sampling, and afterwards released. In *I. graellsii*, morph frequency can be unambiguously estimated even in immature specimens (Fig. 1). In *I. elegans, rufescens* females are easily distinguished. For the remaining phenotypes, we considered that all immature specimens with a black mark on the dorsum of the eighth abdominal segment were *infuscans* females, although this might slightly underestimate *infuscans* frequency because 14% of *infuscans* females did not develop this mark in the laboratory (see Results).

Statistical tests

Observed and expected phenotypic frequencies were compared using χ^2 tests of goodness-of-fit (Sokal & Rohlf, 1995). The sex ratio was calculated to test whether gynochrome genotypes are lethal in males.

RESULTS

Inheritance of colour morphs

The segregation of female phenotypes in all progenies is shown in Table 1. Results indicate that females can produce offspring of one, two or three phenotypes and that when two phenotypes are present they segregate in proportions of 3:1 or 1:1. In a single crossing of the third generation we obtained all phenotypes in a proportion of 2:1:1.

These results are compatible with the hypothesis that the inheritance of this polymorphism is controlled by a single autosomal locus with three alleles and a hierarchy of dominance ($p^a > p^i > p^\circ$). According to this hypothesis the androchrome morph has three possible genotypes (p^ap^a, p^ap^i, p^ap°), the *infuscans* morph two (p^ip^i, p^ip°) and the *rufescens-obsoleta* morph is homozygous for the recessive allele ($p^\circ p^\circ$). Table 1 shows the deduced genotypes based on this hypothesis. Observed frequencies did not significantly differ from the expected frequencies. Some males were used in several crossings (male 9.1.2 in three crossings with all female phenotypes), and in all cases the observed segregation of female morphs was in agreement with the proposed mode of inheritance.

The sex ratio did not differ from 1:1 in any family, which suggests that none of the genotypes is lethal in males. Females that died before sexual maturation were assigned to the most probable phenotype, taking into account that all females with a black mark on the eighth abdominal segment are *infuscans* (see below). This is an underestimate of the *infuscans* frequency, because 13 out of 92 *infuscans* females (14%) developed the *infuscans* phenotype in the absence of a black mark on the eighth abdominal segment. These more speculative frequencies (Table 1) are also compatible with the proposed inheritance system.

Hybrid crossings

The segregation of female morphs obtained from hybrid crosses is in agreement with a single genetic system controlling the polymorphism in both species (Table 2). As a consequence of high mortalities in hybrid progenies, we only obtained 46 females in the first generation and 56 females in the second generation. The observed morph frequencies corroborate the mode of inheritance as the same in *I. elegans* and *I. graellsii*.

All immature hybrid *infuscans* females ($N = 4$) showed the black mark on the eighth segment just as *infuscans* females of *I. graellsii* have. However, in the hybridization back crosses, one *infuscans* female ($N = 14$) did not present this mark, as some *infuscans* females of *I. elegans* did not. Regarding thorax coloration, we observed several differences in the immature *infuscans* and androchrome morphs between both species. In *I. graellsii,* the thorax presents a whitish coloration in the first 2–4 days of adult life (Cordero, 1990). In *I. elegans* the coloration of the thorax of these morphs is violet (Parr, 1973; Cordero *et al*., 1998). In the immature hybrid females, thorax coloration was whitish in the first generation, but violet in the second.

Morph frequencies in natural populations

In all sampled field populations of both species all three female morphs occurred (with one exception, likely a consequence of small sample size). Androchrome frequencies were highly variable in *I. elegans* in north-west Spain: range 4–91% (Table 3, Fig. 2). In contrast, androchrome frequencies for populations of *I. graellsii* from the same area were always below 50% (5–40%).

The highest frequencies of androchromes for *I. graellsii* were found in three localities (Xuño, Corrubedo and Vixán) geographically near to populations with the highest androchrome frequencies for *I. elegans*. These three populations were compared with Lanzada, O Rosal, Campus and O Castelo in a contingency table (all populations sampled between 1999 and 2002 and with a sample size of 28–68 females; see Table 3). Results indicate highly significant differences between populations ($\chi^2 = 52.9$, d.f. = 12, $P < 0.001$). The lowest frequency of androchromes in *I. elegans* was found in Doniños (see Fig. 2), a locality near to two localities of hybridization (Monetti *et al*., 2002). We have obtained the first data

Table 2. Frequencies of female morphs in hybrid progenies between *Ischnura graellsii* and *I. elegans*. Bold letters after the female code indicate colour morph (A, androchrome; I, *infuscans*). Females of the first generation are field collected specimens of *I. graellsii* that were mated to males of *I. elegans* from the first laboratory generation (see Table 1 for their mothers code). Of the second-generation crossings, female D is a field collected *I. graellsii* specimen that was mated to a hybrid male. The other females are hybrids from the first generation, named after their mother's code adding an ordinal, and were mated to males of *I. elegans* from the second or third laboratory generation (see Table 1 for their mothers code). Other data as in Table 1

	Parental genotypes		Individuals			Expected (%)			Observed				
Crossing	♀	♂	♂	♀	%♂	A	I	O	A	I	O	χ^2	*P*
First generation													
♀1 **I** × ♂10.1	$p^a p^{i/\circ}$	$p^a p^i$	7	8	47	50	50		1 (4)	3 (4)		0.00	1.000
♀2 **I** × ♂1.1	$p^i p^{i/\circ}$	$p^a p^a$	5	5	50	100			4 (5)			–	–
♀3 **A** × ♂1.1	$p^a p^?$	$p^a p^a$	10	6	63	100			6 (6)			–	–
♀4 **I** × ♂1.2	$p^a p^{i/\circ}$	$p^a p^a$	11	14	44	100			8 (14)			–	–
♀5 **A** × ♂11.1	$p^a p^i$	$p^a p^a$	10	13	43	100			11 (13)			–	–
Second generation													
♀D **I** × ♂2.1	$p^i p^i$	$p^a p^i$	5	7	42	50	50		3 (6)	1 (1)		1.00	0.317
♀2.2 **A** × ♂2.1.1	$p^a p^i$	$p^i p^{i/a}$	3	3	50	50	50		1 (1)	2 (2)		0.33	0.564
♀5.1 **A** × ♂B.4	$p^a p^i$	$p^a p^\circ$	15	18	47	75	25		10 (12)	6 (6)		1.33	0.248
♀5.2 **A** × ♂B.3	$p^a p^i$	$p^a p^\circ$	21	10	68	75	25		7 (7)	3 (3)		0.13	0.715
♀*1.1 **A** × ♂9.2–5.3	$p^a p^i$	$p^a p^i$	26	19	58	75	25		13 (17)	2 (2)		1.09	0.297

*Female 1.1 was mated to two males of the same genotype.

on morph frequency in two populations of *I. graellsii* from southern Iberia (Table 3), and in both cases data are very similar to northern populations.

DISCUSSION

Inheritance of colour morphs

The mode of inheritance of body coloration reported for *I. graellsii*, i.e. control by an autosomal locus with three alleles and hierarchical dominance (Cordero, 1990), is also supported for *I. elegans*. Yet, in *I. graellsii*, the colour phenotype of all females can be determined immediately after emergence (Cordero, 1992b). This is because the *infuscans* morph shows a black mark on the dorsum of the eighth abdominal segment that allows easy separation from the androchrome morph. Moreover, the antehumeral lines are much broader in *infuscans* than in androchromes (Cordero, 1992b). In contrast, in *I. elegans* 14% of *infuscans* females lack the black mark and cannot be distinguished from androchromes until they develop the mature coloration. The absence of the violet colour phase in some androchromes (Fig. 1) remained unnoticed in previous work (but see Longfield, 1949), and might have important consequences for the interpretation of variability in behaviour and fitness correlates in androchromes.

The first generation of hybrid females were more similar to their mother phenotype (*I. graellsii*) in body coloration and black marks on the eighth abdominal segment, but when the proportion of *I. elegans* genes increased in the second generation, as a consequence of crossing hybrid females with males of *I. elegans*, they became more similar to *I. elegans*.

Dominance hierarchies of species with a studied mode of inheritance indicate dominance of the androchrome allele in *I. elegans* and in *I. graellsii* (Cordero, 1990), but recessivity of this allele in *I. damula*, *I. demorsa* and *C. tenellum* (Johnson, 1964; Johnson, 1966; Andrés & Cordero, 1999). Following the general assumption that for a polymorphic locus the ancestral allele is recessive to those that have evolved subsequently (a principle known as Haldane's sieve; Clarke *et al.*, 1985) this suggests two alternative ancestral situations depending on the species. To resolve whether female colour polymorphisms originated several times many more species should be studied and insights into the phylogenetic relationships should be achieved.

Hybridization and female morph frequencies

Our results indicate that the genetic system controlling the inheritance of colour morphs is identical in

Table 3. Frequencies of female morphs in natural populations of *Ischnura elegans* and *I. graellsii* in Galiza (north-west Spain) and southern Iberia (last two populations). The Universal Transverse Mercator (UTM) coordinates are shown. *N*, sample size; A, androchrome; I, *infuscans*; O, *rufescens-obsoleta/aurantiaca*. Frequencies based on less than 50 females are less likely to correctly estimate morph frequency (Cordero Rivera & Andrés, 2001)

					Observed frequencies (%)			
Population	UTM	Species	Date	*N*	A	I	O	Source
Foz	29TPJ4123	*I. elegans*	vi.2001	70	62.9	35.7	1.4	This paper
Cedeira	29TNJ7030	both + hybrid	vi.2001	67	22.4	71.6	6.0	This paper
Doniños	29TNJ5515	*I. elegans*	vii.1987	34	5.9	88.2	5.9	This paper
		I. elegans	vi.2001	51	3.9	92.2	3.9	This paper
Laxe	29TNH0085	*I. elegans*	vi.2001	26	50.0	50.0	0.0	This paper
		I. elegans	vii.2002	34	41.2	55.9	2.9	This paper
Traba	29TMH9681	*I. elegans*	vi.2000	15	66.7	33.3	0.0	This paper
Carnota	29TMH9241	*I. elegans*	vi.2000	36	80.6	16.7	2.8	This paper
		I. elegans	vi.2001	69	91.3	5.8	2.9	This paper
Louro	29TMH9234	*I. elegans*	vi.2000	55	85.5	10.9	3.6	This paper
		I. elegans	vii.2000	35	85.7	14.3	0.0	This paper
		I. elegans	v.2001	51	84.3	11.8	3.9	This paper
Muro (Xuño)	29TMH9720	*I. graellsii*	vi.2002	53	39.6	52.8	7.5	This paper
Vixán	29TMH9810	*I. graellsii*	vii.1989	37	29.7	64.9	5.4	Cordero (1990)
		I. graellsii	vi.2002	52	34.6	51.9	13.5	This paper
Corrubedo	29TMH9514	*I. graellsii*	viii–ix.1988	94	18.1	70.2	11.7	Cordero (1990)
		I. graellsii	vii–viii.1989	97	11.3	78.4	10.3	Cordero (1990)
		I. graellsii	vii.1999	28	21.4	60.7	17.9	Andrés *et al*. (2000)
O Castelo	29TNH2568	*I. graellsii*	vii.1999	42	11.9	76.2	11.9	Andrés *et al*. (2000)
A Lanzada	29TNH1000	*I. graellsii*	vii.1999	75	14.7	76.0	9.3	Andrés *et al*. (2000)
		I. graellsii	vi.2002	33	18.2	75.8	6.1	This paper
		I. graellsii	viii.2002	40	15.0	80.0	5.0	This paper
Lourizán	29TNG2695	*I. graellsii*	viii–ix.1986	29	20.7	75.9	3.4	Cordero (1990)
		I. graellsii	v.1987	65	16.9	76.9	6.2	Cordero (1990)
		I. graellsii	viii–ix.1987	672	13.7	75.7	10.6	Cordero Rivera & Egido Pérez (1998)
		I. graellsii	v–vi.1988	85	8.2	78.8	12.9	Cordero (1990)
		I. graellsii	ix.1988	107	17.8	71.0	11.2	Cordero (1990)
Salcedo	29TNG2896	*I. graellsii*	vii–viii.1985	523	18.0	69.2	12.8	Cordero (1990)
Barra	29TNG1179	*I. graellsii*	viii–ix.1996	766	14.4	76.1	9.5	Cordero Rivera & Egido Pérez (1998)
Campus	29TNG2568	*I. graellsii*	vii–viii.1995	440	5.0	78.4	16.6	Cordero Rivera & Egido Pérez (1998)
		I. graellsii	vii.1999	68	1.5	89.7	8.8	Andrés *et al*. (2000)
O Rosal	29TNG1744	*I. graellsii*	viii.1986	46	6.5	78.3	15.2	Cordero (1990)
		I. graellsii	viii–ix.1990	1097	10.0	77.9	12.0	Cordero Rivera & Egido Pérez (1998)
		I. graellsii	vii.1999	68	8.8	85.3	5.9	Andrés *et al*. (2000)
Castro Verde	29SBN8974	*I. graellsii*	iv.2003	48	18.8	72.9	8.3	This paper
Doñana	29SQC1908	*I. graellsii*	vi.2003	77	10.4	76.6	13.0	This paper

the two studied *Ischnura* species. Both field and laboratory studies (Monetti *et al*., 2002) show that these species hybridize. Females of *I. graellsii* readily mate in the laboratory with males of *I. elegans*, but the resulting hybrids are incapable of intermating or mating with males of *I. graellsii*. Nevertheless, hybrid males are able to mate with *I. graellsii* females and hybrid females to mate with *I. elegans* males (R. A. Sánchez-Guillén & A. Cordero Rivera, unpubl. data).

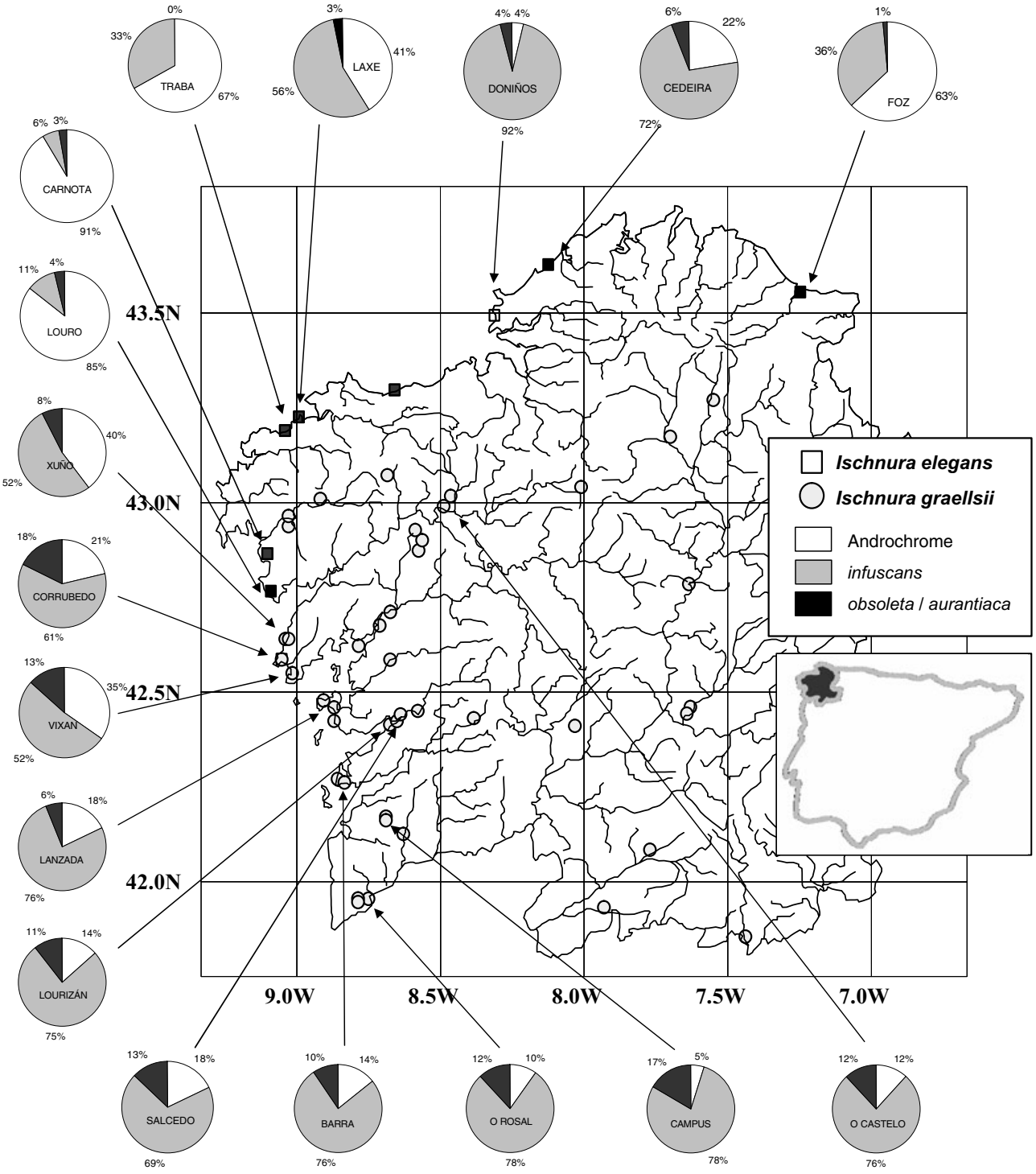

Figure 2. Colour morph frequencies for natural populations of *Ischnura elegans* and *I. graellsii* in north-west Spain. Black squares indicate localities where both species have hybridized (Foz and Louro) or are currently hybridizing (Cedeira).

If hybrids are fertile and back-cross with one of the parental species, hybridization can result in genetic introgression, which may increase the genetic variability of the introgressed species (Grant & Grant, 1992, 1994). Extensive hybridization may even lead to extinction of one or both parental species (Rhymer, Williams & Braum, 1994; Allenford & Leary, 1998). The dynamics of hybridization is substantially influ-

enced by the details of mate choice and the genetic system (Klingenberg, Spence & Mirth, 2000). For instance crosses between *Limnoporus dissortis* and *L. notabilis* (Gerridae) produce surviving offspring that are almost exclusively male (Spence, 1990). Moreover, because females prefer to mate with smaller males, hybridization and introgression occurs primarily through matings of the relatively large *L. notabilis* females with the smaller *L. dissortis* or hybrid males (Spence, 1990).

Unidirectional mating and observations on body coloration and pattern in offspring of hybrid crosses suggest the introgression of genes of *I. elegans* into populations of *I. graellsii*. This is suggested by estimations of phenotypic morph frequencies for populations of *I. elegans* and *I. graellsii*. The highest androchrome frequency in *I. graellsii* is found in populations that are near populations of *I. elegans* where androchromes are the most frequent morph (Fig. 2).

Probably, *I. elegans* is a recent immigrant in the area (the first record of this species in north-west Spain is from 1984, whereas *I. graellsii* is known in the area since 1917). Furthermore this species is dispersing through the area; several individuals of *I. elegans* were found among hundreds of specimens of *I. graellsii* in at least five coastal localities (Xuño, Corrubedo, Vixán, Lourizán and Barra; Table 3). Furthermore, many individuals from Cedeira were clearly hybrid (based on body coloration and anal appendages morphology) when sampled (June 2001), and there is evidence for past hybridization in Foz and Louro, two localities that now only have *I. elegans* populations (Monetti *et al*., 2002). Finally, in 2003 we found a high proportion of hybrids and a mixture of *I. graellsii* and *I. elegans* in two (formerly) populations of *I. graellsii* (Cordero, pers. observ.). Founder effects might explain the initially high frequencies of androchromes in *I. elegans*, a species that shows striking variability in morph frequencies in different countries (Hinnekint, 1987; Banham, 1990; Van Gossum *et al*., 1999). Alternatively, this prevalence of androchromes in north-west Spain could also be explained if this morph were more likely to disperse. However, female morphs of *I. elegans* and *Coenagrion puella* do not differ in dispersal rates (Conrad *et al*., 2002).

As explained above, our results suggest a relationship between hybridization (areas marked as black squares in Fig. 2) and frequency of androchromes in both species. Unfortunately we lack historical records of morph frequency to test this hypothesis. Owing to the unidirectional mating that produces the absorption of *I. graellsii* by *I. elegans* (Monetti *et al*., 2002), this last species also might have absorbed the typical frequencies of female morphs of *I. graellsii* in places where genes of this last species were prevailing, for instance Cedeira and Doniños. At other localities genes of *I. elegans* might have been incorporated into *I. graellsii* populations and the frequency of androchromes is rising as a consequence. But if founder effects caused most *I. elegans* immigrants to be androchromes, then androchrome frequencies in *I. elegans* should be decreasing in at least some populations. An alternative explanation for the geographical patterns in morph frequencies is that a common environmental variable is affecting populations of both species and increasing the frequency of androchromes in the same geographical area (note that all areas of hybridization are coastal localities; Fig. 2). Molecular evidence showed that morph frequencies are under stabilizing selection in north-west Spain in *I. graellsii* (Andrés *et al*., 2000) and *Ceriagrion tenellum* (Andrés *et al*., 2002). Nevertheless, the environmental variables contributing to this stabilizing selection remain elusive. Future studies of natural morph frequencies of both *I. elegans* and *I. graellsii* in different regions in the Iberian Peninsula, adjacent countries of Europe and northern Africa should reveal whether female morph frequencies in north-west Spain are aberrant. For the moment, the only two populations of *I. graellsii* studied from southern Spain and Portugal (Table 3) are very similar to northern populations.

Recent studies on the maintenance of female polymorphism in odonates underlie a relationship between population density and morph frequency, and morph-specific fitness correlates (Cordero Rivera & Egido Pérez, 1998; Sirot & Brockmann, 2001; Andrés *et al*., 2002; Sirot *et al*., 2003). Banham (1990) found that androchromes of *I. elegans* mate less often, store a smaller amount of sperm and have lower egg load than gynochromes, and suggested that mating frequency is determined by egg load rather than colour morph: females that have matured at least 150 eggs mate irrespective of colour phenotype. The cause for the lower egg load of androchromes is unknown (Banham, 1990). According to male mate choice experiments, males predominantly prefer to mate with the most common female morph in the population (Miller & Fincke, 1999; Van Gossum *et al*., 2001a). However, female morphs differ in mating avoidance tactics (Van Gossum *et al*., 2001b; Sirot *et al*., 2003) and mating frequencies (Banham, 1990; Cordero *et al*., 1998). Female morph fitness correlates are influenced not only by morph frequency, but also by population density and sex ratio (Van Gossum *et al*., 2001b). Changes in female morph frequency due to introgression or an environmental variable will therefore have effects on female morph fitness correlates. As a consequence, hybridization is likely to have important implications for the maintenance of multiple female morphs only during short periods when two species with contrasting androchrome frequencies start to hybridize.

In summary, we have found that a single genetic system controls female colour morphs in both species and hybrids. Furthermore we have evidence for past and present hybridization between both species (Monetti *et al*., 2002), and this is likely producing introgression of *elegans* genes into *graellsii* populations. These facts together are probably contributing to the temporary maintenance of a broad spectrum of morph frequencies in geographically near populations. Hybridization, the first mechanism proposed for the maintenance of this polymorphism (Johnson, 1975), was not supported in previous studies (Cordero, 1992a; Cordero & Andrés, 1996), but nevertheless seems important for the temporal maintenance of this polymorphism under limited circumstances.

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